The underlying mechanisms for nitrogen and phosphorus removal in high rate algal ponds used to treat brewery effluent; Harvesting algae using filter-feeding fish; The use of brewery effluent in agricultural crop production; and Duckweed as wastewater treatment solution

Report to the
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by

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Executive summary

1. Introduction

Project Eden is an experimental facility at Ibhayi Brewery in Port Elizabeth, which consists of an integrated algal ponding system, a constructed wetland, aquaculture, hydroponic and crop production facilities. It was designed to treat brewery effluent using alternative technologies that add value to the products that are produced as part of the process.

The initial phases of the project have demonstrated a technology that offers a more stable and environmentally sustainable alternative to conventional activated sludge systems, with the potential of adding considerable value to the process. The system produced clean water available for reuse or use in downstream activities, algae, fish and vegetables. The primary objectives, which were to provide evidence that the concept works and preliminary optimization data, were achieved and the data that supports the delivery of these objectives were presented in the WRC project that preceded this one (WRC Report TT 601/14).

A number of gaps for future research were identified in the earlier work which are addressed in the current study:

1. We needed to develop a better understanding of community-structure and underlying-mechanisms of the processes of nutrient removal in the algal ponds.
2. Removing the algae was identified as a bottleneck and it was suggested that an alternative approach where algal biomass is converted directly into filter-feeding-fish biomass be investigated.
3. The use of duckweed as an alternative method to high rate algal ponding (HRAP) in lowering nutrient concentrations in brewery effluent using duckweed, and the use of this plant as a fish feed supplement.
4. The use of HRAP treated brewery effluent as a water and nutrient source for hydroponic crop production, which started during the project that preceded this one.
5. The use of HRAP treated brewery effluent as water and nutrient source in crop irrigation and the effect that this water source has on soil.

2. Microbial community structure and mechanisms of nutrient removal

Tertiary treatment of wastewater using high rate algal ponds (HRAPs) is achieved through aerobic degradation of organic matter by bacteria and fungi. This aerobic degradation results in the release of inorganic substances such as carbon dioxide (CO_2), phosphate (PO_4) and ammonia (NH_3). The concentrations of these inorganic substances are lowered in the effluent through combinations of bacterial nitrification, algal assimilation, NH_3 volatilization, continuation of the abiotic nitrogen and phosphorus cycles and PO_4 precipitation. When the HRAPs used in wastewater treatment are operating optimally, they can effectively remove nitrogen and phosphorus from the effluent. The overall aim of his work was to identify the organisms in the pond under different conditions and to monitor the change in nutrient concentration under these conditions, to develop a better understanding of which mechanisms were at work in the HRAP.

Microalgae in the HRAP lowered the nitrogen and phosphorus concentration in the brewery effluent through algal assimilation and probably made the greatest contribution under conditions conducive to microalgal growth, where algal biomass and chlorophyll concentrations increased. Similarly, bacterial nitrification also played a role and was responsible for the oxidation of NH_4-N into NO_2-N

and NO_3-N in the HRAP. This was supported by the presence of nitrifying bacteria identified through metagenomics and the elevated concentrations of NO_2-N and NO_3-N in the effluent when microalgae and bacteria consortium were cultured at pH 7.0 and 8.5. The laboratory trials, where ammonia concentrations were lowered in the absence of microorganisms, and the high temperatures and pH of the HRAP, suggest that as much as 50% of the ammonia in the HRAP might have been removed from the system due to abiotic mechanisms, such as ammonium volatilization and the continuation of the abiotic nitrogen cycle.

Microalgal growth, increases in chlorophyll (Chl) *a* concentration and nutrient removal from the post-AD brewery effluent were influenced by pH and temperature. Summer conditions (with its longer day length, higher temperatures, and subsequently faster rates of chemical reaction) and slower flow rates (i.e. higher HRTs) favoured algal productivity and the performance of the HRAP in lowering NH_4-N in the effluent. Under these circumstances, algal assimilation contributed considerably to effluent treatment but the high pH of the ponds suggests that a large portion of the nitrogen was probably removed through ammonium volatilization. The partial removal of PO_4-P, on the other hand, appeared to be related to algal assimilation. During winter and at increased flow rates, algal productivity and nutrient reduction efficiency was decreased substantially. However, under these conditions there were shifts in the bacteria community structure that saw the proliferation of faster growing species such as the Proteobacteria, which have a high affinity for nutrients and include some nitrifying bacteria. This suggests that the mechanism of nutrient removal in the HRAP in winter and at increased flow rates (i.e. conditions that do not favour algal productivity) could shift in favour of bacterial nitrification. However, many bacterial nitrifiers are slow growing and, as such would be washed out at reduced hydraulic retention times, and the relative abundance of known nitrifiers among the identified species was still low in winter and at high flow rates; more research is needed to identify the bacterial species that are present under these conditions and that appear to be responsible for lowering nutrients in the effluent. The hypothesis that there is probably a shift in favour of bacterial nitrification in winter is supported by the lower pH values recorded in autumn and winter, which are (a) more favourable for bacterial nitrification and (b) less favourable for ammonium volatilization than the higher pH values seen in spring and summer which would be mediated by algal photosynthetic activity.

3. Algal "harvesting" using fish

Removing microalgal cells remains a challenge in producing an effluent that meets discharge requirements when using high rate algal ponds (HRAP) in wastewater treatment. Filter feeding fish can remove microalgae from effluent treatment ponds; however, due to the high organic content of brewery effluent and substantial fluctuations in pH and oxygen concentration associated with high algal concentrations, it remains to be seen if fish are able to cope with such a dramatic change in their environment and to determine the rate of removal. It has been hypothesised that pre-treatment in HRAP might contribute towards this end.

The overall aim of this study was to investigate the use of phytoplankton-feeding fish as in-situ filter feeders, as an affordable, alternative method that reduces algal biomass in treated brewery wastewater.

Mozambique tilapia ingested microalgae from the HRAP effluent and algae were present in a large proportion of the intestine of all the fish in these experiments. However, based on isotopic δ15N values, the algal biomass did not appear to contribute substantially to nutrient assimilation in fish tissue, since these values differed from those of the algae by an estimate of two trophic levels. It is

possible that the fish were unable to digest and assimilate the nutritive value contained within the microalgal cells that were ingested, due to reduced digestion efficiency as a result of environmental stress, such as high pH and free-ammonia.

These fish can be used to reduce the biomass of algae in HRAP effluent at a rate of at least 200 mg algae/mL effluent/g fish/day. However, the absolute rate at which algal biomass was removed by the tilapia in this study was confounded by the production of algae in the fish culture tanks. The relative rate of algal removal demonstrated that withholding formulated feed does not improve the rate of filter feeding. On the contrary, fish fed a commercial formulated feed removed significantly more algal biomass from HRAP effluent than those that had access to microalgae only. On its own, the algae had insufficient nutrients and the fish's growth, energy reserves and general health were compromised when additional feed was withheld and this probably reduced their filter feeding efficiency.

This work has demonstrated the potential for using fish to remove algae from HRAP pond effluent, but more research is needed before recommendations can be made for the application of this method of removing algae from treated effluent.

4. Duckweed as an effluent cleaning agent and a feed source for fish

Integrated algal ponding systems are being used to treat brewery effluent, but harvesting the algae remains a constraint in this process. Duckweed could potentially be used as an alternative to algae as it has been used for wastewater renovation, is easy to harvest (being a macrophyte) and has been used to supplement animal feed due to its high protein and carbohydrate content. This study aimed to evaluate the nutrient removal efficiency of duckweed grown on brewery effluent and its quality as a tilapia feed supplement.

Duckweed was grown in 40 L containers filled with brewery effluent or a nutrient solution that represents river water (control). Duckweed and its associated micro-organisms where efficient at removing dissolved nitrogen and phosphorus from brewery effluent. They removed on average 3.14 ± 0.54 mg/L/d of dissolved nitrogen and 1.38 ± 0.25 mg/L/d of dissolved phosphorus which equated to an average of 95.28 ± 1.89% of total dissolved nitrogen and 90.79 ± 4.11% of dissolve phosphorus.

Tilapia (18.32 ± 1.94 g/fish) growth was compared when fish were fed either commercial feed, duckweed grown in river-like water or brewery effluent or a combination of feed and duckweed. After 42 days fish fed brewery effluent grown duckweed only and river-like duckweed only where smaller (23.53 ± 1.15 g; 22.82 ± 3.88 g) than fish fed commercial feed supplemented with brewery effluent grown duckweed (37.78 ± 2.00 g) or river-like duckweed (39.38 ± 2.94 g) and commercial feed only (41.46 ± 4.53 g; ANOVA, $p = 0.0001$). There was no significant difference in the weight of tilapia fed commercial feed only and tilapia fed commercial feed supplemented with brewery effluent grown duckweed and river-like duckweed (ANOVA, $P > 0.05$).

In conclusion, duckweed grows on brewery effluent and removes dissolved nitrogen and phosphorus. Future work needs to focus on direct comparisons between the rate of efficiency with which nitrogen and phosphorus are lowered in duckweed and microalgal ponding systems. Tilapia readily eat brewery effluent grown duckweed, but it cannot be used as a full replacement for commercial tilapia feed. Duckweed makes for a good feed supplement at levels up to 50% of the feed.

5. Treated brewery effluent as a hydroponic water and nutrient source

Brewery effluent subject to anaerobic digestion and treatment in a primary-facultative pond contained sufficient nutrients to support the growth of *Lycopersicum escolentum* "Moneymaker" tomatoes. The adjustment of the effluent pH with phosphoric acid to between pH 6.0 and 6.5 significantly increased plant growth compared to those in unaltered effluent. The pH adjusted effluent-grown plants grew to a mean height of 831 ± 21 mm and a dry biomass weight of 42.3 ± 2.8 g compared to the unaltered pH effluent plants which grew to a height of 411 ± 21 mm and a weight of 7.7 ± 0.7 g after 49 days. Similarly, initial fruit production was higher for plants grown in pH adjusted effluent compared with those with no pH control. Effluent treatment in high-rate algal ponds (HRAP) prior to use in the hydroponic tomato system had no apparent benefits. The effluent-grown plants did not perform as well as plants grown in inorganic-fertilizer and municipal water. This was probably due to plant stress related to brewery effluent alkalinity, ammonium nutrition and nitrogen limitation, for example, and requires further investigation.

6. The use of treated brewery effluent as a water and nutrient source in irrigated crop production, and its effect on the soil

To determine if brewery effluent treated in AD, primary facultative pond (PFP), HRAP and CW systems might be suitable for crop irrigation, test crops of cabbage (*Brassica oleracea* cv. Star 3301) were produced in soil, and were irrigated with effluent drawn from different source.

Cabbage grew significantly larger when irrigated with post-AD, post-PFP or post-HRAP effluent, compared to those irrigated with post-CW effluent or the control plants which was irrigated with water only. Brewery effluent can be used to improve conventional crop yields, due to the addition of organic nutrients in the treated brewery effluent. However, the yield of crops grown using brewery effluent was 13% lower than cabbage plants irrigated with water that was supplemented with an inorganic fertiliser. The relatively high conductivity (3019.05 ± 48.72 $\mu s/cm^2$) of treated brewery effluent may be the main factor for the reduced cabbage yields, combined with lower nutrient levels in the effluent compared to that of the inorganic fertiliser treatment. Post-HRAP and post-CW brewery effluent were the least suitable for irrigated crop production due to the higher conductivity and lower nutrient content of these treated effluents.

After three months, soils irrigated with post-AD and post-PFP brewery effluent had a significantly higher sodium content and sodium adsorption ratio (3919 ± 94.77 mg/kg and 8.18 ± 0.17) than soil irrigated with a commercial nutrient solution (920.58 ± 27.46 mg/kg and 2.20 ± 0.05; $p<0.05$), which remains a cause of concern. However, this was not accompanied by a deterioration in the soil's hydro-physical properties, nor a change in the metabolic community structure of the soil. Nonetheless, after prolonged irrigation with treated brewery effluent, sodium is likely to build up in the soil in the long-term and this can be expected to be accompanied by a deterioration in the soil physical structure (but this remain to be tested).

Brewery effluent improved crop production compared to crops grown using conventional water only and there the use of this effluent did not have a negative influence on soil character; however, the long-term effect of salt accumulation in the soil needs to be considered and should be the focus of future research. The benefits of developing this nutrient and water resource for use in downstream agriculture could contribute to cost-reductions at the brewery, more efficient water, nutrient and energy management and job creation, all with the potential of improving food security in local communities.

7. Recommendations for application of this technology in agriculture

The work presented here has, again, demonstrated the value of alternative effluent treatment technologies and it has demonstrated that they can result in alternative downstream activities such as hydroponic vegetable production or conventional crop irrigation. Tomato plants grown in treated brewery effluent did not grow as well as those grown using inorganic hydroponic solution, but the manipulation of the pH of treated effluent resulted in a significant improvement in growth. Furthermore, the production of cabbages grown in the soil and irrigated with treated brewery effluent was significantly greater than those irrigated with a conventional water source. In both hydroponic tomatoes production and the irrigation of cabbage crops grown in the soil, the use of brewery effluent as a nutrient source was inferior to the use inorganic fertilizes; however, the compromised growth rates need to be considered in relation to the added value associated with the potential of producing an organic product.

Millions of litres of organic effluent are discharged to municipal sewers every day in South Africa and around the world. The technology that we are developing here and the results of this research demonstrate the potential of recovering the nutrients and water in this effluent for downstream use in agriculture.

8. Recommendations for the brewery

The use of HRAP as an alternative, environmentally sustainable, low-tech, low-maintenance, resilient, dynamic and self-regulating, onsite effluent treatment solution remains an option for treating brewery effluent, particularly if the effluent is to be made available for reuse or for use in downstream agriculture. However, there are challenges that need to be addressed regarding minimizing nutrient pollution and finding a solution for managing the alkalinity of the effluent that comes from the anaerobic digester. The alkalinity of the brewery wastewater remains one of the key concerns moving forward if it is to be used as nutrient supply for hydroponics, and this requires further investigation in the future. The alkalinity is less of a concern in conventional crop irrigation since the soil tends to act as a pH buffer. However, the potential build-up of salts in the soil, when using treated brewery effluent as a water and nutrient source in crop production, is another challenge that the breweries will face if the treated brewery effluent is to be used to irrigate crops.

Altering the up-stream management practices of the anaerobic digester and cleaning practices in the brewery could potentially address both the alkalinity and the problem associated with salts in the effluent. For example, alternative detergents could be used in the brewery, or effluent streams could be split, and alternative methods of pH control could be used in the anaerobic digester. These approaches should be considered in the structural design of future breweries and alternative cleaning procedures should be considered if brewery effluent is going to be made available for downstream use in agriculture in the future.

9. Recommendations for future research

While this work has contributed to our understanding of the shifts in community structure and associated mechanisms, it has also identified a gap in our knowledge: The majority of the organisms in high rate algal ponds (HRAP) under certain conditions remain “unknown” (in some instances about 50% of the bacteria and close to 75% of the eukaryotes were “unknown”) and for us to fully understand the mechanisms responsible for nutrient dynamics in these ponds these organisms need to be characterised. This must be done in future research.

Controlled grazing experiments, which would provide further insight on the impact of zooplankton grazing on microalgae and bacteria, is another area that requires further investigation. This would further enhance the understanding of the microbial shifts that take place under different conditions.

There is potential for using fish to remove algae from HRAP pond effluent. However, we have not reached the point where we can make recommendations to apply this research; further research is required. This future research needs to focus on developing methods/technologies aimed at mitigating the negative effects that HRAP water chemistry has on fish physiology; for example, adjusting pond effluent chemistry prior to exposing fish to the water or possibly using alternative fish species that are better adapted to withstand the extreme environmental conditions of HRAP effluent. There are species of fish that are morphologically more adapted to remove algae that have settled out of suspension; either way, future work should also focus on making sure that fish have greater access to algae by increasing the portion of the algal biomass that forms a biofloc that is either in suspension or settled. This work also needs to be designed to ensure that filtration rates of the fish can be adequately estimated, taking algal productivity and the effect that algal settlement and microalgal grazers have on algal biomass into account.

Similarly, more research into the use of duckweed in nutrient removal from brewery effluent is needed before practical recommendations can be made. The rate of nutrient removal by duckweed is highly dependent on temperature and it was interesting to observe that phosphate removal by duckweed is probably more consistent than phosphate removal by algae; however, the experiments here were not designed to make direct comparisons between algal and duckweed systems, but this could be looked at in future work. Duckweed was successfully used as a fish feed supplement and it was substantially easier to harvest the duckweed compared with unicellular algae.

When brewery effluent was used to produce cabbages in the soil, it did not negatively influence soil character; however, we are still concerned that the long-term build-up of salts in the soil might compromise the use of this effluent as a water and nutrient source. Although the built-up salt could be addressed by changing the cleaning chemicals used upstream in the brewery, it could also be addressed by investigating the use of halophytic plants as part of the treatment process. Future work could also focus on the use of crops that are known to reduce the build-up of salt in the soil.

Finally, brewery effluent can be used as a water and nutrient source in hydroponic vegetable production, with the added advantage of a hydroponic product that has the potential of organic certification. However, the growth of hydroponic crops in brewery effluent is inferior to that of hydroponic crops grown using inorganic fertilisers. Future research should be carried out to: (a) identify alternative crops that might be better suited to brewery effluent as a nutrient source; and (b) optimise the use of brewery effluent as a potential organic nutrient source in hydroponic crop production.

10. Conclusion

The drop in nutrient concentration in the high rate algal ponds used to treat brewery effluent was due to a combination of mechanisms that include algal assimilation, bacterial nitrification, ammonia volatilization and the abiotic nitrogen and phosphorus cycles. The microbial species in the ponds were characterised under various environmental conditions such as different flow rates (i.e. hydraulic retention times) and different times of year, and the corresponding mechanisms responsible for the changes in nutrient concentration were investigated under different environmental conditions too. It is the shift in population structure and corresponding mechanism of

nutrient removal and dynamic nature of these communities and their ability to change rapidly that makes these systems so adaptable and able to treat dynamic effluent streams.

The benefits of fully understanding the mechanisms of nutrient removal from HRAP ponds used to clean brewery effluent and further developing this nutrient and water resource for downstream reuse could contribute to cost-reductions at breweries and other similar water users. It is also likely to result in more efficient water, nutrient and energy management, and the creation of downstream job opportunities with the potential of improving food security in local communities. This research continues to contribute towards social, economic and environmentally sustainable water management practices.

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CONTENTS

Acronyms and Abbreviations

AD - Anaerobic digester
AFP - Air filled porosity
AS - Activated sludge
AWCD - Average well colour development
BE - Brewery effluent
bwt - body weight
CCI - Chlorophyll concentration index
CEC - Cation exchange capacity
CFU - Colony forming units
Chl - Chlorophyll
COD - Chemical oxygen demand
COD_F - Chemical oxygen demand – filtered to 8.0 µm
CW - Constructed wetland
DAFF - Department of Agriculture, Fisheries and Forestry
DIFS - Department of Ichthyology and Fisheries Science, Rhodes University
DO - Dissolved oxygen
DWS - Department of Water and Sanitation (previously Department of Water Affairs – DWA)
EBRU - Institute of Environmental Biotechnology Research, Rhodes University
EC - Electrical conductivity
H - Shannon-Weaver index
HRAP - High rate algal pond
HRT - Hydraulic retention time
NS - Nutrient solution
OD - Optical density
PAR - photosynthetically active radiation
PCR - Polymerase chain reaction
PFP - Primary facultative pond
R - Richness
SAB - South African Breweries Ltd
TDS - Total dissolved solids

1. Introduction

The Department of Ichthyology and Fisheries Science at Rhodes University and SAB Ltd constructed the Project Eden facility as an experimental effluent treatment site at Ibhayi Brewery in Port Elizabeth. The sight consisted of an integrate algal ponding system, a constructed wetland, an aquaculture facility and a hydroponic facility and it was designed to treat brewery effluent using alternative technologies that extract value from the products that are produced as part of the treatment process.

We partnered with the Water Research Commission (WRC) and our initial project was successful in achieving its goals of: (a) demonstrating a technology that offers a more stable and environmentally sustainable alternative to conventional activated sludge systems, and (b) showing that water and nutrients can be recovered for reuse or for use in downstream activities. This was done using little more than the sun's energy and natural systems. We adopted a multidisciplinary approach where a unique integration of existing technologies was used: Industrial effluent was treated using (1) anaerobic-digestion (AD), (2) high-rate-algal-ponding (HRAP) and (3) constructed-wetlands (CW) to make water and nutrients available for (4) algal, (5) vegetable, (6) fish and (7) fish-feed production. The primary objectives, which were to provide evidence that the concept works and preliminary optimization data, were achieved. The data that supports the delivery of these objectives were presented in WRC project K5/2008 (Jones *et al.* 2014).

A number of gaps for future research were identified in the earlier work, two of which are addressed in the current study:

1. We needed to develop a better understanding of community-structure and underlying-mechanisms of the processes of nutrient removal in the algal ponds (Chapter 2); and

2. Harvesting the algae was identified as a bottleneck and it was suggested that an alternative approach where algal biomass is converted directly into filter-feeding-fish biomass be investigated (Chapter 3).

This report includes an additional three deliverables that did not form part of the WRC K5/2284 list of deliverables and these have been included here for various reasons. Nearly all of the students that joined this program brought with them a freestanding student bursary, so it was possible to increase the capacity and subsequently the outputs of the project; furthermore, the work covered in these additional deliverables contribute to the overall aim of our Rhodes University/SAB Ltd/WRC research program:

3. The use of duckweed as an alternative method to HRAP in lowering nutrient concentrations in brewery effluent using duckweed, and the use of this plant as a fish feed supplement (Chapter 4). This work formed part of the program's broader aims and was indirectly supported by K5/2284.

4. The use of HRAP treated brewery effluent as a water and nutrient source for hydroponic crop production started during the WRC K5/2008 that preceded this project. This was an additional piece of work that was not required to complete the K5/2008 list of deliverables and these data were collected after the completion of K5/2008 final report. We have taken the opportunity to report these findings here (Chapter 5).

5. The use of HRAP treated brewery effluent as water and nutrient source in crop irrigation and the effect that this water source has on soil (Chapter 6). Potential exists to beneficiate brewery effluent and to adjust effluent characteristics using algal ponding so that it can be used in crop irrigation. Again, this did not form part of the original list of deliverables but contributes to the overall goal of this program.

2. Mechanisms of nutrient removal in high rate algal ponds

This section of the project is a continuation of the work that was carried out for the Water Research Commission (WRC) at Rhodes University (Jones *et al.* 2014) that characterised and optimised the removal of nutrients from brewery effluent using algal ponding, with an emphasis on adding value to the treatment process (i.e. beneficiation). This previous work did not focus on the mechanisms of nutrient removal and the microbial community structure responsible for these mechanisms so the current project was designed to address this gap.

The WRC funded this research. In addition, the student received a freestanding bursary from the Department of Agriculture that covered her student fees and contributed to her living costs. The SAB Ltd made the research site available and the cost of the metagenomic work (which was not budgeted for in the WRC project) was covered by the Department of Agriculture. The metagenomic work is reported here as it contributes to work funded by the WRC.

MSc student Mmathabo Mogane completed this work and the majority of this section of the report. Parts of this report have been taken directly from her draft MSc thesis (Mogane 2016) and parts of this report are being prepared for publication as they appear here.

2.1.1 Rationale

The Ibhayi Brewery is billed by the municipality for treating its waste effluent, and there are considerable cost savings if there is a reduction in the chemical oxygen demand (COD) content of the effluent (Cilliers 2012). The on-site treatment facility uses a drum filter as a primary treatment that separates solid items from the brewery effluent (Cilliers 2012). Subsequently, this primary treated effluent stream is split: one portion is discharged directly to the municipal sewer and the remaining portion further undergoes secondary treatment in an anaerobic digester to biodegrade its organic matter content (Cilliers 2012). The purpose of the anaerobic digestion process is to reduce the amount of COD reporting to the municipal wastewater treatment plant, because charges are levied by the local authority based on COD in the effluent stream (Cilliers 2012). The post-anaerobically digested (post-AD) brewery effluent stream is further treated in an activated sludge digester, an aeration basin, a clarifier and then discharged into the municipal sewer after chlorination (Cilliers 2012).

A portion of the effluent leaving the Ibhyai Brewery anaerobic digester is diverted to a small wastewater treatment pilot plant for further treatment (i.e. Project Eden), which is the focus of this study. Here, further treatment of the post-AD brewery effluent is the subject of an on-going piloting study which uses both microalgae and bacteria biocatalysts to lower the ammonium-nitrogen (NH_4-N) and phosphate-phosphorus (PO_4-P) present in the post-AD brewery effluent to acceptable levels for discharge into the environment (DWA 2004). The microalgae-bacteria assemblages are cultured in shallow open ponds circulated by paddle wheels which provide turbulent energy to ensure mixing of the biocatalysts with the effluent. These ponds are called high rate algal ponds (HRAPs) because of their high biological productivity. In HRAPs, organic matter in the effluent is aerobically degraded by a consortium of bacteria and fungi (Aguirre *et al*. 2011; Kshirsagar 2013). Aerobic degradation of organic matter by bacteria and fungi results in the release of CO_2, ammonia (NH_3) and PO_4 which are assimilated into biomass by autotrophic microalgae populations in the HRAPs (Aguirre *et al*. 2011; Kshirsagar 2013).

The HRAPs at the Ibhayi Brewery site are considered as a potential solution for brewery effluent treatment. Cilliers (2012) found that the HRAPs were efficient in the removal of NH_4-N and PO_4-P from the post-AD brewery effluent. However, the microbial complexes and the underlying

mechanisms responsible for the removal of NH_4-N and PO_4-P from the post-AD brewery effluent were not fully described or understood.

A better understanding of which microalgae-bacteria consortia are present in the HRAPs and how NH_4-N and PO_4-P are removed from the post-AD brewery effluent under different environmental conditions can give an idea of how the HRAPs function. An understanding of the functioning of HRAPs would provide wastewater treatment plants with the management tools to optimise the treatment of effluent when using an algal ponding system.

It is known that abiotic, biotic and operational factors affect HRAPs productivity and performance in nutrient removal (Madigan & Martinko 2006). Abiotic factors that have a major role in influencing the performance of the microbial community in the HRAPs includes the intensity of the photosynthetically active radiation (PAR) on microalgae growth, the effects pH on nutrient solubility and availability, the effects of temperature on microbial metabolism and productivity, the effect of dissolved CO_2 on pH levels and the concentrations of dissolved O_2 which are required by bacteria for the degradation of organic matter in an aquatic system (Johnson 2010). Operational factors are those elements which can be manipulated and used to manage the performance of the HRAPs. Such factors include pond depth and its influence on light penetration, which has an impact on the light regime to which photosynthetic organisms are exposed. Other operational factors include the effects of turbulence on nutrient availability and exposure to light intensity and the hydraulic retention time (HRT) of effluent in a pond (Johnson 2010). Biotic variables include competition between microorganisms for resources, zooplankton grazing on microalgae/bacteria and the effect of infectious pathogens, such as parasites, on microalgae (Larsdotter 2006, Johnson 2010; Markou & Geogakakis 2011). All the above factors affect the microbial community structure and can result in shifts in their relative abundance (Addy & Green 1996).

2.1.2 Aims and objectives

The purpose of this study was to create a better understanding of the biotic and abiotic changes occurring in the HRAPs at different times of the year and at different HRTs. It was also aimed at creating a better understanding of the underlying mechanisms responsible for the removal of NH_4-N and PO_4-P from the post-AD brewery effluent using HRAP. The specific objectives were to:

- establish the identity of microorganisms present in a HRAP using light microscopy and metagenomics work, describe the microbial community structure (microalgae and bacteria) and nutrient removal from the post-AD brewery effluent in an HRAP at different times of the year;
- establish the identity of microorganisms present in HRAPs using light microscopy and metagenomics work, describe the microbial community structure (microalgae and bacteria) and nutrient removal from the post-AD brewery effluent in an HRAP while operating at different HRTs; and
- determine the effects of environmental parameters, such as temperature and pH on the nutrient removal from the post-AD brewery effluent.

2.2 Materials and methods

2.2.1 Seasonal variation of microorganisms and physicochemical parameters in a high rate algal pond

About 1000 L/d of effluent was drawn from the Ibhayi Brewery anaerobic digester tank and decanted into 5000 L anaerobic buffer tank. The anaerobic buffer tank discharged effluent into a circular

primary facultative pond (PFP) with a depth and volume of 108 cm and 16730 L. It was situated inside a greenhouse covered with clear polycarbonate sheeting (Rhino Plastics Pty Ltd). Effluent from the PFP decanted into a splitter box, which divided the effluent into two streams. Effluent from each side of the splitter box flowed by gravity into two parallel HRAP systems (i.e. HRAP train-A and HRAP train-B). Each train consisted of two ponds in series, i.e. HRAP A1 and HRAP A2 (A-train), and HRAP B1 and HRAP B2 (B-train). These ponds were made of a green polyvinylchloride pond liner supported by a galvanised metal frame (Figure 2.1). The first pond of each train was approximately 25.0 cm deep, with a surface area of 14.8 m^2 and a volume of 3700 L/pond (Cilliers 2012). Effluent was gravity fed into the second pond of each train, which had a depth of 11.5 cm, with a surface area of 15.0 m^2 and a volume of 1700 L/pond (Cilliers 2012). Each pond had a paddle wheel, which continuously stirred the effluent and maintained the algal cells in suspension. Post-HRAP A2 and post-HRAP B2 effluent was decanted into a 500 L sump placed below the ground.

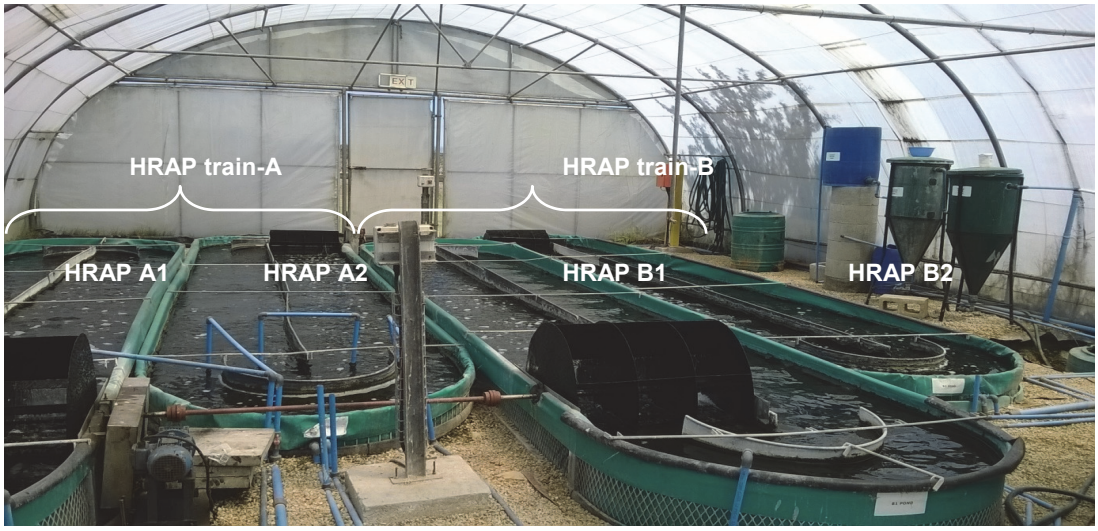

Figure 2.1 The high rate algal ponds (HRAPs) consist of two parallel trains, each train includes two ponds run in series where the numbers 1 and 2 represents the first and second ponds in system A and B respectively.

The HRAP A train was operated at a flow rate of 1000 L/d for 12 months. The system was treated as a continuous flow system during daylight hours (08:00-16:00 h) and then ran as a batch culture for the remaining 16 h of the day, while paddle wheels remained operational throughout. Temperature and pH of the effluent were measured from the post-AD effluent, in the PFP and HRAP A2 (Section 2.2.4). The microbial community structure, algal biomass and Chl *a* concentrations were analysed from water samples collected in the HRAP A2 during this period (Section 2.2.4). The NH_4-N, NO_2-N, NO_3-N, PO_4-P and COD_F were analysed from post-AD, post-PFP and post-HRAP A2 effluent (Section 2.2.4).

Data were presented as a monthly mean with a standard error. Linear regression analysis was used to compare some of the water quality parameters with algal biomass or Chl *a* concentration, which was determined at $p \leq 0.05$.

2.2.2 The microbial community structure and physicochemical parameters in high rate algal ponds at different inflowing effluent rates

The flow rate of post-PFP effluent into the HRAP A train was maintained at 1000 L/d (i.e. an hydraulic retention time, HRT: 5.4 days) as a control/reference for 21 days, while that into HRAP B train was progressively increased from 1000 to 2200 L/d over the same period (Figure 2.2). Effluent flowed through HRAP B2 at 1000 L/d for seven days before data were collected for the first time, which took

place on day zero (Figure 2.2). The flow rate was subsequently increased to 1400 L/d (HRT: 3.9 days) from day zero to day seven, at which time the second sample was taken at day-7 (Figure 2.2). Flow rate was then increased to 1800 L/d (HRT: 3.0 days) from day seven to day 14, and then to 2200 L/d (HRT: 2.5) from day 14 to day 21, with samples taken at the end of each seven-day period. Data were collected from the HRAP A2 at the same intervals, only the flow into HRAP A2 was maintained at 1000 L/d (Figure 2.2).

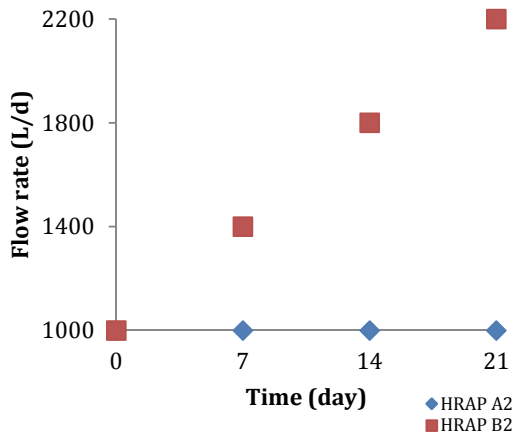

Figure 2.2 The flow rate of post-primary facultative pond (post-PFP) effluent into the parallel high rate algal ponds (HRAP A2 and HRAP B2). The hydraulic retention time at 1000, 1400, 1800 and 2200 L/d was 5.4, 3.9, 3.0 and 2.5 days, respectively.

Temperature and pH of the effluent were measured from the post-AD effluent, in the PFP, HRAP A2 and HRAP B2 (Section 2.2.4). The microbial community structure, algal biomass and Chl *a* concentrations were analysed from water samples collected in the HRAP A2 and HRAP B2 (Section 2.2.4). The NH_4-N, NO_2-N, NO_3-N, PO_4-P and COD_F were analysed from post-AD, post-PFP, post-HRAP A2 and post-HRAP B2 effluent (Section 2.2.4).

Data collected from the HRAP B2 were described using linear regression (Statistica, version 12) where the independent variable included the range of flow rates tested in this experiment. The slope of the linear regression model was determined at $p \leq 0.05$. Graphs were generated using Microsoft Excel (Version 2007).

2.2.3 The removal of nitrogen and phosphorus from anaerobically digested brewery effluent using microorganisms taken from the high rate algal ponds cultured under different temperatures and pH

The experiment was carried out in a controlled environment room. The effects of temperature (20.0 and 30.0°C) and pH (7.0, 8.5 and 10.0) on the growth of microorganisms and the removal of nutrients from the post-AD brewery effluent were investigated over a period of six days.

Glass tanks were half filled with tap water were used as water baths. Thermostatically controlled heaters were used to heat up and maintain the water at the selected temperature. The effluent used had the same chemical properties, and other environmental conditions in the laboratory were standardized. The temperature and pH were monitored frequently to ensure they were within the desired range. Fluorescent bulbs and strip lights were used as a source of illumination providing an average PAR of 77.61 μmol/cm/s. A timer switch was used to maintain a photoperiod of 12 h darkness and 12 h light.

Glass, round-bottom flasks were filled with microalgae culture and carbon dioxide (CO_2) was used to lower the pH in the cultures. Another experiment that served as a control used hydrochloric acid (HCl) to lower the pH. If the CO_2 or HCl decreased the pH too much, sodium hydroxide (NaOH) was used to bring the pH to the required level. The control of HCl pH adjustment method was used to account for the masking effects of bubbled CO_2 on algae growth. The flasks treated with CO_2 and HCl were represented at the three pH levels (7.0, 8.5, 10.0), and it was also represented in triplicate (i.e. three flasks per pH treatment). The flasks were continuously aerated to keep the algal cells in suspension and covered with a perforated transparent wrap to allow sufficient illumination while reducing influx of contaminates (Figure 2.3).

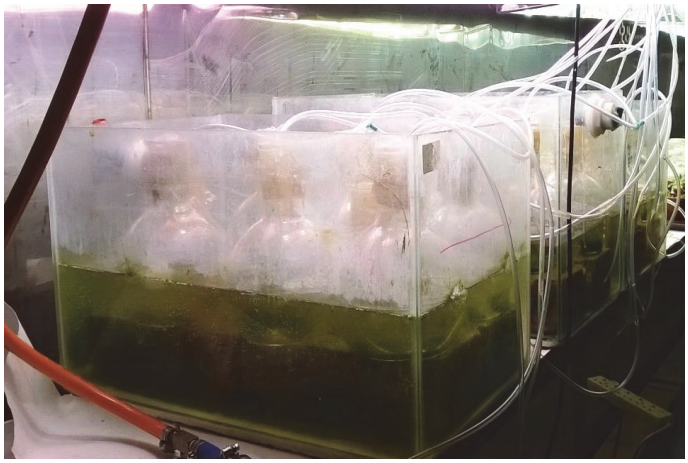

Figure 2.3 Glass tanks (water baths) with flasks containing microalgae culture.

To demonstrate the loss of NH_4-N from the effluent through mechanisms other than microbial activity, an additional control treatment was included for the NH_4-N data set. This included a flask of autoclaved growth medium that was not inoculated with microorganism isolated from the HRAP.

The microbial community structure was analysed from the water sample taken at the start of the experiment (Section 2.2.4), while Chl *a* concentrations were measured at two days intervals (Section 2.2.4). The NH_4-N, NO_2-N, NO_3-N and PO_4-P concentrations were analysed from all the flasks daily (Section 2.2.4).

Data were analysed with multivariate analysis of variance (MANOVA; Statistica, version 12.0) at $p \leq 0.05$. Graphs were plotted using Microsoft Excel (Version 2007). Some of the collected data were fitted with a slope using a linear regression model (Statistica, version 12.0). The slope of the linear regression model was determined at $p \leq 0.05$.

2.2.4 Materials and methods common to all experiments

Microalgae collected in the water samples were examined under a light microscope. The identity of microalgae was established visually with the aid of algae identification guidelines provided by Belcher & Swale (1976), Taylor *et al*. (2007) and Bellinger & Sigee (2010).

Microorganisms were also identified using metagenomics. Water samples were filtered through a 0.20 µm membrane filter (Sterivex, Millipore Catalog # SVGPL10RC). The deoxyribonucleic acid (DNA) was extracted using a commercially available DNA extraction kit (PowerWater ®Sterivex DNA isolation kit; MO BIO Laboratories, Inc.). The genomic material was amplified through polymerase chain reaction (PCR) and sequenced using Illumina sequencer (Inqaba Biotechnical Industries Pty Ltd). The primer pair 566F and 1200R was used to amplify the 18S rRNA region while the primer pair

27F and 518R was used to amplify 16S rRNA region. For every sample, reads yield were analysed using a Basic Local Alignment Search Tool (BLAST) programme. The reads percentage represented the relative abundance of the species.
To determine algal biomass, water samples of a standard volume were filtered through a glass fibre filter paper (Whatman GF/F) with a pore size of 0.70 µm and a diameter of 47.00 mm was weighed. The filter paper with algal cells was dried in an oven at 80.0℃ for 24 h. After 24 h, the filter paper with algae was weighed. The weight of dry algal matter was obtained by subtracting the weight of the filter paper from that of filter paper with algae, and the biomass was calculated by dividing this mass by the volume of the original water sample.

The water samples were also filtered through a 0.70 µm glass fibre filter paper (Whatman GF/F) to determine the chlorophyll (Chl) *a* concentration (Arar & Collins 1997). Filter papers with algal cells were kept in a freezer at -20.0℃ until Chl *a* extraction (Arar & Collins 1997). The Chl *a* concentrations was determined according to Jeffrey & Humphrey (1975) method using a trichromatic equation (Kim *et al*. 2014).

Temperature was measured together with pH values using an electronic probe (Hanna, model: HI 98129, United Kingdom). The raw pH values were anti-logged and then the average and standard errors were calculated, where after these values were logged so that data were presented as pH.

Ammonium-nitrogen (hypochlorite ion method – EPA 350.1, US Standard Methods 4500 – NH_3 D), nitrite-nitrogen (N-[1-naphtyl] ethylenediamine dihydrochloride – NO_2-N; EPA 354.1, US Standard methods 4500-NO_2^- B), nitrate-nitrogen (NO_3-N; 2,6-dimethylphenol DMP method, US Standard methods 4500-NO_3^- B), phosphate-phosphorus (ammonium vanadate and ammonium heptamolybdate method, US Standard methods 4500 PC) and the chemical oxygen demand (COD_F; potassium dichromate method – EPA 410.4, US Standard Methods 522OD) were measured using commercial test kits (Merck Pty Ltd) and a spectrophotometer (Pharo 100 Spectroquant, Merck).

2.3 Results

2.3.1 Seasonal variation

Microbial community structure in the high rate algal pond
The microalgae community structure varied seasonally, with more taxa observed during summer and fewer taxa in winter (Table 2.1, Figure 2.4). However, some species such as *Chlorella* sp., *Scenedesmus* sp. and *Chlamydomonas* sp. were present in the pond throughout the year (Table 2.1). There was also a shift in the bacterial community and its relative abundance at different seasons (Figure 2.5).

Table 2.1 Microorganisms, particularly microalgae in a high rate algal pond (HRAP A2) over 12 months identified using a light microscopy. The tick (√) indicates the presence of a particular microorganism in the HRAP.

	Time (month-year)											
	2013			2014								
Microalgae species	**October**	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**
Scenedesmus sp.	√	√	√	√	√	√	√	√	√	√	√	√
Chlorella sp.	√	√	√	√	√	√	√	√	√	√	√	√
Diatoma sp.	√	√	√	√	√	√	√	√	√	√	√	√
Chlamydomonas sp.	√	√	√	√	√	√	√	√	√	√	√	√
Microcystis sp.	√	√	√	√	√	√	√	√	√	√	√	√
Phormidium sp.	√	√	√	√	√	√	√	√	√	√	√	√
Synechococcus sp.	√	√	√	√	√	√	√	√	√	√	√	√
Arthrospira sp.	√	√	√	√	√						√	√
Pediastrum sp.	√	√	√	√	√						√	√
Navicula sp.		√	√	√							√	√
Rotifer		√	√	√	√							
Nitzschia sp.		√	√	√	√							√
Haematococcus sp.		√	√	√	√	√	√	√	√			
Euglena sp.		√	√	√								
Nematoda			√	√	√							
Macrothrix									√			
Oocystis sp.	√	√										
Ostracod	√											
Oscillatoria sp.			√	√	√							
Stigeoclonium sp.	√	√	√	√								√
Fritschiella sp.		√										
Chroococcus sp.	√	√	√									
Tribonema sp.	√	√	√									
Spirogyra sp.				√								

Microalgae biomass and chlorophyll a concentrations

Algal biomass in the HRAP increased from October to February, a period that covered the austral summer (Figure 2.6). The algal biomass then decreased during winter (July), and then, began to rise again in spring (September). Chlorophyll *a* concentration, as expected, followed similar trends to algal biomass (Figure 2.6). The algal biomass and Chl *a* concentrations demonstrated seasonal trends.

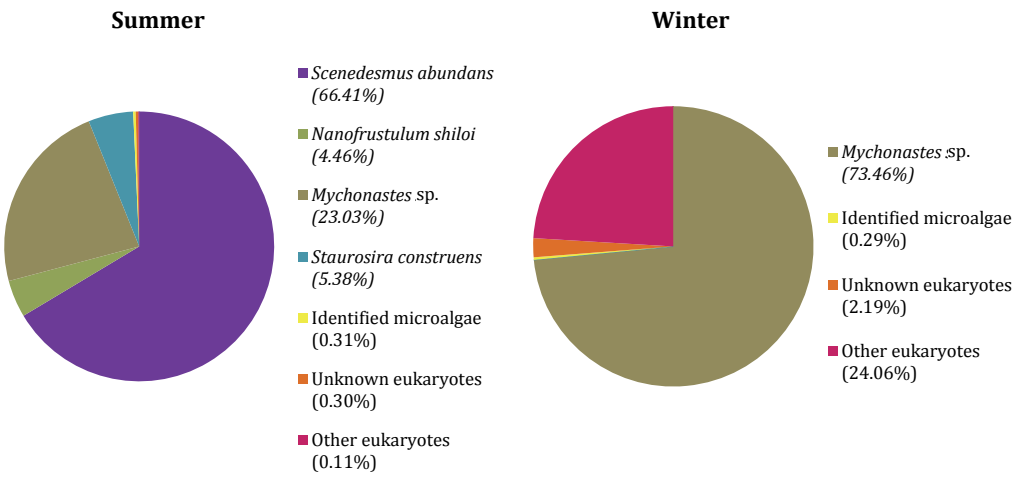

Figure 2.4 The microalgae community structure and their relative abundance as percentage (%) in a high rate algal pond (HRAP A2) with post-primary facultative pond (post-PFP) effluent flowing into HRAP A1, and then HRAP A2 at a rate of 1000 L/d during summer, winter and spring identified using metagenomics. Other eukaryotes refer to non-algal species which were also amplified with the universal primer pair 566F and 1200R.

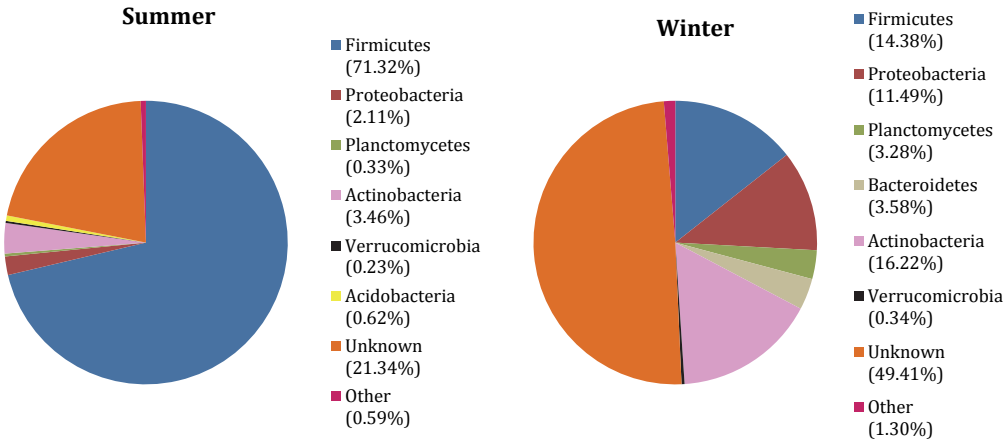

Figure 2.5 The bacterial community structure at a phylum level and its relative abundance as percentage (%) in a high rate algal pond (HRAP A2) with post-primary facultative pond (post-PFP) effluent flowing into HRAP A1, and then HRAP A2 at a rate of 1000 L/d during summer, winter and spring.

Water quality parameters

The monthly mean temperatures of effluent emerging from the anaerobic digester into the PFP and then the HRAP A2 showed a decrease along the effluent treatment system (Figure 2.7). In the post-AD, PFP and HRAP A2 effluent, the lowest monthly mean temperatures were recorded during winter, while the highest mean temperatures were recorded in summer.

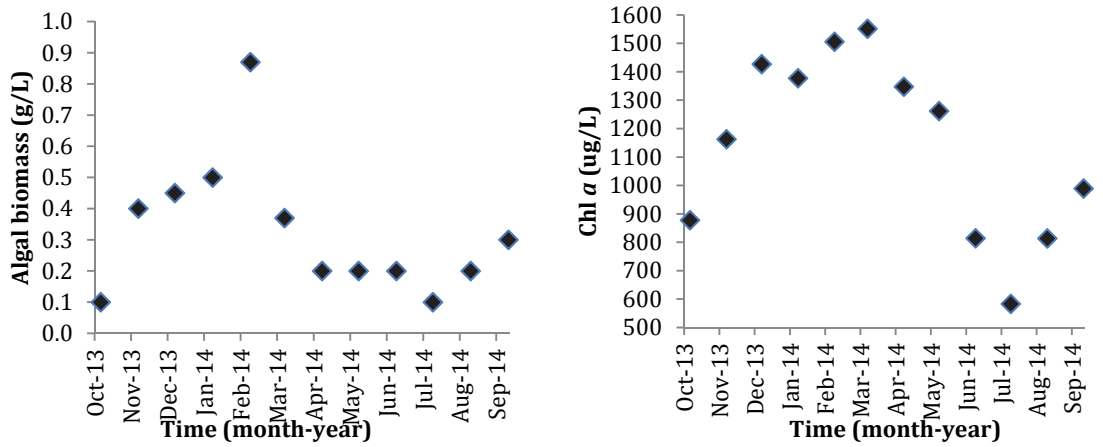

Figure 2.6 The algal biomass and chlorophyll *a* (Chl *a*) concentration in high rate algal pond (HRAP A2) over 12 months (n = 12).

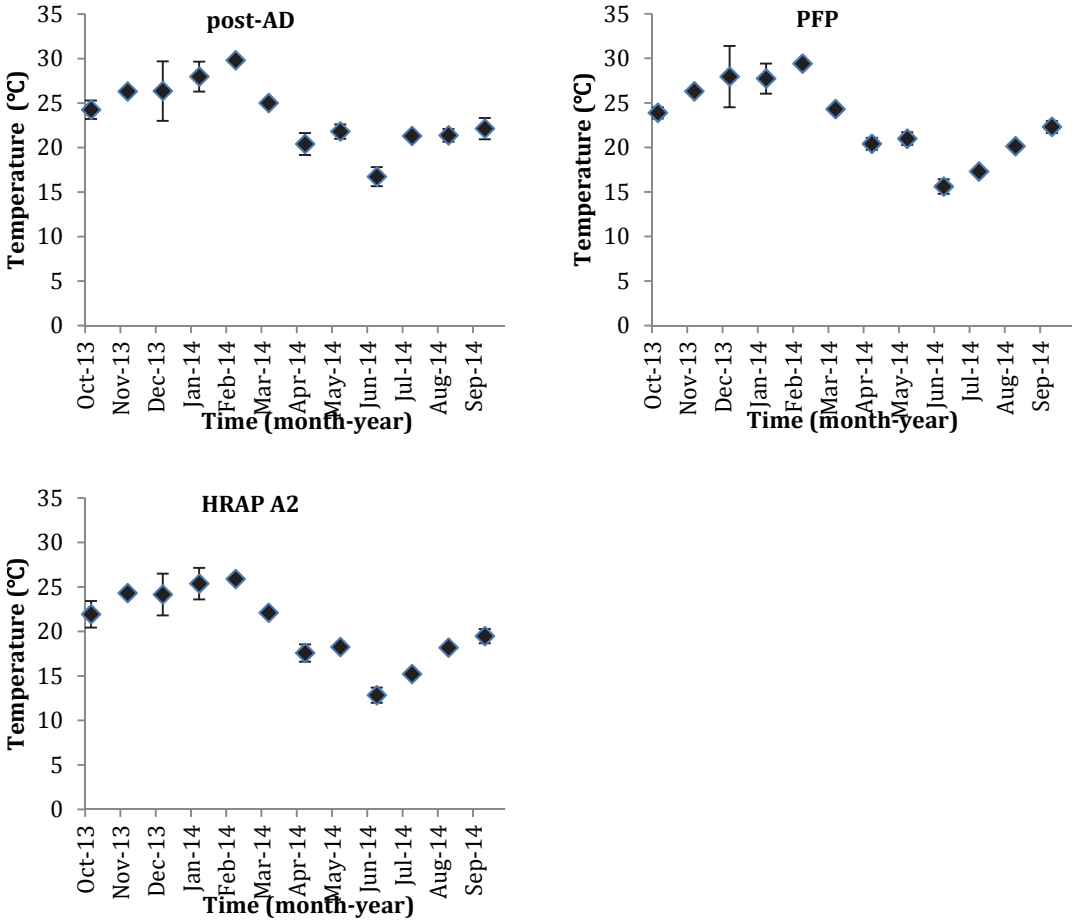

Figure 2.7 The monthly mean (± standard error) temperature in post-anaerobically digested (post-AD) brewery effluent, primary facultative pond (PFP) effluent and high rate algal pond (HRAP A2) effluent over 12 months.

The pH values in the post-AD and PFP effluent were lower than in the HRAP A2 effluent (Figure 2.8). The values ranged from 6.39 ± 0.61 to 9.00 ± 0.00 in post-AD effluent and PFP effluent. In the HRAP A2 treated effluent, the pH ranged from 7.25 ± 0.75 to 10.23 ± 0.21 with the highest average pH values recorded during spring and summer.

The removal efficiencies of NH_4-N oscillated between 93 and 99% throughout the year, with the lowest measurement recorded in June with a removal efficiency of 93% (Figure 2.9).

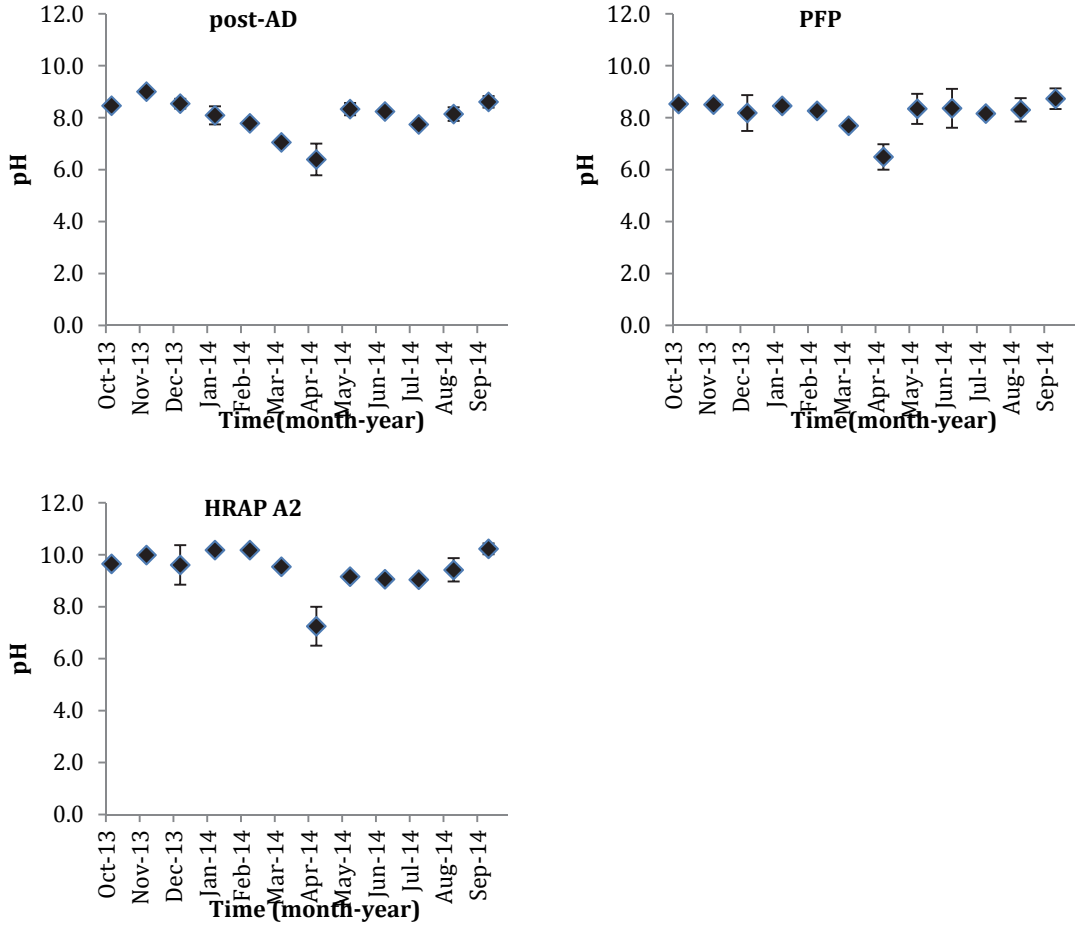

Figure 2.8 The monthly average (± standard error) pH in post-anaerobically digested (post-AD) brewery effluent, primary facultative pond (PFP) effluent and high rate algal pond (HRAP A2) effluent over 12 months.

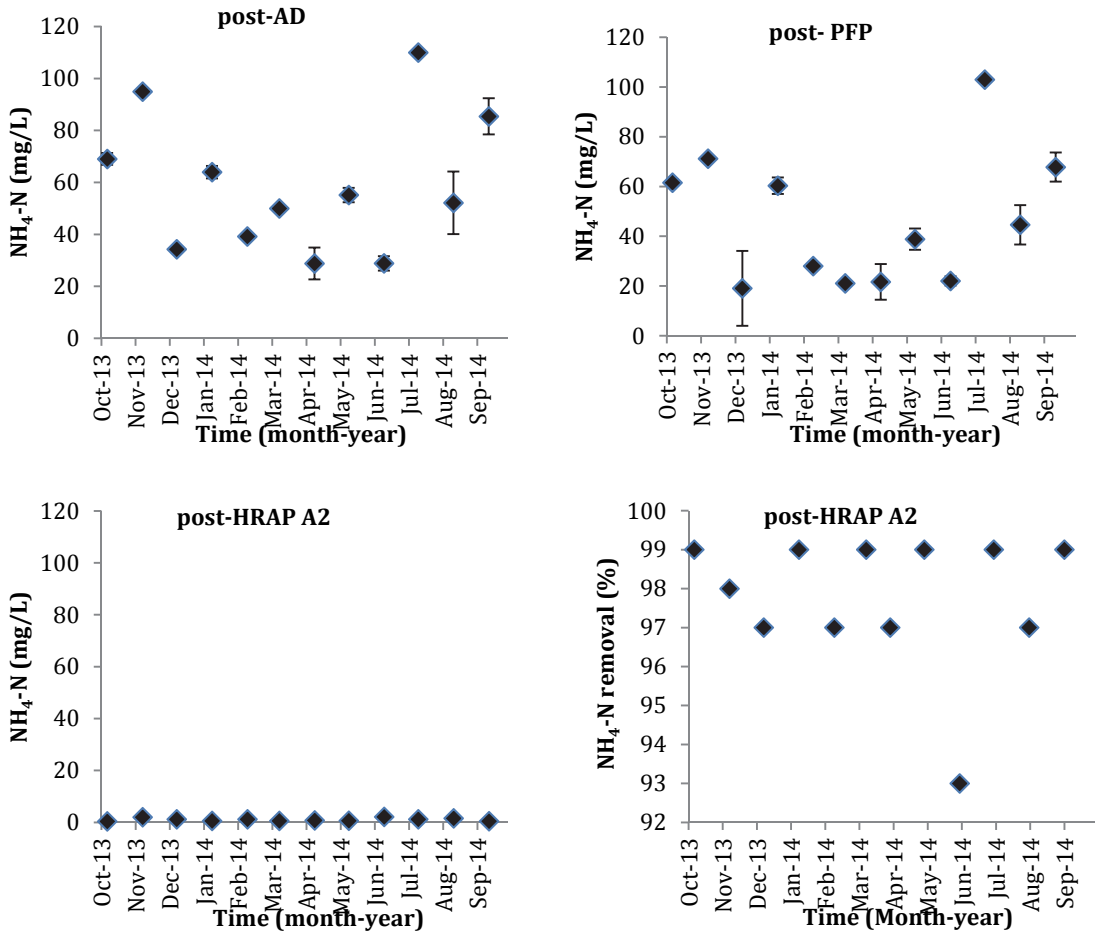

Figure 2.9 The monthly mean (± standard error) ammonia-nitrogen (NH_4-N) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal pond (post-HRAP A2) effluent over 12 months.

The lowest monthly mean measurement of NO_2-N of 0.46 ± 0.15 mg/L, was recorded in early summer (November) while the highest measurement, of 2.38 ± 0.64 mg/L, was recorded in mid-winter (June) (Figure 2.10). There appeared to be a seasonal signal in the removal efficiencies of NO_2-N. Generally, higher efficiencies were recorded in the warmer months of the year, up to 84%, while the lower removal efficiencies coincided with the cooler months of the year down to 49%. However, the lowest and highest measurements recorded of 30% in September and 86% in October appeared as outliers and broke with the observed trend in seasonal associated removal efficiencies.

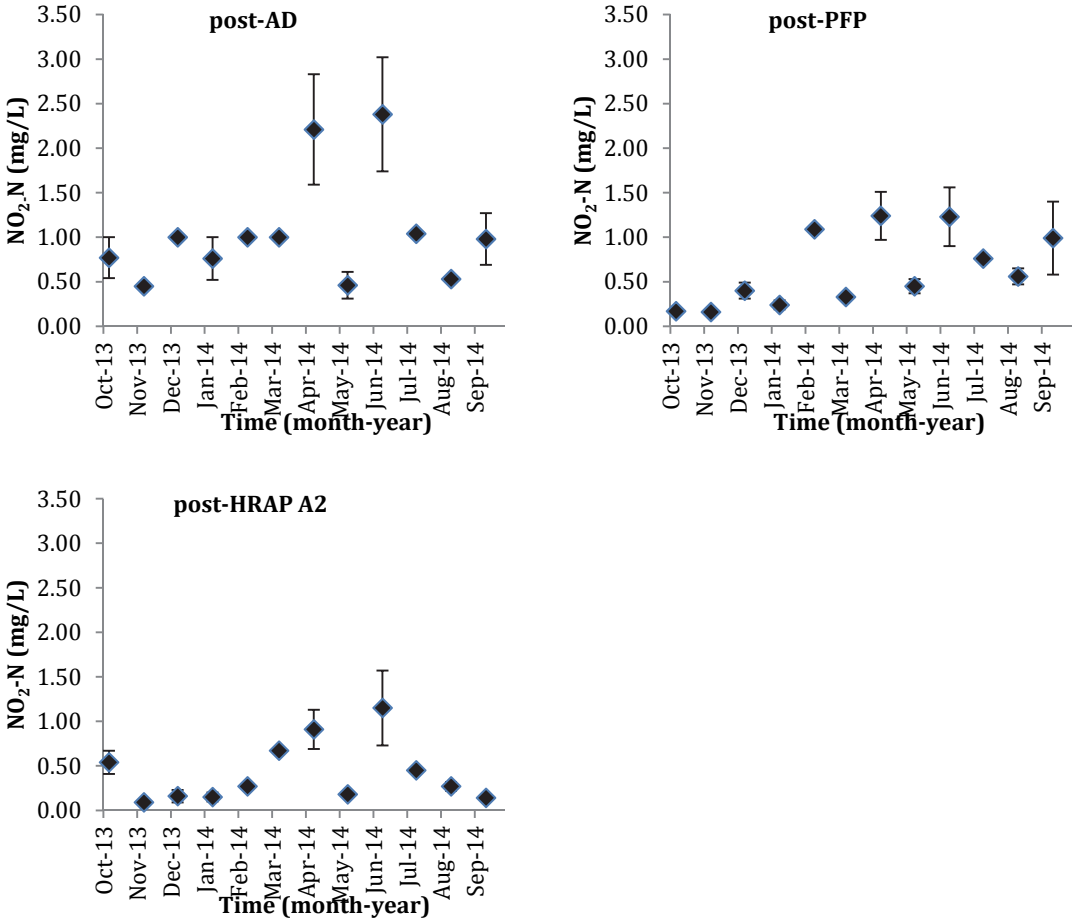

Figure 2.10 The monthly mean (± standard error) nitrite-nitrogen (NO_2-N) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal pond (post-HRAP A2) effluent over 12 months.

The highest concentrations of NO_3-N recorded in the post-AD effluent was 37.47 ± 0.00 mg/L in winter (July) and the lowest concentrations measured in this effluent was 0.41 ± 0.00 mg/L in late spring, November (Figure 2.11). Similar seasonal trends were also observed in the post-PFP and post HRAP A2 effluents, in the former, 37.87 ± 0.00 mg/L was measured in winter (July) and 0.59 ± 0.00 mg/L was measured in late spring (November), while in the latter effluent, 43.21 ± 2.83 mg/L was measured in winter (August) and 4.15 ± 0.00 mg/L was measured in late spring (November). The highest increase in NO_3-N in the post-HRAP A2 effluent relative to the post-AD effluent occurred in winter.

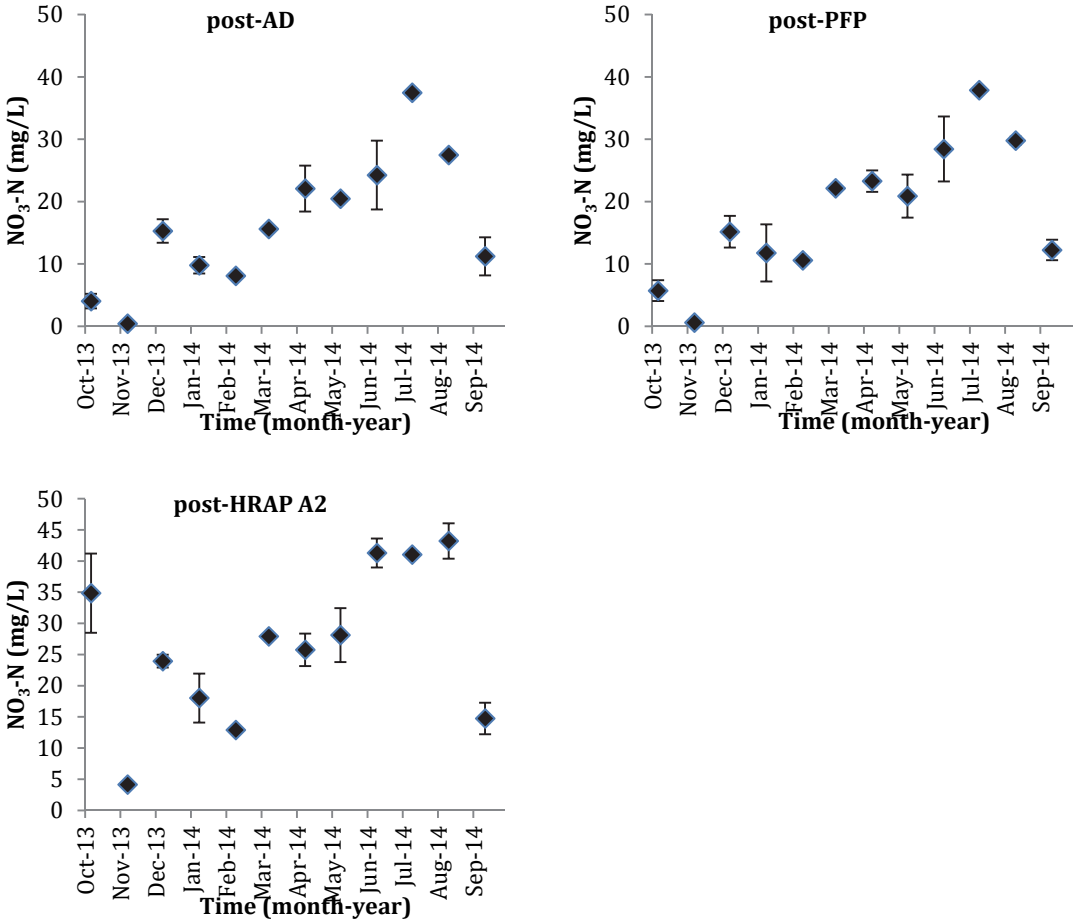

Figure 2.11 The monthly mean (± standard error) nitrate-nitrogen (NO_3-N) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal pond (post-HRAP A2) effluent over 12 months.

The monthly mean concentration of PO_4-P from the post-AD effluent to the post-PFP effluent decreased slightly (Figure 2.12). In the post-HRAP A2 effluent, PO_4-P removal efficiency was between 20 and 70%, however, the removal efficiencies had no clear seasonal trends throughout the year.

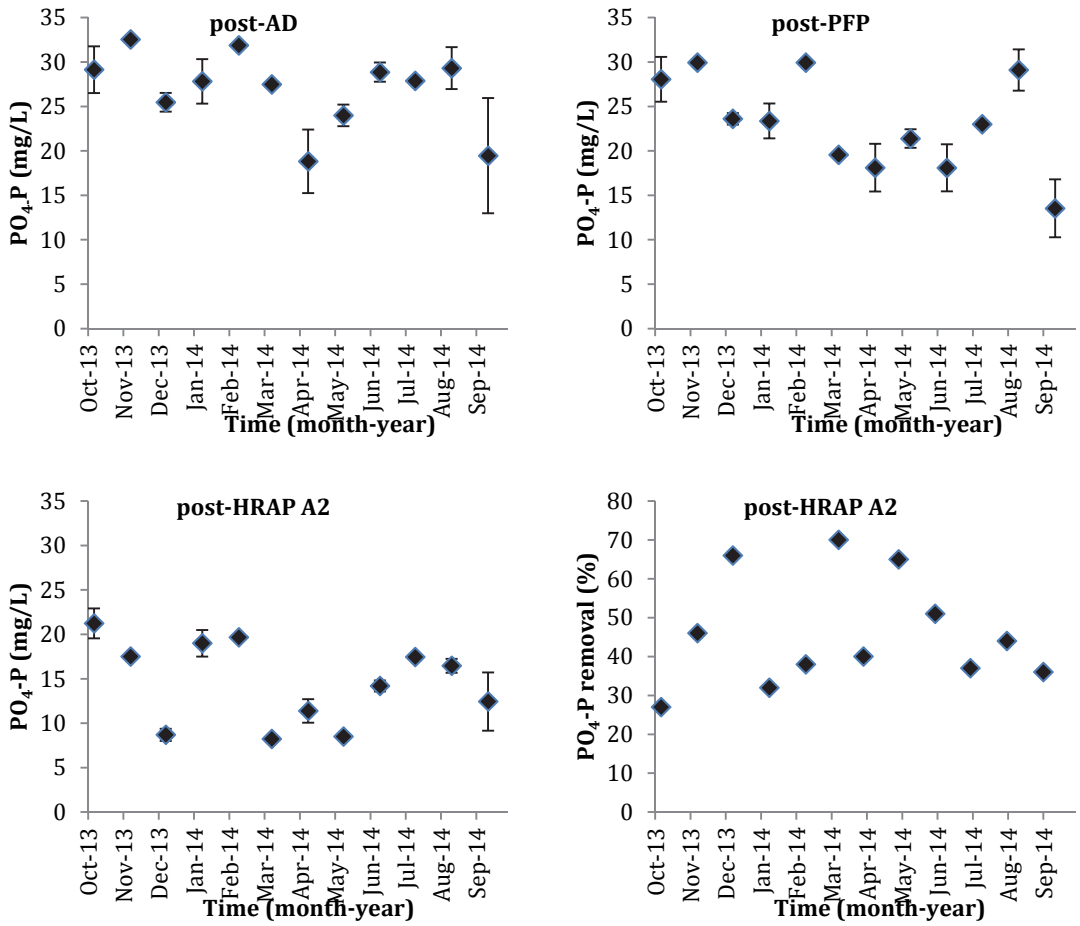

Figure 2.12 The monthly mean (± standard error) phosphate-phosphorus (PO_4-P) concentration in post-anaerobically (post-AD) digested brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal pond (post-HRAP A2) effluent over 12 months.

In the post-AD and post-PFP effluent, there was no apparent seasonal trend in the monthly mean COD_F (Figure 2.13). In the post-HRAP A2 effluent, COD_F values also did not seem to have any seasonal trends, however, the highest mean value of COD_F was recorded in summer (January).

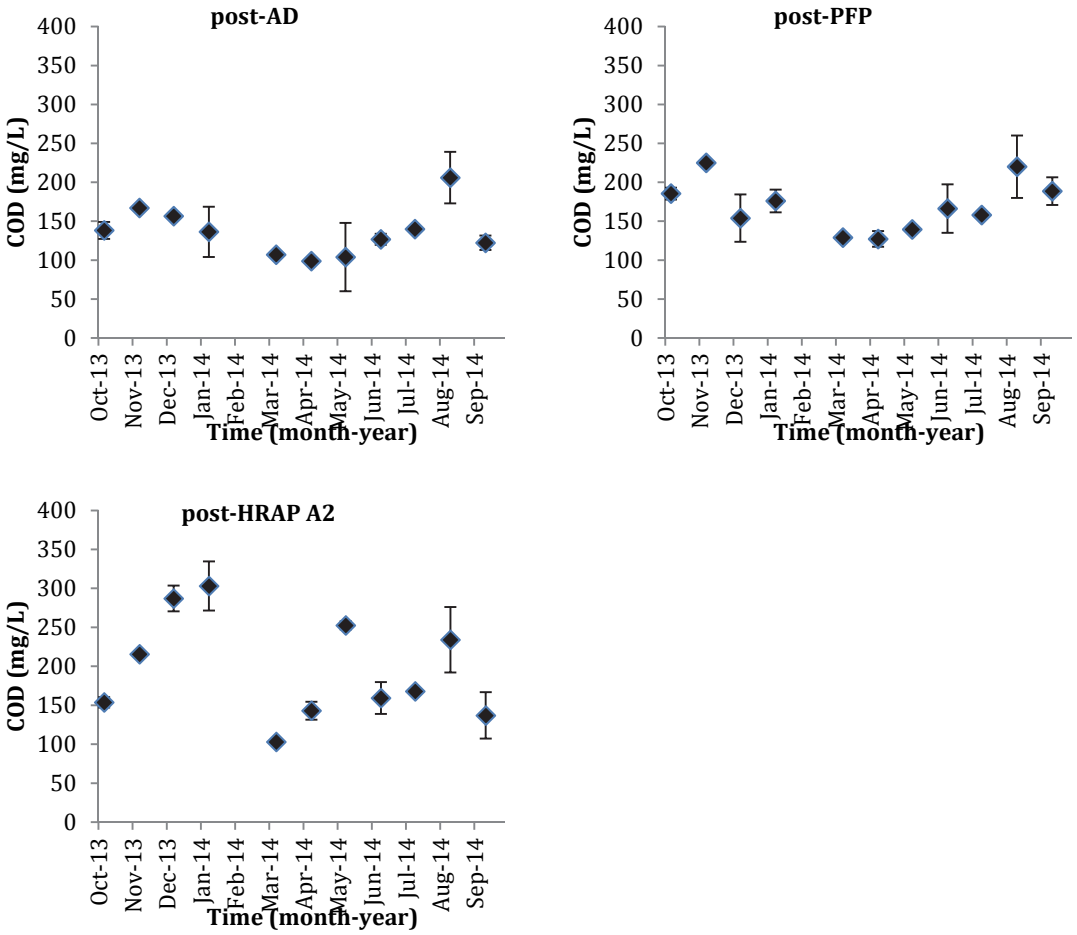

Figure 2.13 The monthly mean (± standard error) chemical oxygen demand (COD_F) in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal pond (post-HRAP A2) effluent over 12 months.

2.3.2 Flow rates

The microbial community structure in high rate algal ponds

An increase in the flow rate of effluent from 1000 to 2200 L/d resulted in shifts in the microalgae species composition and their relative abundance in the HRAP B2 as the flow rate of effluent into the pond increased (Table 2.2, Figure 2.14). The bacterial community composition and their relative abundance in the HRAP B2 also shifted as the flow rate of post-AD brewery effluent into it was progressively increased (Figure 2.15).

Table 2.2 Microalgae species in high rate algal ponds (HRAP A2 and HRAP B2) with post-primary facultative pond (post-PFP) effluent flowing into HRAP A at a rate of 1000 L/d while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d over 21 days. Algal cells were identified using light microscopy.

	HRAP A2 1000 L/d	**HRAP B2** 1000 L/d	**HRAP A2** 1000 L/d	**HRAP B2** 1400 L/d	**HRAP A2** 1000 L/d	**HRAP B2** 1800 L/d	**HRAP A2** 1000 L/d	**HRAP B2** 2200 L/d
Scenedesmus sp.	√	√	√	√	√	√	√	√
Chlorella sp.	√	√	√	√	√	√	√	√
Diatoma sp.	√	√	√	√	√	√	√	
Chlamydomonas sp.	√	√	√	√	√	√	√	
Microcystis sp.	√	√	√	√	√	√	√	
Phormidium sp.	√	√	√	√	√	√	√	
Synechococcus sp.	√	√	√	√	√	√	√	
Pediastrum sp.	√		√		√		√	
Arthrospira sp.	√	√	√	√	√		√	

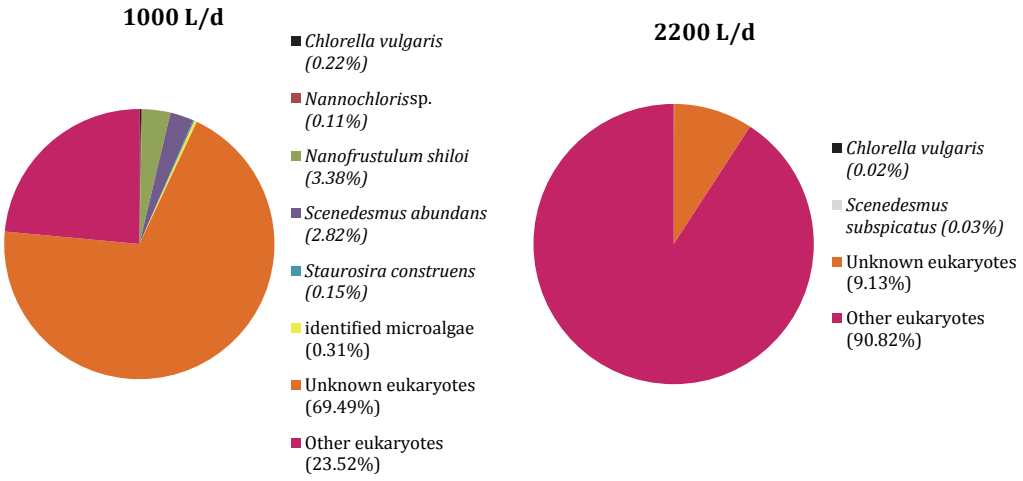

Figure 2.14 Microalgae species and their relative abundance as percentage (%) in a high rate algal pond (HRAP B2) with post-primary facultative pond (post-PFP) effluent flowing into the HRAP B train at a flow rate of 1000 L/d and 2200 L/d identified using metagenomics. Other eukaryotes refer to non-algal species which were also amplified with the universal primer pair 566F and 1200R.

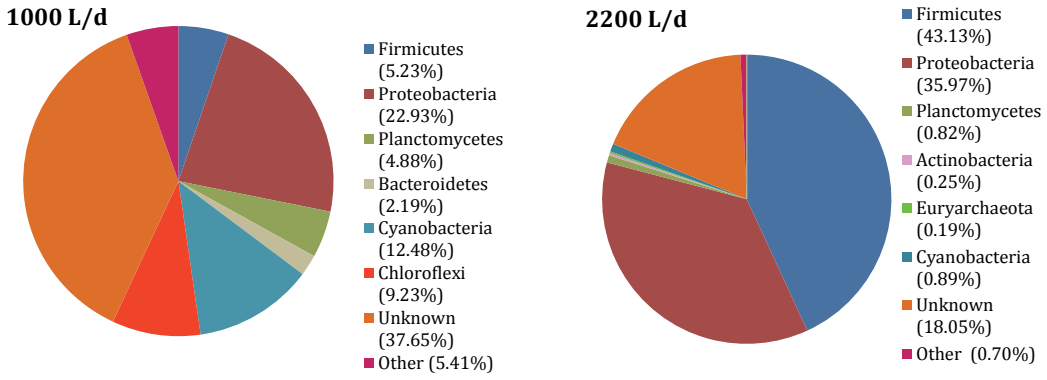

Figure 2.15 The bacterial community structure at a phylum level and their relative abundance as percentage (%) with post-primary facultative pond (post-PFP) effluent flowing into HRAP B2 at a rate of 1000 L/d and 2200 L/d.

Microalgae biomass and chlorophyll a concentrations

The algal biomass in HRAP A2 (1000 L/d) increased, while in HRAP B2 (1000-2200 L/d), the biomass decreased over the period of the experiment (Figure 2.16). Similar trends were observed with the Chl *a* concentration (Figure 2.17).

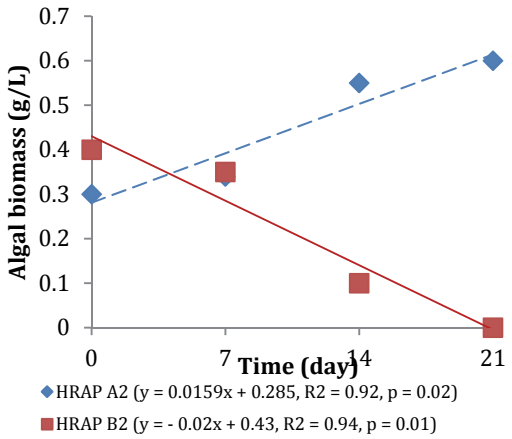

Figure 2.16 Algal biomass in high rate algal ponds (HRAP A2 and HRAP B2) with post-primary facultative pond (post-PFP) effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

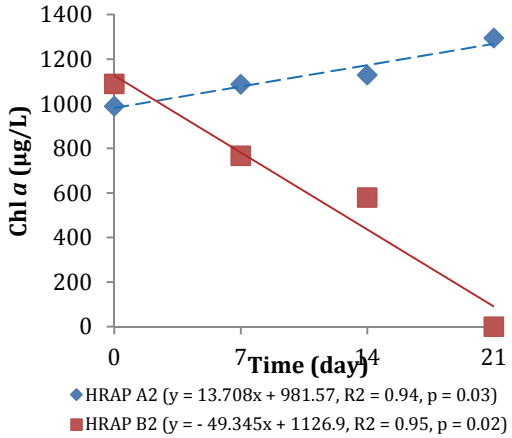

Figure 2.17 Chlorophyll (Chl) *a* concentration determined using a trichromatic equation in high rate algal ponds (HRAP A2 and HRAP B2) with post-primary facultative pond (post-PFP) effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

Water quality parameters

The temperature was on average about 2.0°C lower in the HRAPs than in the post-AD effluent and PFP (Figure 2.18). The increase in flow rate did not appear to influence the temperature in the HRAPs.

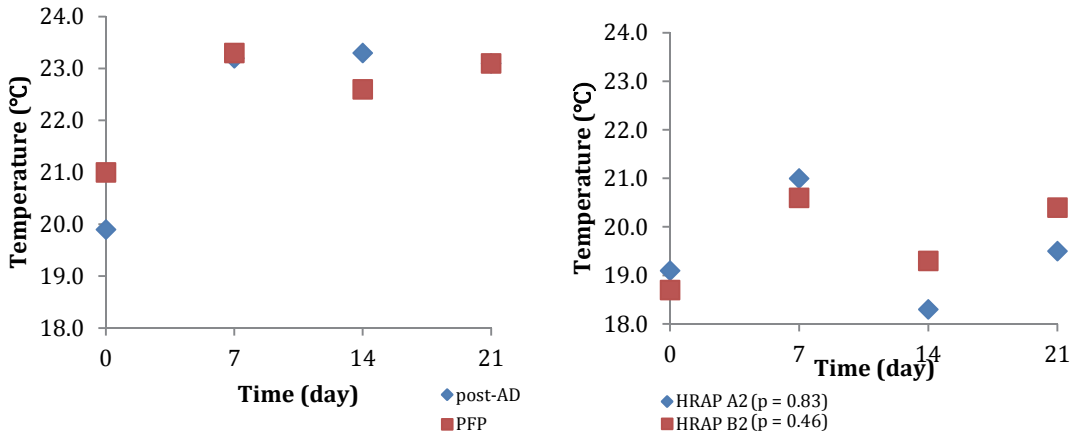

Figure 2.18 Temperature value in post-anaerobically digested (post-AD) brewery effluent, primary facultative pond (PFP) effluent and high rate algal ponds (HRAP A2 and HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

The pH of the post-AD and PFP effluent was lower than that recorded in the HRAPs (Figure 2.19). In the HRAP A2 and HRAP B2, the pH did not differ.

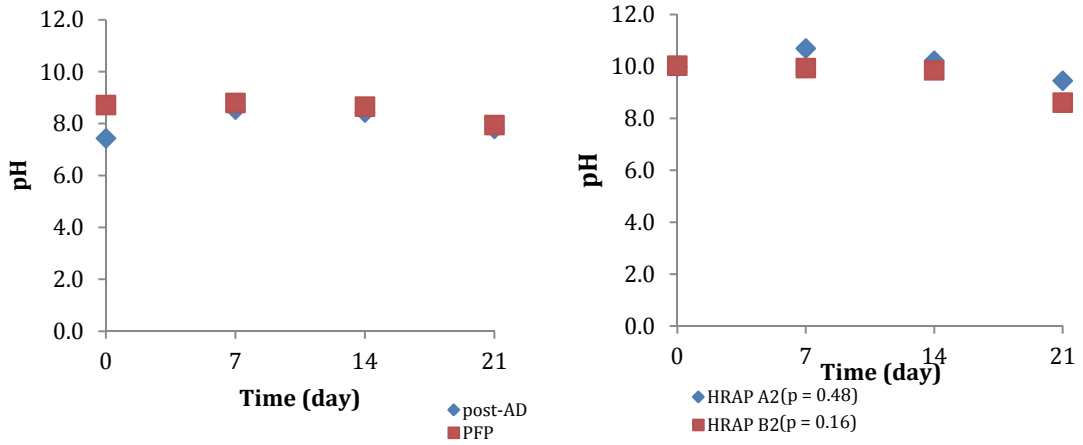

Figure 2.19 The pH values in post-anaerobically digested (post-AD) brewery effluent, primary facultative pond (PFP) effluent and high rate algal ponds (HRAP A2 and HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while (HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

The concentration of NH_4-N decreased from the post-AD effluent into the post-PFP effluent (Figure 2.20). Ammonium-nitrogen concentrations further decreased in the post-HRAPs effluent. The NH_4-N concentrations remained constant in the HRAP A2 that was operated at 1000 L/d. In the HRAP B2 where the effluent flow rate was increased, the NH_4-N concentrations were also constant, ranging from about 0.5 mg/L at 1000 L/d to 70.0 mg/L at 2200 L/d.

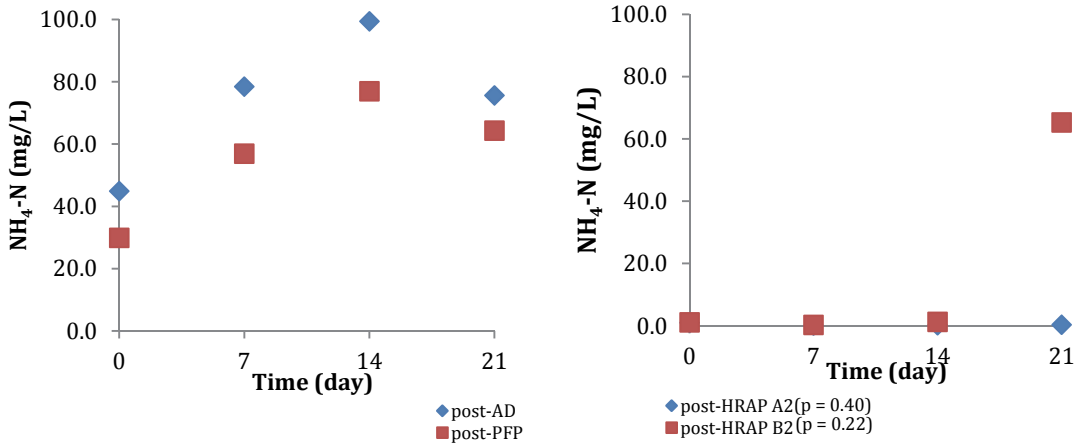

Figure 2.20 Ammonium-nitrogen (NH_4-N) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal ponds (post-HRAP A2 and post-HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

Nitrite-nitrogen concentrations decreased from the post-PFP effluent to the post-HRAPs effluent (Figure 2.21). The NO_2-N concentration increased when the flow rate was increased in HRAP B2, whereas there was no significant change in NO_2-N concentration when the flow was maintained at 1000 L/d.

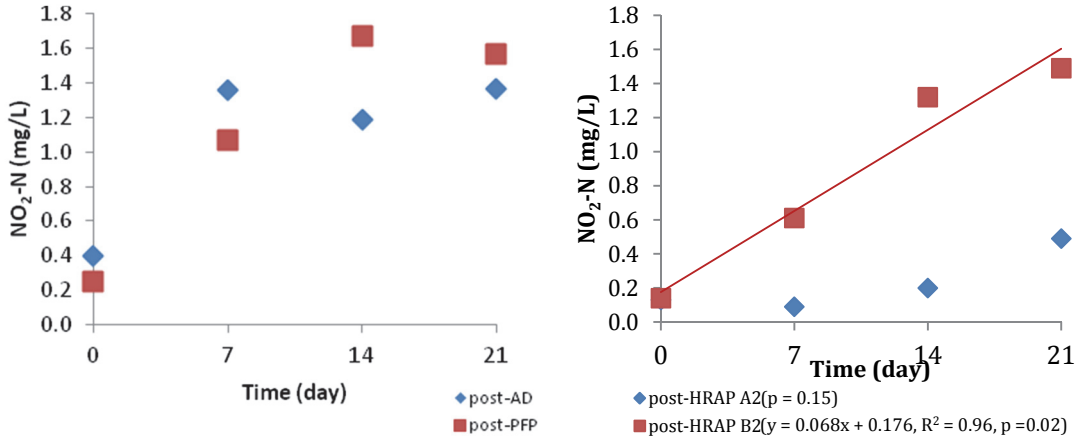

Figure 2.21 Nitrite (NO_2-N) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal ponds (post-HRAP A2 and post-HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

The NO_3-N concentrations also decreased from the post-PFP effluent to the post-HRAP B2 effluent where the flow rate was increased from 1000 to 2200 L/d, however, there was no significant difference in NO_3-N removal (Figure 2.22). In the HRAP A2 maintained at 1000 L/d, the concentrations of NO_3-N increased over time ($p = 0.04$).

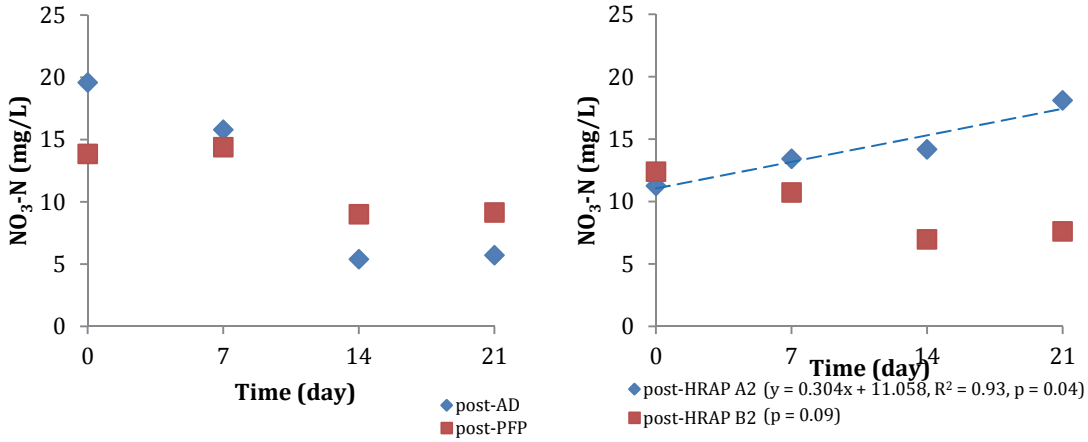

Figure 2.22 Nitrate (NO_3-N) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal ponds (post-HRAP A2 and post-HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

Phosphate-phosphorus concentrations decreased from the post-AD into the post-PFP effluent (Figure 2.23). The PO_4-P concentrations further decreased in the HRAPs. The removal of PO_4-P in the HRAP B2 decreased with an increase in the effluent flow rate, while in the HRAP A2, the removal of PO_4-P remained constant.

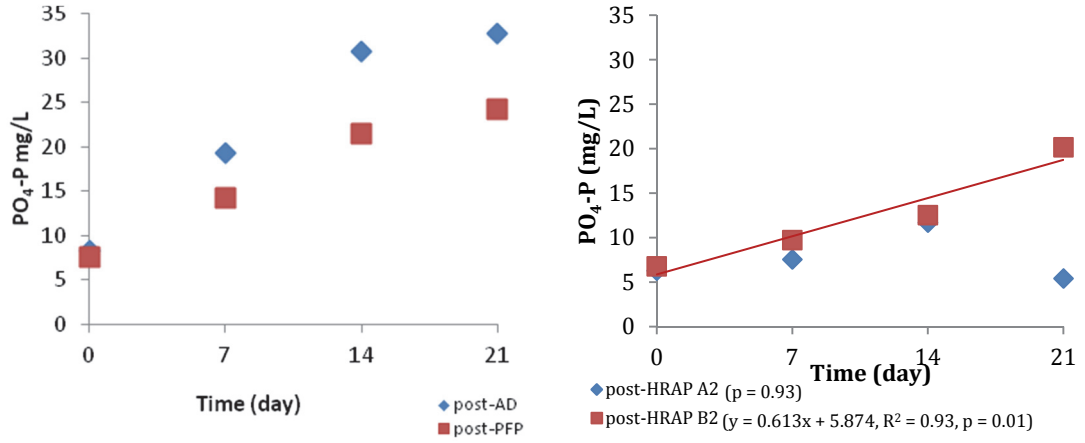

Figure 2.23 Phosphate-phosphorus (PO_4-P) concentration in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal ponds (post-HRAP A2 and post-HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

The COD_F in the post-AD effluent was lower than in the post-PFP effluent on a number of occasions (Figure 2.24). In the post-HRAP A2 effluent at a constant flow of 1000 L/d, the COD_F increased over the 21 day trial, while in post-HRAP B2 effluent, the COD_F remained constant over the same period as the flow rate increased from 1000 L/d to 2200 L/d.

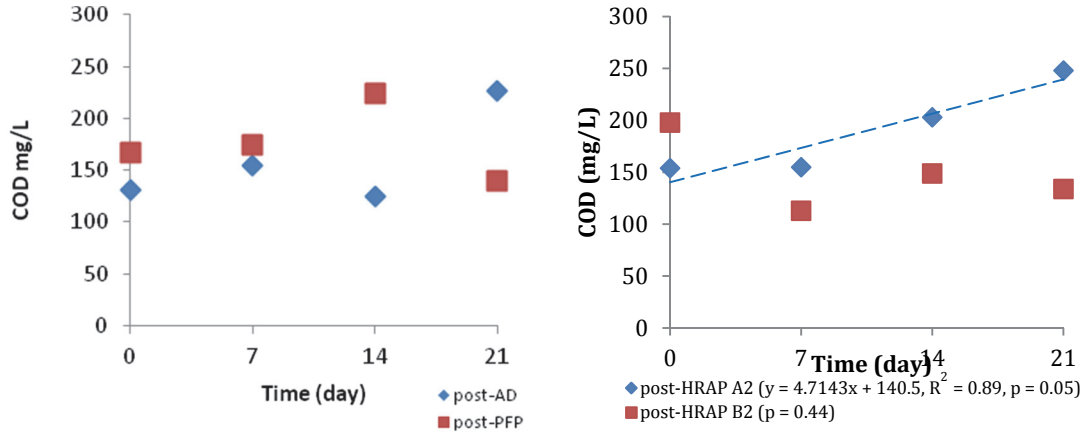

Figure 2.24 Chemical oxygen demand (COD_F) in post-anaerobically digested (post-AD) brewery effluent, post-primary facultative pond (post-PFP) effluent and high rate algal ponds (post-HRAP A2 and post-HRAP B2) effluent with post-PFP effluent flowing into HRAP A at a constant rate of 1000 L/d for 21 days, while HRAP B flow rates were progressively increased from 1000 L/d to 1400, 1800 and 2200 L/d at day 0, day 7, day 14 and day 21 respectively.

2.3.3 Temperature and pH

Microalgae-bacteria assemblages

Most of the microalgal species present in the algal community that was investigated belonged to the Chlorophyta, while few species were from the Cyanophyta (Figure 2.25, Table 2.3). The remaining algal species were distributed among the other phyla. In the bacterial community, Firmicutes were the dominant phylum with a relative abundance of 14.38% while Verrumicrobia had the lowest relative abundance of 0.34% (Figure 2.26).

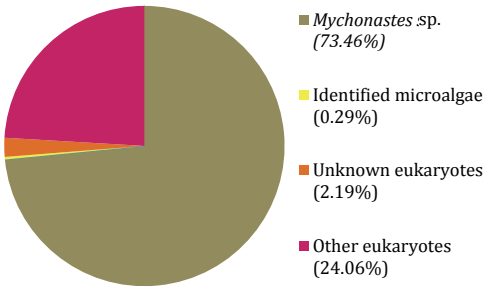

Figure 2.25 The microalgae community structure and their relative abundance as percentage (%) identified using metagenomics at the beginning of the experiment. Other eukaryotes refer to non-algal species which were also amplified with the universal primer pair 566F and 1200R.

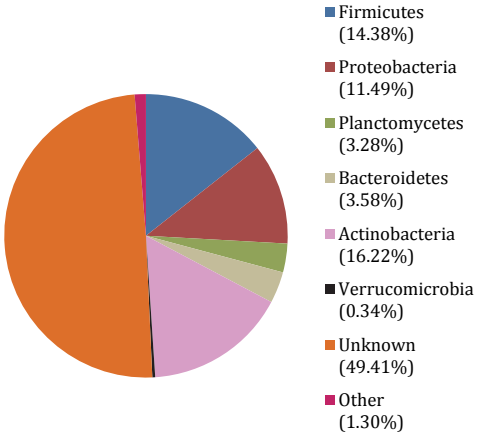

Figure 2.26 The bacterial community structure identified at the beginning of the trial and their relative abundance as percentage (%).

Chlorophyll a concentrations

The Chl *a* concentrations increased from day zero to day six (Figure 2.27, Figure 2.28). The Chl *a* concentrations showed a significant difference with an increase in temperature from 20.0 to 30.0°C ($p < 0.001$) in both pH adjustment methods. Also, with an increase in pH from 7.0 to 8.5 and 10.0, the Chl *a* concentrations were significantly different ($p < 0.001$) in both pH adjustment methods. When microalgae were cultured at 20.0°C, the concentration of Chl *a* was the greatest at pH 8.5, then followed by pH 10.0 and pH 7.0 (Figure 2.27). Similar trends were observed at 30.0°C, the Chl *a* concentration was also higher at pH 8.5, and followed by pH 10.0 and pH 7.0 (Figure 2.28).

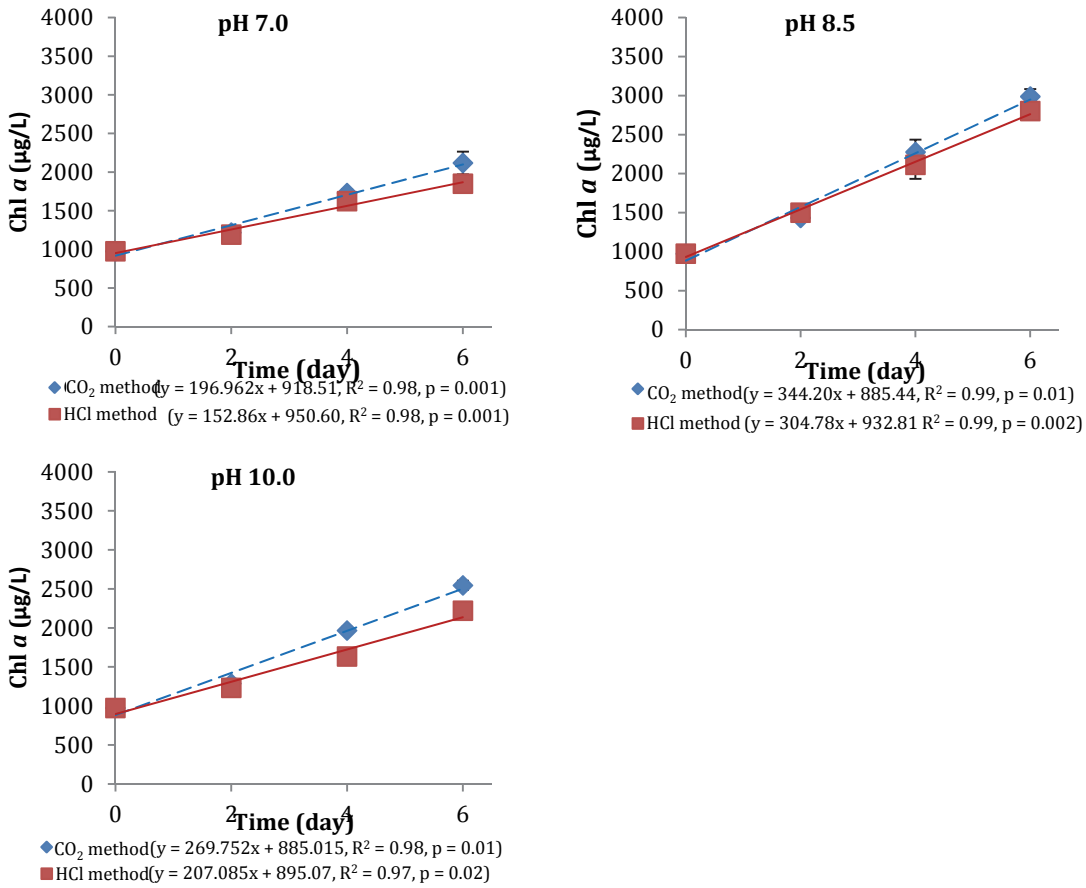

Figure 2.27 Changes in the mean (± standard error) chlorophyll (Chl) *a* concentration of microalgae cultured at 20.0°C in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

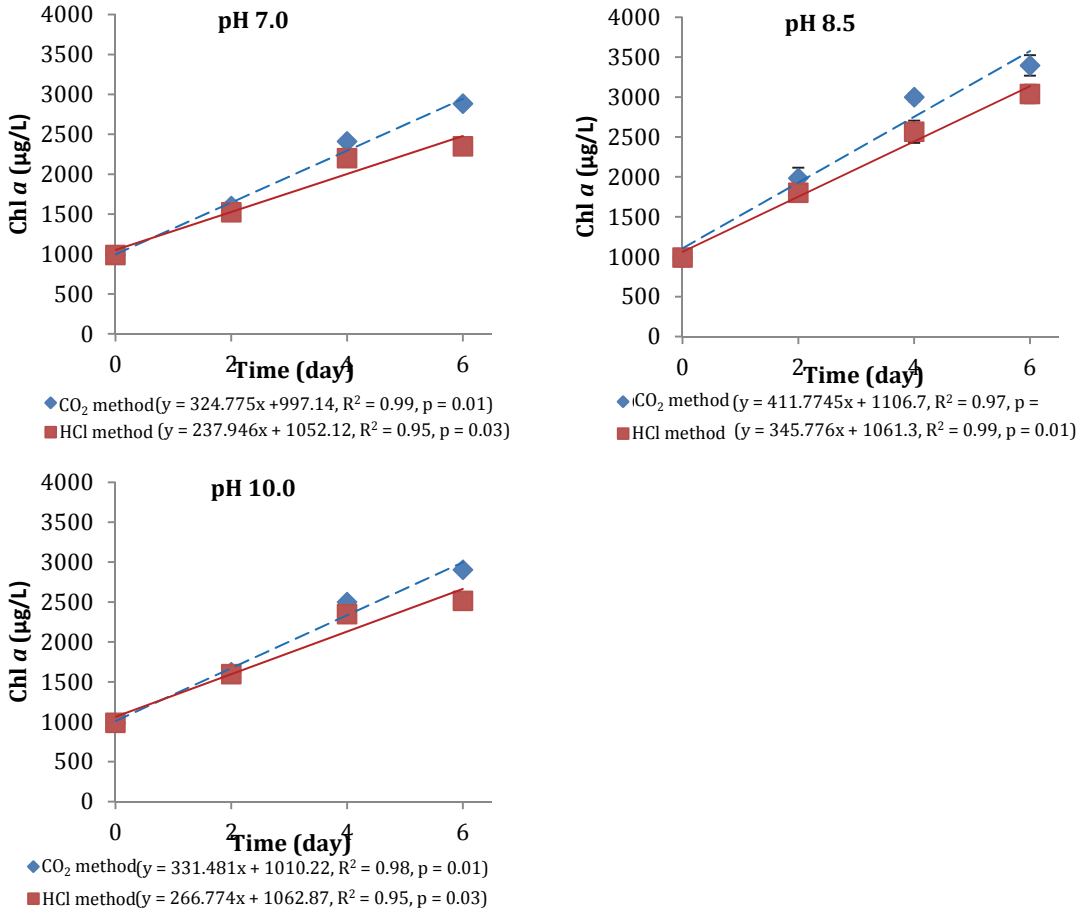

Figure 2.28 Changes in the mean (± standard error) chlorophyll (Chl) *a* concentration of microalgae cultured at 30.0°C in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

Water quality parameters

The temperature in the water baths fluctuated between 19.5 and 21.0°C when the trial was run at 20.0°C, with an overall mean (± standard error) of 20.59 ± 0.63°C. At 30.0°C, the temperature varied within a mean range of 29.0 to 32.0°C with an overall mean of 31.06 ± 0.97°C.

There was a significant difference ($p < 0.001$) in the removal of NH_4-N when temperature was increased from 20.0 to 30.0°C in both pH adjustment treatments (Figure 2.29, Figure 2.30). There was also a significant difference ($p < 0.001$) in the removal of NH_4-N at different pH levels in both pH adjustment treatments. At 20.0°C, there was about 65 to 85% decrease in NH_4-N at pH 7.0, 8.5 and 10.0 in the CO_2 and HCl treatments (Figure 2.29). In both pH adjustment treatments, more NH_4-N was removed through microbial activity at pH 7.0, 8.5 and pH 10.0 rather than through volatilization. At 30.0°C, a greater portion of about 82 to 84% of the NH_4-N in the microalgae cultures at pH 7.0 and 8.5 was removed through microbial activity in the CO_2 and HCl treatments (Figure 2.30). At pH 10.0, a greater amount of NH_4-N was removed through volatilization in both pH adjustment treatments.

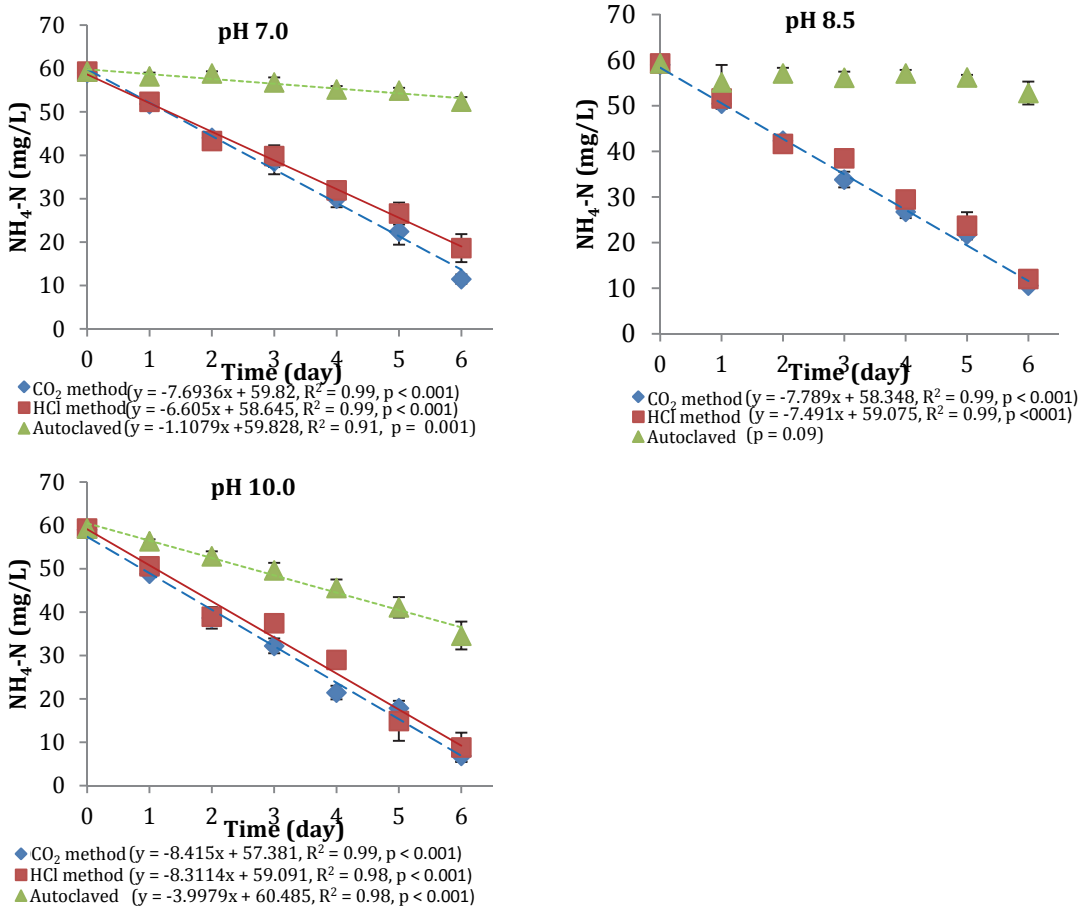

Figure 2.29 Changes in the mean (± standard error) ammonium-nitrogen (NH_4-N) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 20.0°C in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods and in the autoclaved medium at pH 7.0, 8.5 and 10.0.

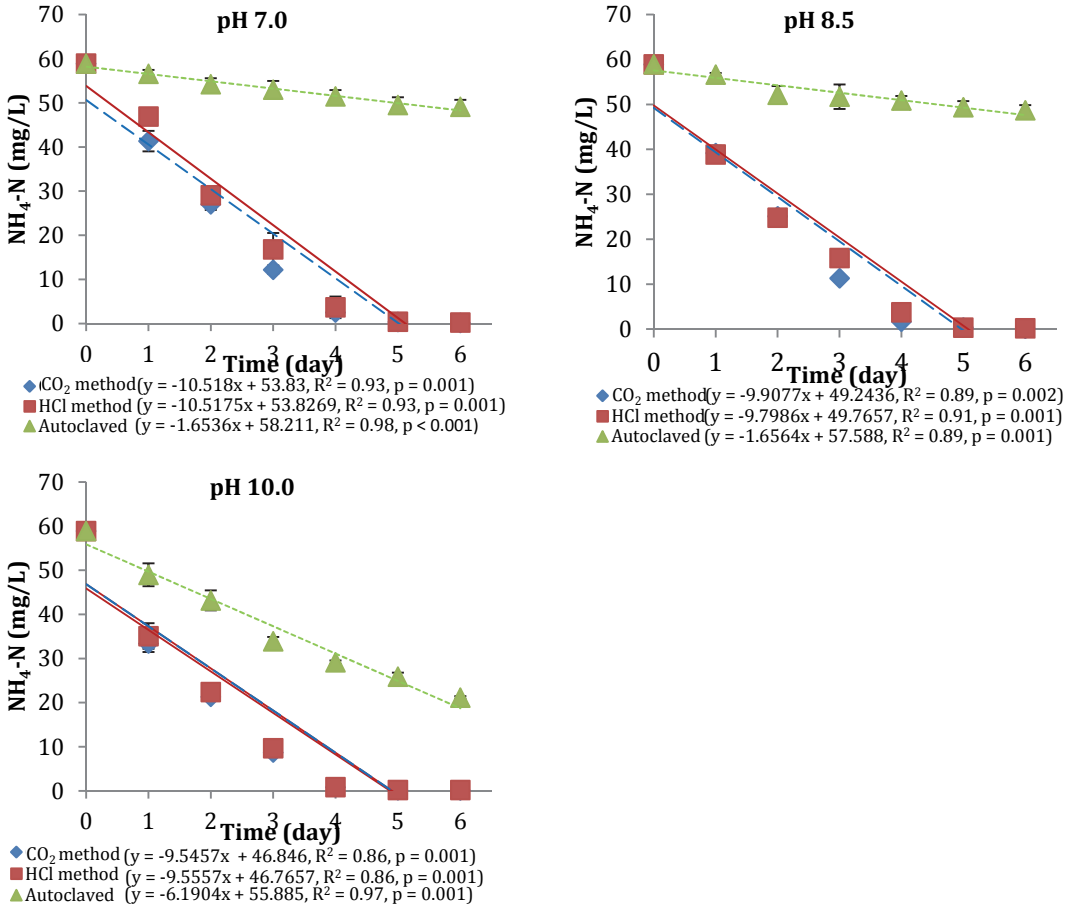

Figure 2.30 Changes in the mean (± standard error) ammonium-nitrogen (NH_4-N) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 30.0℃ in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods and in the autoclaved medium at pH 7.0, 8.5 and 10.0.

The NO_2-N concentrations were significantly different ($p < 0.001$) at different pH levels in both pH adjustment methods. Nitrite-nitrogen was greater at pH 7.0 and 8.5 than at pH 10.0 (Figure 2.31, Figure 2.32). An increase in temperature from 20.0 to 30.0℃ elevated NO_2-N concentrations ($p < 0.001$) in both pH adjustment methods.

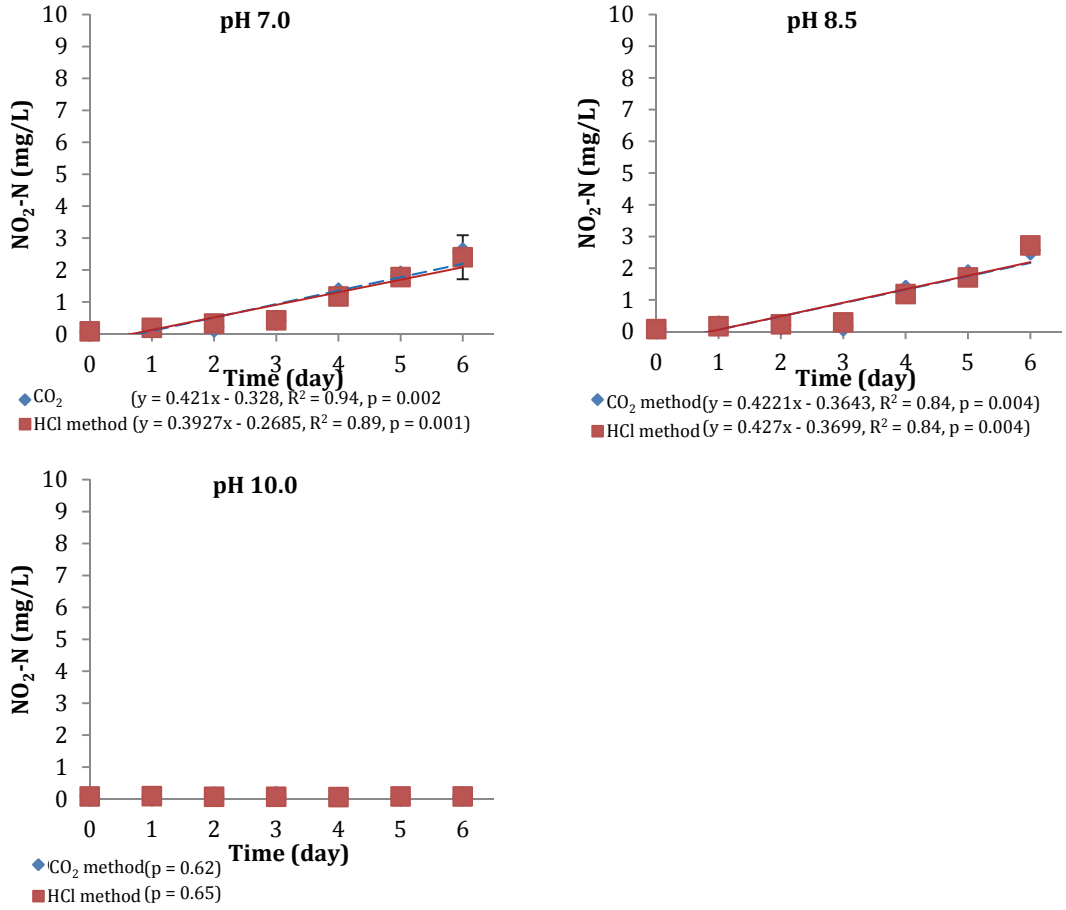

Figure 2.31 Changes in the mean (± standard error) nitrite-nitrogen (NO_2-N) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 20.0℃ in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

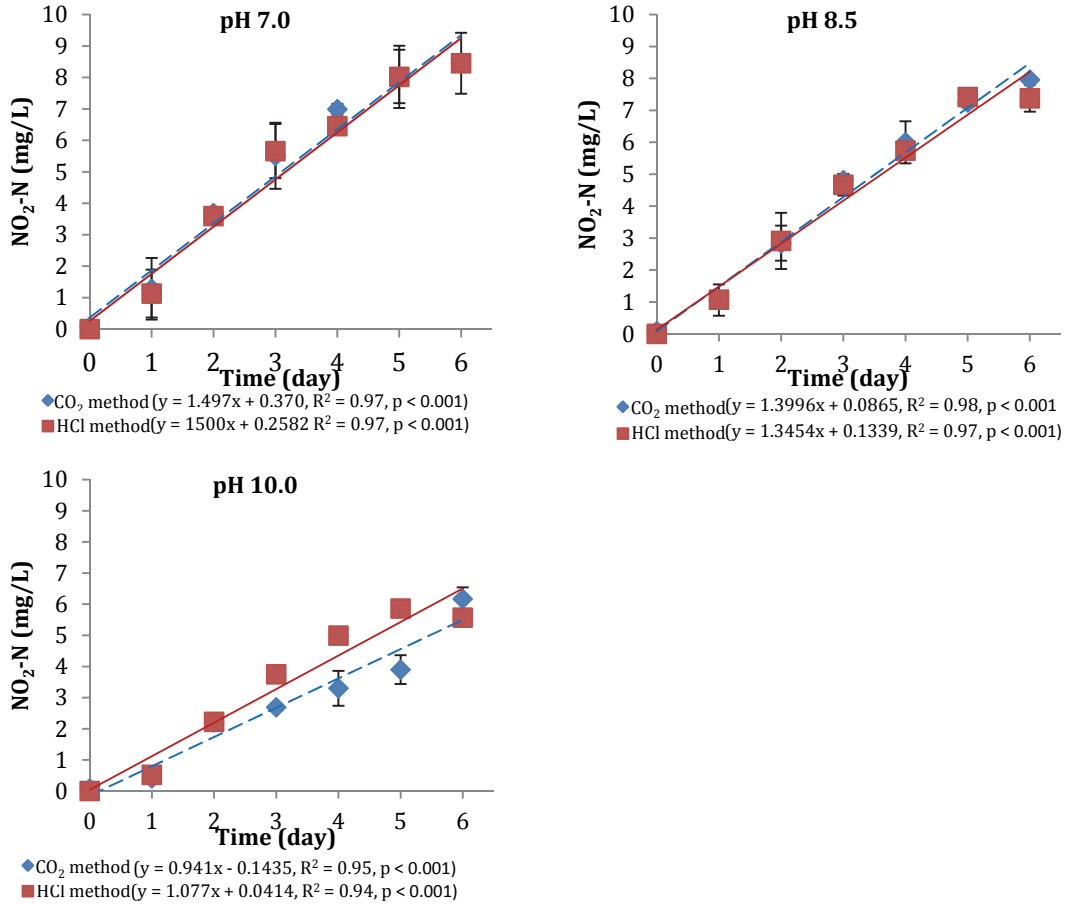

Figure 2.32 Changes in the mean (± standard error) nitrite-nitrogen (NO_2-N) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 30.0°C in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

At 20.0 and 30.0°C, NO_3-N concentrations were significantly different ($p < 0.001$) in both pH adjustment treatments (Figure 2.33, Figure 2.34). At 20.0°C, the concentration of NO_3-N in the post-AD brewery effluent increased more at pH 7.0 and 8.5 in both pH adjustment treatments (Figure 2.33). However, at pH 10.0, there was only a slight increase in NO_3-N. Similar trends were observed when microalgae cultures were grown at 30.0°C. At pH 7.0 and 8.5, the concentrations of NO_3-N also increased more than at pH 10.0 (Figure 2.34).

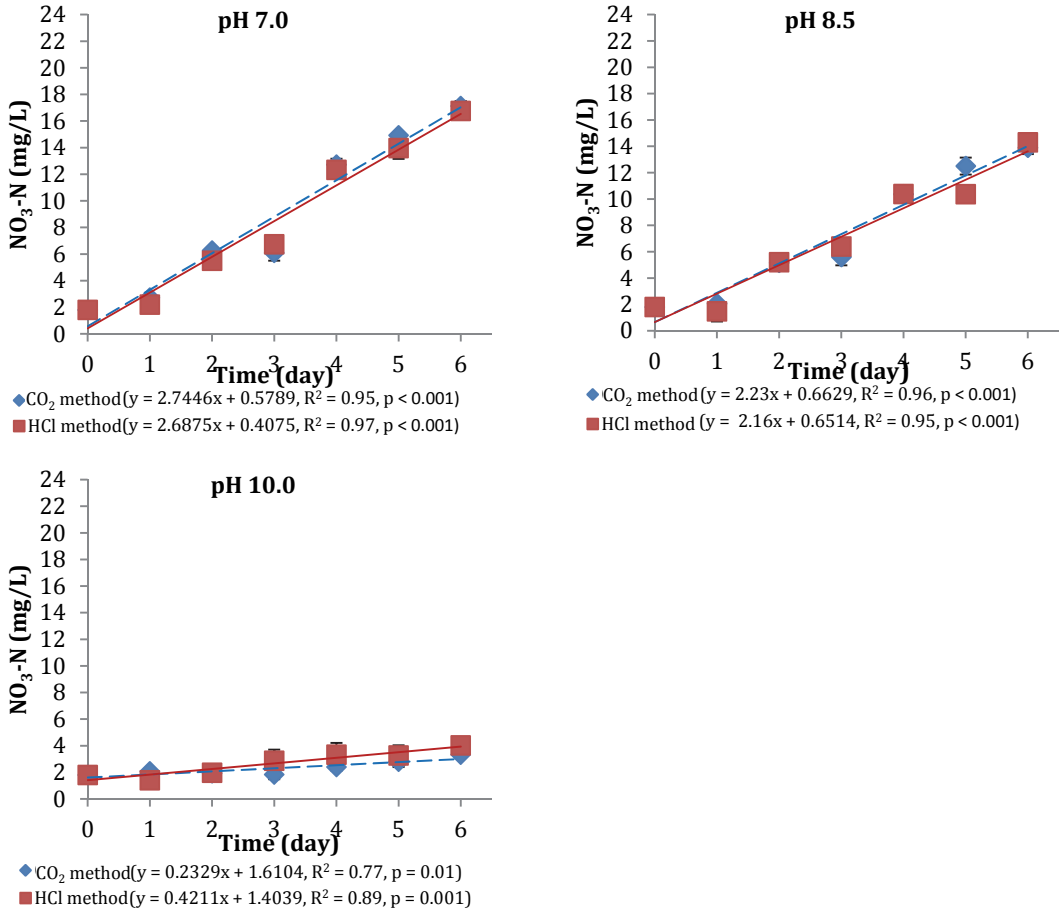

Figure 2.33 Changes in the mean (± standard error) nitrate-nitrogen (NO_3-N) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 20.0℃ in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

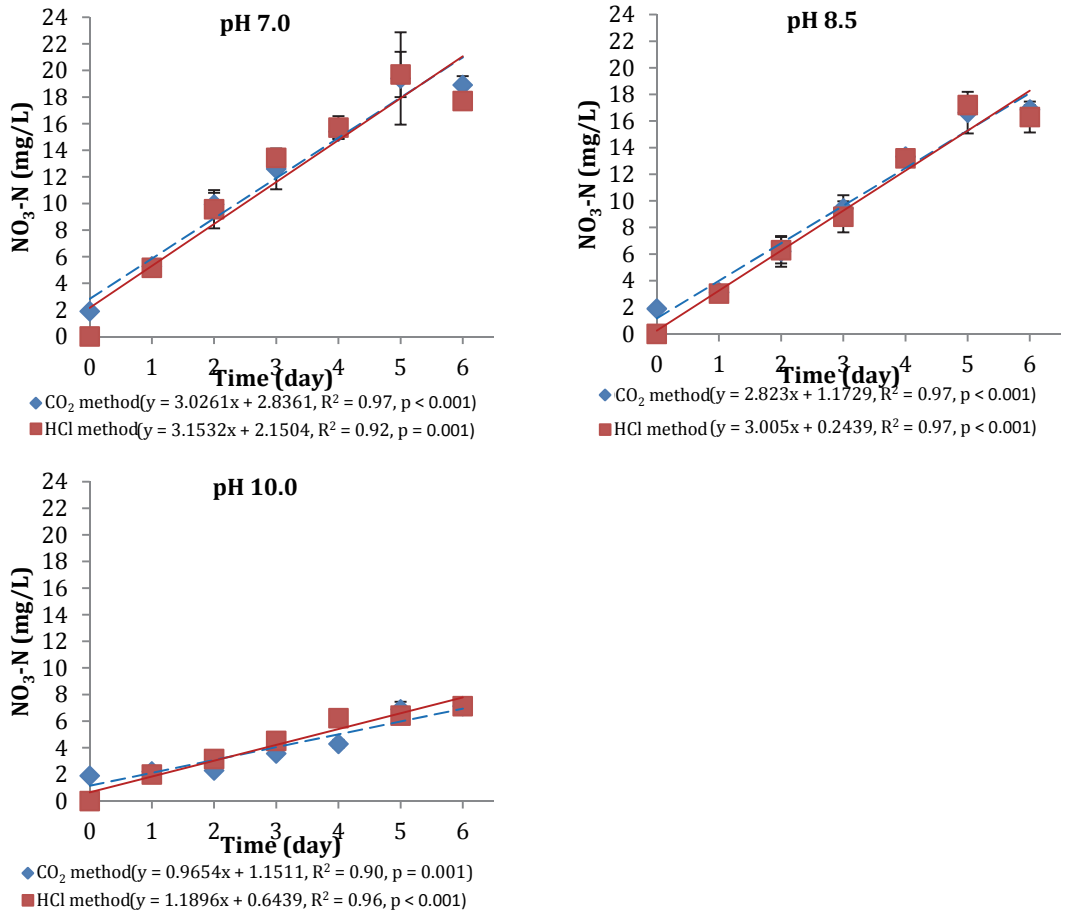

Figure 2.34 Changes in the mean (± standard error) nitrate-nitrogen (NO_3-N) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 30.0℃ in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

There was a significant difference ($p < 0.001$) in the removal of PO_4-P with an increase in temperature. About 72 to 76% of the PO_4-P was removed when microalgae were cultured at 20.0℃ in the CO_2 and HCl treatments at pH 7.0, 8.5 and 10.0 (Figure 2.35). At 30.0℃, more than 90% of the PO_4-P was removed at pH 7.0, 8.5 and 10.0 in both pH adjustment treatments (Figure 2.36). There was also a significant difference ($p = 0.02$) at different pH levels in PO_4-P removal when microalgae cultures were treated with CO_2, however, in the HCl treatment there was no difference ($p = 0.44$) in PO_4-P removal at different pH levels.

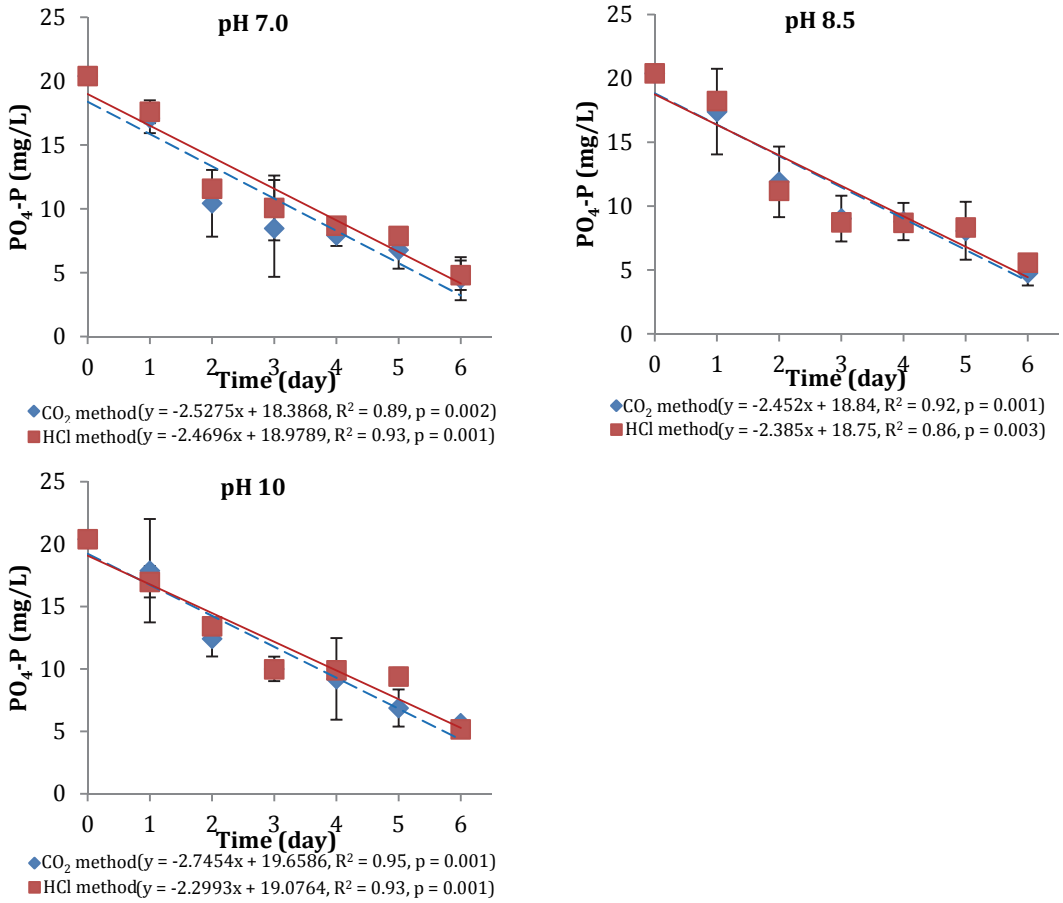

Figure 2.35 Changes in the mean (± standard error) phosphate-phosphorus (PO_4-P) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 20.0°C in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

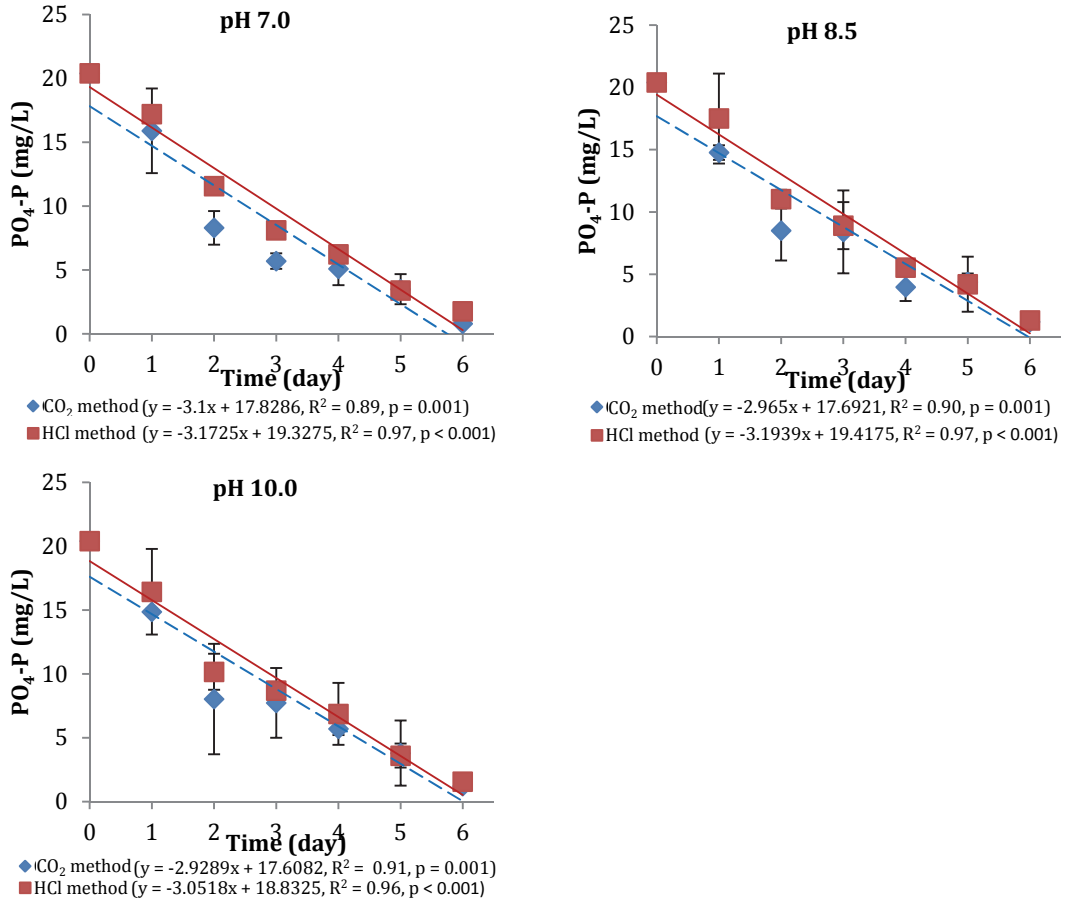

Figure 2.36 Changes in the mean (± standard error) phosphate-phosphorus (PO_4-P) concentration in the post-anaerobically digested (post-AD) brewery effluent treated in microalgae cultures grown at 30.0℃ in carbon dioxide (CO_2) and hydrochloric acid (HCl) pH adjustment methods at pH 7.0, 8.5 and 10.0.

2.4 Discussion

The microbial community structure

Seasonal variation of the microbial community structure and their relative abundance in HRAP A2 was probably due to the seasonal variation of temperature and pH (Affan *et al*. 2005). An increase in the flow rate of post-AD brewery effluent into the HRAP B train resulted in a washout of some microalgae species in HRAP B2, thus fewer species were observed at a flow rate of 2200 L/d (El-Sayed *et al.* 2010). In HRAP B2, shortening of the retention time might have not provided some algal species sufficient time to grow (Table 2.2), however, fast growing microalgae such as *Chlorella* sp. and *Scenedesmus* sp. are able to grow even at a shorter HRT (Johnson 2010). Shifts in the bacterial community structure in the HRAP B2 were probably due to an increase in the incoming post-AD effluent into the HRAP B2 (increasing dilution rate) which carries exogenous bacteria into the pond, thus contributing to the community structure (Albaggar 2014). In general, a shorter HRT is species selective, in a sense that it favours the growth of some bacteria, while others are washed out of a pond (Albaggar 2014). Variations in the microbial community structure at different seasons and flow rates affected the algal biomass in the ponds.

Microalgae biomass and chlorophyll a concentration

The seasonal variation in temperature affected microalgae growth in the HRAP, thus the seasonal changes in algal biomass and Chl *a* concentration. This was demonstrated here when an increase in temperature from 20.0 to 30.0℃ favoured microalgae growth. An increase in temperature enhances microalgae metabolism and the rate of all related chemical reactions, which in turn increases algal biomass (Sevrin-Reyssac 1998; Zimmo 2003). Beside temperature, other factors affecting microalgae growth such as solar radiance, day length and zooplankton grazing pressure also vary seasonally (De Pauw & van Vaerenbergh 1983). In winter, when temperature and solar PAR is lower and day length shorter than in summer, the growth rate of microalgae decreases (De Pauw & van Vaerenbergh 1983; Assemany *et al*. 2015). A shorter day length deprives algal cells from receiving maximum solar PAR and temperature during the day, reducing the rate of photosynthetic reactions, which in turn reduces microalgae growth (De Pauw & van Vaerenbergh 1983; Assemany *et al*. 2015). However, during summer, the longer day length exposes algal cells to sufficient light and temperature, thus a greater biomass is produced during this time (Assemany *et al*. 2015). During summer, zooplankton were seen grazing on microalgae under a microscope, providing evidence that grazing could have contributed to the low gain in algal biomass than expected from November 2014 to January 2015. Davis *et al*. (2012) reported that the grazing rates of *Daphnia galeata* and *Daphnia retrocurva* were responsible for about 85% of the grazing rate and this resulted in a decline in algal biomass in the western Lake Erie. In the HRAP B2, where post-AD brewery effluent flow rates were progressively increased, the reduction in the standing algal biomass coincided with increasing dilution rates of the effluent. At 2200 L/d, algal cells were washed out of the HRAP due to the shortening of the HRT (Sacasa Castellanos 2013). A reduction in the HRT of the pond reduced algal biomass and Chl *a* concentrations in the HRAP. Variations in the algal biomass at different seasons (temperature and pH) and at different effluent flow rates affected the reduction of nutrients from the post-AD brewery effluent.

Mechanisms of lowering nitrogen concentrations in effluent

Microbial activity, which includes algal assimilation and bacterial nitrification, was one of the mechanisms responsible for lowering NH_4-N concentrations in the post-AD brewery effluent (Sevrin-Reyssac 1998; Zimmo 2003). An increase in temperature enhances cellular metabolism (and the rate of related chemical reactions), which in turn increases nutrient uptake within the pH range of 7.0-8.5 (Sevrin-Reyssac 1998; Zimmo 2003). This was demonstrated in the seasonal variation experiment during summer, when the reduction efficiency of NH_4-N was greater than in winter. This corresponded with an increase in algal biomass during summer and a decrease in winter. It was evident in the flow rate experiment that the standing algal biomass in the HRAP B2 influenced the change in nutrient concentration at 2200 L/d, when the algal biomass was close to zero due to algal cell washout, there was no apparent removal of PO_4-P. It was also shown that an increase in temperature from 20.0 to 30.0℃ enhanced nutrient reduction efficiency and favoured the growth of microalgae. De Pauw & van Vaerenbergh (1983) also reported that the growth rate of *Oscillatoria agardhii* increased with an increase in temperature from 20.0℃, to 25.0 and 30.0℃. Maynard *et al*. (1999) and Camargo Valero & Mara (2007) reported that microalgae nutrient uptake can be the main mechanism lowering NH_4-N concentration in wastewater during summer.

Bacterial nitrification was probably responsible for the oxidation of NH_4-N into NO_2-N and NO_3-N. This can be supported by the presence of nitrifying bacteria identified through metagenomics and the elevated concentrations of NO_2-N and NO_3-N in the effluent when microalgae and bacteria consortium were cultured at pH 7.0 and 8.5. It is known that nitrification occurs within this pH range (Khin & Annachhatre 2004; Munezvenyu 2008). In the seasonal variation and flow rate experiments,

when the pH was between 7.0 and 8.5, nitrification could have been one of the mechanisms responsible for lowering the NH_4-N concentration. However, nitrifiers are generally slower-growing bacteria and washout before heterotrophic bacteria, so bacterial nitrification could have been compromised at the faster flow rates.

Another underlying mechanism responsible for the lowering NH_4-N in wastewater is the volatilization of the unionised ammonia (NH_3-N) into the atmosphere and the continuation of the abiotic nitrogen cycle in the absence of microorganisms. Volatilization of NH_4-N occurs at higher temperatures during summer when the pH is above 9.0 (Sevrin-Reyssac 1998; Zimmo 2003). This was demonstrated when, at pH 10.0, the volatilization of NH_3-N appeared to be greater at 30.0℃ than at 20.0℃. However, the loss of nitrogen from wastewater through NH_3-N volatilization in algal ponds is considered less significant, compared to the nitrogen removed through biological activity (Camargo Valero & Mara 2010). A considerable portion of the NH_4-N was removed from the effluent through NH_3-N volatilization or through other abiotic mechanisms in the autoclaved medium at pH 10.0, which was not as apparent when the pH was lower. Camargo Valero & Mara (2010) also reported the volatilization of NH_3-N from effluent with no microbial activity when the pH was above 8.5. Algal assimilation and nitrification were certainly responsible for lowering nitrogen in the HRAP ponds used to treat brewery effluent at Ibhayi Brewery (i.e. the seasonal variation and flow rate experiments), but the pH of these ponds suggest that a large portion, i.e. close to 50% of the ammonium removed, could have been due to ammonia volatilization and due to the continuation of the abiotic nitrogen cycle in the absence of microorganisms.

The lowering of PO_4-P concentration in wastewater was probably due to algal. In the seasonal experiment, there was no apparent link between the drop in PO_4-P and algal biomass or pH. However, in the flow rate, temperature and pH experiments, there was a link between the decrease in PO_4-P and algal biomass. The growth of microalgae was favoured by an increase in temperature and corresponded with an increase in the reduction efficiency of PO_4-P. This was also observed by Powell *et al*. (2008), when the reduction efficiency of PO_4-P increased with an increase in temperature from 15.0 to 25.0℃ in microalgae cultures. This is an indication that the decrease in PO_4-P was due to microalgal assimilation. This hypothesis is further supported by the finding that the rate of partial removal of phosphorus did not increase with an increase in pH (i.e. when pH was lowered using HCl), but there was a significant increase in the rate at which PO_4-P concentration was lowered with an increase in the addition of CO_2 which was used to lower the pH. The addition of CO_2 in microalgae culture is known to increase algal biomass and thus results in algal assimilation (Park *et al.* 2011), which was seen here too with increased chlorophyll production when more CO_2 was added to the cultures. The increased carbon in the CO_2 dose and resultant increase in nutrient assimilation might have been responsible for the lower PO_4-P concentration when CO_2 was used.

The chemical oxygen demand

In the HRAP A2, the highest COD_F recorded in December and January, overlapped with the period of elevated algal biomass and Chl *a* concentrations. In algal ponds, microalgae use CO_2 as a source of carbon and in turn releasing photosynthetic organic products such as glycolic acid into the water body which also contributes to the escalating levels of COD_F in HRAPs and this makes it difficult to reduce COD in algal ponds (Wang *et al*. 2009). Total dissolved solids (TDS) also contribute to the COD in a sense that when TDS in ponds increases, the COD also rises (Akan *et al*. 2010). In the HRAP B2 where the flow rate of post-AD brewery effluent was progressively increased, the constant COD in the HRAP B2 could have been attributed to a decrease in the algal biomass. It is known that microalgae release glycolic acid into water which contributes to dissolved COD (Wang *et al*. 2009;

Kim *et al*. 2014), however, in the HRAP B2 where the flow was increased, this was probably not the case, and thus a constant COD level was maintained.

2.5 Conclusion

Microalgae growth and nutrient removal from the post-AD brewery effluent were influenced by temperature and pH. The outcomes of this study demonstrated that summer conditions and slower flow rates (i.e. higher HRTs) favoured algal productivity and the performance of the HRAP in lowering NH_4-N in the effluent. Under these circumstances, algal assimilation contributed considerably to effluent treatment but the high pH of the ponds suggests that a large portion of the nitrogen was probably removed through ammonium volatilization. The partial removal of PO_4-P, on the other hand, appeared to be related to algal assimilation. During winter and at increased flow rates, algal productivity and nutrient reduction efficiency was decreased substantially. However, under these conditions there were shifts in the bacteria community structure that saw the proliferation of faster growing species such as the Proteobacteria, which have a high affinity for nutrients and include nitrifying bacteria. This suggests that the mechanism of nutrient removal in the HRAP in winter and at increased flow rates (i.e. conditions that do not favour algal productivity) could shifts in favour of bacterial nitrification. However, the relative abundance of known nitrifiers among the identified species was still low in winter and at high flow rates; more research is needed to identify the bacterial species that are present under these conditions and that appear to be responsible for lowering nutrients in the effluent. The hypothesis that there is probably a shift in favour of bacterial nitrification in winter is further supported by the lower pH values recorded in autumn and winter, which are (a) more favourable for bacterial nitrification and (b) less favourable for ammonium volatilization than the higher pH values seen in spring and summer which would be mediated by algal photosynthetic activity.

The largest portion of the bacterial species in winter where “unknown” and majority of the algae in the HRAP in winter and at slow flow rates were also “unknown. This highlights the need to identify these organisms in future research. Controlled grazing experiments, which would provide further insight on the impact of zooplankton grazing on microalgae and bacteria, is another area that requires further investigation since this would further enhance the understanding of the microbial shifts that take place under different conditions.

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3. Harvesting algae using phytoplanktivorous fish

This project formed the basis of MSc student Miss Melisa Mayo's thesis (Mayo 2016) and parts of this report are taken directly from the draft version of that thesis and from a publication manuscript that is being prepared. The work forms the basis and motivation for further work that will be carried out on a new Water Research Commission (WRC) Research Project that will start in 2016 (P1004467).

The WRC funded this research (K5/2284), while SAB Ltd made the research site and utilities available for the work. In addition, the student received a freestanding bursary from the National Research Foundation that covered her student fees and contributed to her living costs.

3.1.1 Rationale

Harvesting microalgal cells remains a challenge in producing an effluent that meets discharge requirements when using algal ponding in wastewater treatment. The presence of algae can influence water quality parameters such as pH and chemical oxygen demand (COD) (Park *et al*. 2011). Various methods have been developed to harvest algae, although it still remains relatively expensive (Craggs *et al*. 2011). Examples of conventional methods include centrifugation, coagulation, and flocculation, in-pond chemical precipitation of suspended material, flotation, and filtration (Middlebrooks *et al*. 1974). However, in spite of the costs involved, neither chemical nor physical methods ensure that the contaminants are completely removed (Hardman *et al*. 1993).

Even with the numerous benefits associated with the use of high rate algal ponding (HRAP) systems, it is not "turn-key" technology, and further research and development is needed to optimise certain elements of the system (Cilliers 2012). The harvesting of the very small algal cells that are suspended in the liquid medium remains a constraint to the efficient use of this technology in treating effluent, and it has been this way for decades (Oswald & Golueke 1968). Successful attempts have been made to overcome this by using waste grown algae as an in situ food source for phytoplankton-feeding fish, which filter the algae out of the water (Edwards 1980; Lincoln *et al*. 1978; Shelef *et al*. 1978). However, due to the high organic content of brewery effluent and substantial fluctuations in pH and oxygen concentration associated with high algal concentrations, it remains to be seen if fish are able to cope with such a dramatic change in their environment (Rakocy *et al*. 2000) and to determine the rate of removal.

3.1.2 Aims and objectives

Microalgae are efficient in nutrient removal from brewery effluent; however, harvesting these cells from a liquid medium is both difficult and expensive, and is considered a "bottle neck" in the process. Many fishes are able to feed on microalgae and plankton thus converting microbial biomass into fish biomass. The overall aim of this study was to investigate the use of phytoplankton-feeding fish as in-situ filter feeders, as an affordable, alternative method that reduces algal biomass in treated brewery wastewater. The objectives of the experiment were:

1) to determine if the presence of fish had an impact on the change in algal biomass in HRAP effluent;

2) to determine if the addition of a supplementary commercial feed and/or different concentrations of algae impact on the rate that tilapia removed algae from HRAP effluent; and

3) to characterise the growth and health of fish subject to a high and low algal concentration both with and without the addition of a commercial feed.

3.2 Methods and materials

3.2.1 Experimental system

The experiments were carried out in a greenhouse facility at Ibhayi Breweries in Port Elizabeth, South Africa. Brewery effluent underwent treatment in the Ibhayi Brewery anaerobic digester (AD), followed by treatment in the Project Eden integrated algal ponding system that consisted of a primary facultative pond (PFP) and a series of high rate algal ponds (HRAP; This system is descried in Section 2.2.1, Figure 2.1). Effluent from these HRAP ponds were used as the water source for the experiments presented here.

The fish culture system consisted of 24 circular fish tanks, each with a maximum operating volume of 560 L (Figure 3.1). The tanks formed two closed recirculating system (12 tanks per system) and the water flow rate could be adjusted with the use of a valve into each tank. Each group of 12 tanks in each system drained into a sump and water was circulated back to the tanks via a sand filter and a biological filtration system. Each tank had a constant air supply, and they were all covered by 30% shade cloth.

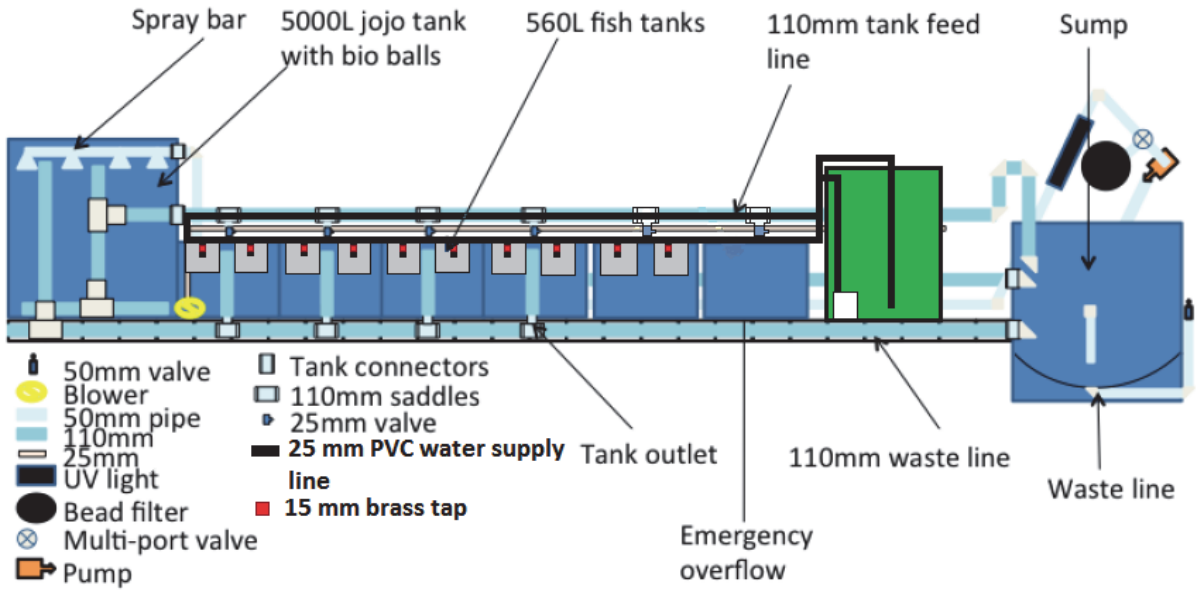

Figure 3.1 Section view of the main aquaculture system which includes the spray bar filter tank, the sump and six of the twelve 560 L fish tanks. This system was filled with post-HRAP water.

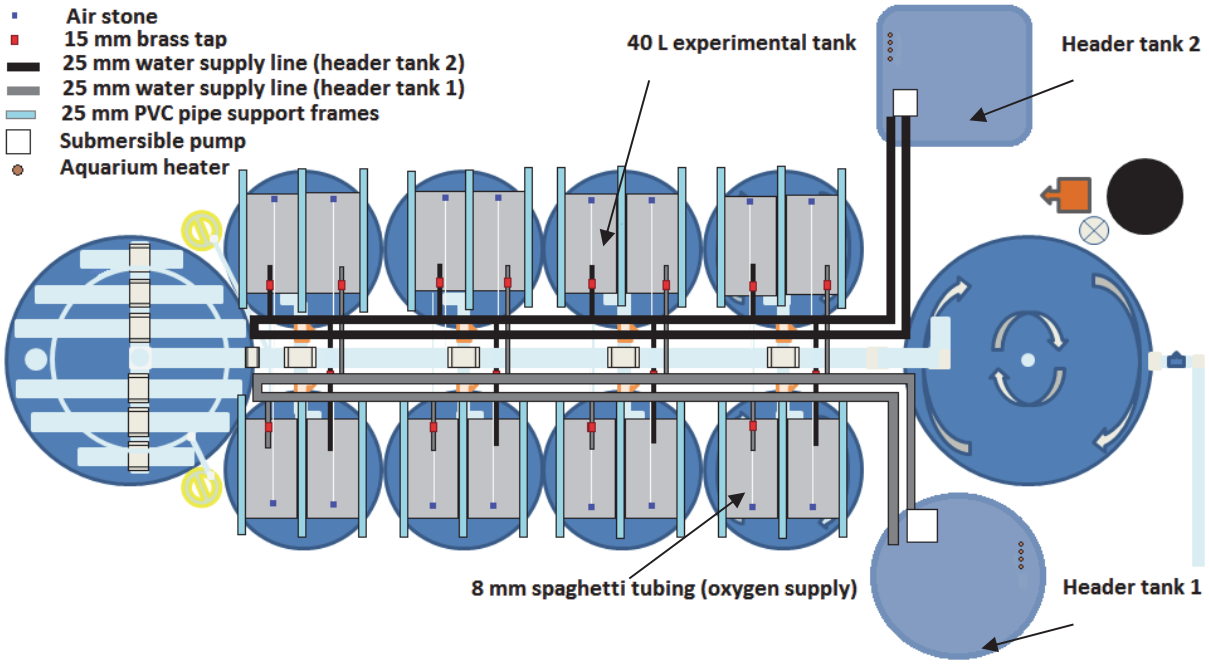

Figure 3.2 Aerial view of the main aquaculture system and sixteen of the twenty 40 L fish culture tanks. Two temporary 1000 L header tanks (1 and 2) supplied post-HRAP water to the experimental tanks. Four of the 560 L tanks have be left out of this diagram to save space and to maintain scale.

Ten tanks from one of the two recirculating systems were used in the experiments that are presented here (Figure 3.2). Twenty 40 L rectangular, fish culture tanks were suspended in the larger 560 L tanks; two small tanks in each large tank (Figure 3.2). The purpose of placing the small tanks into the larger ones was to moderate the temperature in the small tanks due to temperature fluctuations in the greenhouse tunnel; the water in the larger tanks was kept at 25°C by circulating it through a heat pump for the duration of the experiment. In addition, aquarium heaters were placed into each 40 L tank to maintain the temperature at 25°C. Each tank was aerated using an air-blower and an air stone to ensure that oxygen levels were maintained at 75-90% saturation and to circulate the water within the tank. The 20 smaller fish culture tanks did not form part of the recirculating system; each of these tanks had its own inflow and outflow so that each could operate as an individual, flow-through system.

Two 1000 L header tanks were installed into the system (Header Tank 1 and Header Tank 2), each with its own supply line to the 20 smaller fish culture tanks (Figure 3.2). One of the header tanks contained effluent from the HRAP with its full complement of algae (effluent mixed to ensure that the algae remained in suspension), while the second contained effluent from the HRAP from which algae had been allowed to settle out of suspension prior to being placed in the header tank.

3.2.2 Experimental animals and acclimation

Captive bred *Oreochromis mossambicus* were collected from a commercial hatchery (Rivendell Hatchery (Pty) Ltd, Grahamstown). They were acclimated to this system for three months prior to the experiments).

3.2.3 Effect of algal concentration and feed supplementation on rate of algal removal

Effluent was drawn from the HRAP system into the two header tanks, and was subsequently drained into the 40 L fish culture tanks. The tanks were either filled with HRAP effluent that contained algal cells (i.e. unsettled, high algal cell concentration HRAP effluent; Table 3.1) or with HRAP effluent with most of the algal cells removed (i.e. settled, low algal cell concentration HRAP effluent; Table 3.1). Fish were stocked into each tank (5.0 fish/tank) and the fish in half the tanks were fed a supplementary diet of commercial fish feed (Table 3.1); they were fed this feed on a daily basis for the first six weeks and three times per day thereafter. The four treatments resulted in a two-by-two factorial experimental design treatments (i.e. the first experiment included treatments T1 to T4, Table 3.1) and each of these treatments was represented by four replicates tanks of fish (Table 3.1).

3.2.4 Change in algal biomass with and without fish in the tank

The high and low algal cell treatments introduced above that were not fed a supplementary diet of commercial fish feed (i.e. T3 and T4) were also represented without fish (i.e. T5 and T6;), in two replicate tanks each (Table 3.1). These treatments formed a second multifactorial experiment where the high and low algal cell treatments were represented both with and without fish in the tanks (Table 3.1).

After they were initially filled from the two effluent sources mentioned above, all the tanks were subsequently subjected to a water exchange of 25 L/day. That is, 25 L was siphoned out of each tank and replaced with either (a) 25 L of HRAP effluent from which algal cells had been settled out (i.e. in the case of all low algal cell concentration treatments) or (b) with a predetermined volume of HRAP effluent that contained the full complement of algal cells mixed with HRAP effluent from which algal cells had been removed so as to obtain an algal cell concentration of 0.5 mg/L (Table 3.1).

3.2.5 Data collection

Changes in algal biomass and filtration rates

To determine the algal biomass of HRAP-A2, triplicate water samples were collected daily, before being filtered (eight micron filter paper; Whatman 40 Ashless Circles, 47 mm diameter, Cat no. 1440 125), dried and weighed on a four-point digital balance. To do this, faecal matter had to first be removed from the tanks, so five litres of water containing all faecal matter was siphoned from each tank daily. Treatments that did not contain fish were also subject to this process in order to standardize the rate of water exchange for all tanks. The removal of this five litre contributed to the 25 L/day water exchange (Table 3.1).

In the first week of the experiment, a set mass of 0.5 mg/L of microalga was added to the T1, T3, and T5 experimental tanks. The number of litres of highly concentrated algal water added to each tank each day was calculated based on the daily microalgal biomass values measured for pond-A2 (Table 3.1). The remaining amount of water siphoned from each tank was replaced with low concentration microalgal water stored in header tank 1 and 2, by opening the valve and from the respective header tank and allowing the water level to rise to the 40 L water line. Prior to the water exchange, water samples were collected from 22 sampling points: 20 experimental tanks, and header tank 1 and 2. Algal-rich water collected from pond-A2 was then added to each tank and the new algal concentrations calculated (Section 2.2.4).

Table 3.1 Fish were grown in high rate algal pond (HRAP) effluent with either a high or low algal concentration. The low algal concentration treatments was drawn from HRAP effluent where algae had been settled out of solution, whereas the high concentration treatments was drawn from HRAP effluent with the full complement of alga mixed with HRAP effluent were algae had been allowed to settled out of solution. Fish in both these treatments were either fed a supplementary formulated diet or not (i.e. the first experiment will include a multifactor design with treatments T1 to T4). In addition, the high and low algal cell treatments were both represented with and without fish, all without the addition of a formulated feed (i.e. the second experiment included a multifactor design with treatments T3 to T6).

Exp. 1	Exp. 2	Fish/tank	Feed	Algae	Effluent exchange rate (L/tank/day)			No. rep.
					(b) unsettled HRAP	(a) settled HRAP	Total	tanks
T1		5	To satiation	High	x	25 – x	25	4
T2		5	To satiation	Low	0	25	25	4
T3	T3	5	None	High	x	25 – x	25	4
T4	T4	5	None	Low	0	25	25	4
	T5	0	None	High	x	25 – x	25	2
	T6	0	None	Low	0	25	25	2

To determine the amount of microalgae removed by fish in each tank, water samples were again collected from each tank 24 hours later. These values were then subtracted from the original microalgal concentrations. The rate of microalgal removal was determined as a function of the fish biomass within each tank. To optimize the amount of microalgae removed from the water, microalgal biomass data were analysed weekly to determine whether to increase or decrease the set value from 0.5 mg/L for each successive week.

The filtration rate of fish was calculated using Equation 3.1 (Turker *et al*. 2003b):

$$FR\ (mg\ C/\ kg^{-1}) = \frac{(AB_i - AB_o)}{fish\ biomass} \tag{3.1}$$

where AB_i is the algal biomass in incoming water ($mg\ C/L$), AB_o is algal biomass in outgoing water, and fish biomass is kg of wet tissue. This step was repeated twice weekly.

Stable isotope analysis

Two separate microbial biomass samples of 250 mL each were collected from the HRAP-A2 with the use of 250 mL glass beakers and filtered through 8.0 µm glass fibre filter paper. Microbial samples were then dried to a constant weight at 80°C for 24 h. Approximately 10 g of commercial fish food was also collected for analysis. Samples were ground with a mortar and pestle (Melville & Connolly 2003). The mortar and pestle were cleaned with 70% ethanol between samples. Sub-samples of only green pellets and only red pellets as well as a combination of red/green pellets were taken. Three repetitions were completed on the fish pellets and duplicates were performed on each of the two microbial biomass samples. Approximately one to 1.1 mg of the pellets and 6.4 mg of the microbial biomass samples were weighed into tin capsules that were pre-cleaned in toluene.

Whole fish specimens were homogenized by grinding up all tissue contents with a hand-held blender. Approximately two grams of homogenized tissue was collected for one fish specimen from each treatment replicate. Tissue samples were then rinsed in distilled water for 10 s before being dried at 60°C for 48 h. Samples were ground to a fine powder with a mortar and pestle. The mortar and

pestle were cleaned with 70% ethanol between each sample. Lipid extraction was performed on all fish tissue samples. A sub-sample of ground tissue from each of the samples was placed in a 25 mL Pyrex test-tube and covered with 10 mL of a 2:1 ethanol: chloroform mixture. The samples were agitated with an ultra-sonic bath for five minutes before the supernatant was poured off. This was repeated twice to ensure the removal of all lipids within the tissue. The samples were then dried at 70°C overnight prior to weighing. Aliquots of approximately 0.6 to 0.7 mg of fish tissue were weighed into tin capsules that were pre-cleaned in toluene. Three repetitions were completed for each sample (Arrington & Winemiller 2002).

Isotope analysis was done on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (Thermo Fischer, Bremen, Germany), housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria. A laboratory running standard (Merck Gel: $\delta^{13}C$ = -20.57 ‰, $\delta^{15}N$ = 6.8 ‰, C% = 43.83, N% = 14.64) and blank samples were rerun after every 12 unknown samples. All results were referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen isotope values. Results were expressed in delta notation using a per mille scale using the standard Equation 3.2:

$$\delta X(‰) = [(R_{sample} - R_{standard})/R_{standard} - 1] \times 1000 \quad (3.2)$$

where X = ^{15}N or ^{13}C and R represents $^{15}N/^{14}N$ or $^{13}C/^{12}C$ respectively. Analytical precision was <0.2 ‰ for $\delta^{13}C$ and <0.2 ‰ for $\delta^{15}N$ (Melville & Connolly 2003).

Fish morphology, growth and health

The amount of commercial fish food consumed was determined for each tank on a weekly basis and recorded. Fish were weighed to the nearest gram and fork length measured to the nearest millimetre once each week during the first six weeks of the trial and once every three weeks thereafter.

At the end of the experiment, the fish were humanely euthanized in a bath of 2-phenoxyethanol at a concentration of 0.4 mg L^{-1} (Brown 2003). The stomachs and intestinal tract were removed and unravelled. In order to determine whether the fish were ingesting the algae in the tanks and to estimate the proportional quantity, the length of the stomach and intestinal tract were measured (mm), as well as the length of intestinal tract occupied by microalgal mass (mm). The quantity (%) was calculated using Equation 3.3:

$$\frac{\text{Length of intetsinal tract occupied by microalgal mass (mm)}}{\text{Total length of intestinal tract (mm)}} \text{ x } 100 \quad (3.3)$$

Visceral fat index (VFI, %) was calculated using Equation 3.4:

$$VFI\ \% = (W_{vf}) \times (W_{fd})^{-1} \times 100 \quad (3.4)$$

where W_{vf} represents visceral fat weight (g) and W_{fd} stands for eviscerated fish weight (g).

Hepatosomatic index (HSI, %) was calculated using Equation 3.5:

$$HSI\ \% = (W_{liver}) \times (W_{fd})^{-1} \times 100 \quad (3.5)$$

where W_{liver} represents liver weight (g) and W_{fd} stands for eviscerated fish weight.

The first gill arch was removed from the left-hand side of eight fish (139-178 mm). The samples were rinsed with distilled water, placed onto microscope slides and analysed with an dissecting microscope and camera at 1.26 x magnification (Olympus SZX16, Olympus DP72 camera). The following measurements were recorded to the nearest 0.01 mm from each of the gill rakers using a computer programme: (a) length of the gill raker from the base to the tip, (b) gill raker width at a point midway between the gill raker base and the tip, (c) open space or distance between adjacent gill rakers (inter-raker distance) at a point midway between the gill raker base and the tip, and (d) distance between gill rakers (number/mm) at the base of the insertion of the gill raker into the branchial cartilage (Rosen & Hales 1981).

Water chemistry

The chemical oxygen demand (COD_F) and NH_4^+, NO_2^-, NO_3^-, PO_4^-, and Cl^- concentrations were measured weekly (Section 2.2.4) and temperature, pH, EC, dissolved oxygen (DO) and % saturation were measured daily in each culture tank.

3.2.6 Statistical analyses

A two-way/multifactorial analysis of variance (MF-ANOVA) and, in instances where data were collected over a period, a repeated measures multifactorial analysis of variance (RM MF-ANOVA) was performed for the comparison of T1, T2, T3 and T4 data (the first experiment) and for the comparison of T3, T4, T5, and T6 data (the second experiment). To ensure that the data met the assumptions of an ANOVA, a Levene's test was performed to determine the equality of variances and a Shapiro-Wilk's test was performed to confirm the normality of residual values. When the data did not meet the assumptions of an ANOVA, a Mauchley Sphericity test was conducted. When the values still did not meet the assumptions of an ANOVA, they were adjusted using the Greenhouse and Geisser epsilon (G-G) and the Hunyh and Feldt epsilon as correction factors.

3.3 Results

3.3.1 Effect of algal concentration and feed supplementation on rate of algal removal

The structure of Mozambique tilapia's gill make it possible for them to filter some of the microorganisms in the HRAP effluent. The size of the microalgal clumps that were observed in the HRAP effluent ranged from just over 200 µm to over a 1000 µm (Figure 3.3). The mean gill raker length and width of the fish used in this experiment was 1233 ± 95 and 425 ± 37 µm respectively, with mean inter raker distances at the base and midpoint of the gill rakers of 604 ± 32 and 640 ± 23 µm respectively (Figure 3.4).

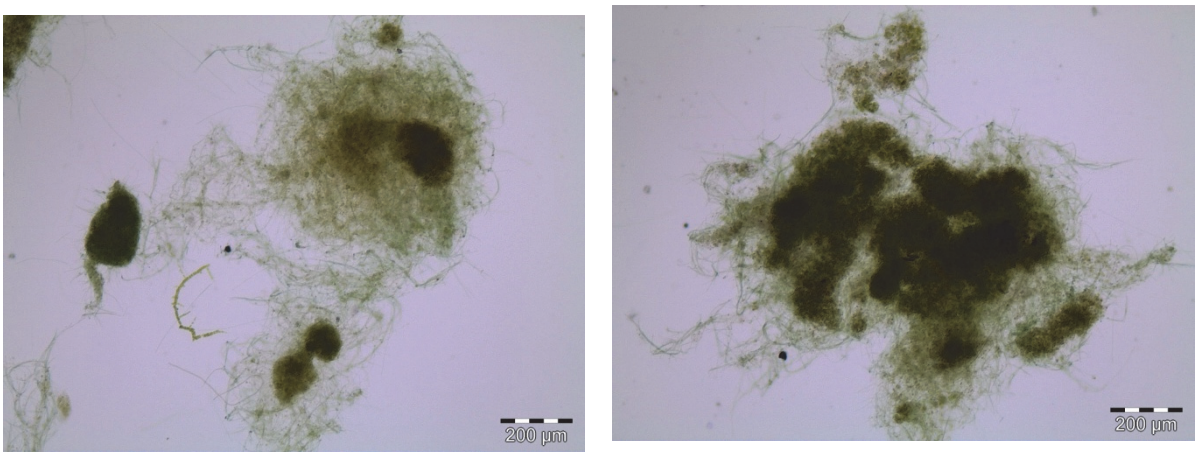

Figure 3.3 Microalgal clumps typically seen in the high rate algal pond used to treat post-anaerobically digested brewery effluent flowing through the system at a rate of 1000L/d (Photographs by Melissa Mayo).

The fish in all the treatments (i.e. Treatment 1 to 4) consumed algae in the HRAP effluent, and there were no difference in the percentage of the stomach content that was filled with algae between treatments (RM ANOVA, $F_{(1\ 12)} = 0.44$, $p = 0.52$). The treatment means ranged from 70.92 ± 20.72 to 98.88 ± 1.12% algae in the stomachs of the tilapia that were sampled. The consumption of algae was also apparent in the green pigmentation of the faeces of all the fish in the experiment.

Algae did not contribute significantly to the isotopic δ15N in fish tissue, even in the absence of a commercial feed. The mean isotopic δ15N and δ13C values obtained from the fish tissue subject to the different treatments were all similar and were all more like the red commercial fish pellet and less like the green commercial pellet and those of the microorganisms harvested from the HRAP (Figure 3.5). The mean isotopic δ13C value of the microorganisms harvested from the HRAP did, however, overlap with those of all the fish tissue samples (Figure 3.5).

The rate of algal removal was not influenced by an interaction between the two main factors (MF RM-ANOVA: $F_{(11,\ 121)} = 1.59$, $p = 0.11$), so the effect of commercial feed on the rate of algal removal was the same for the high and low algal concentration treatments. However, when looking at algal concentration on its own, there was a significant difference between the high and low algal concentration treatments ($F_{(11,\ 121)} = 5.99$, $p < 0.001$), but the differences that were seen showed no regular pattern.

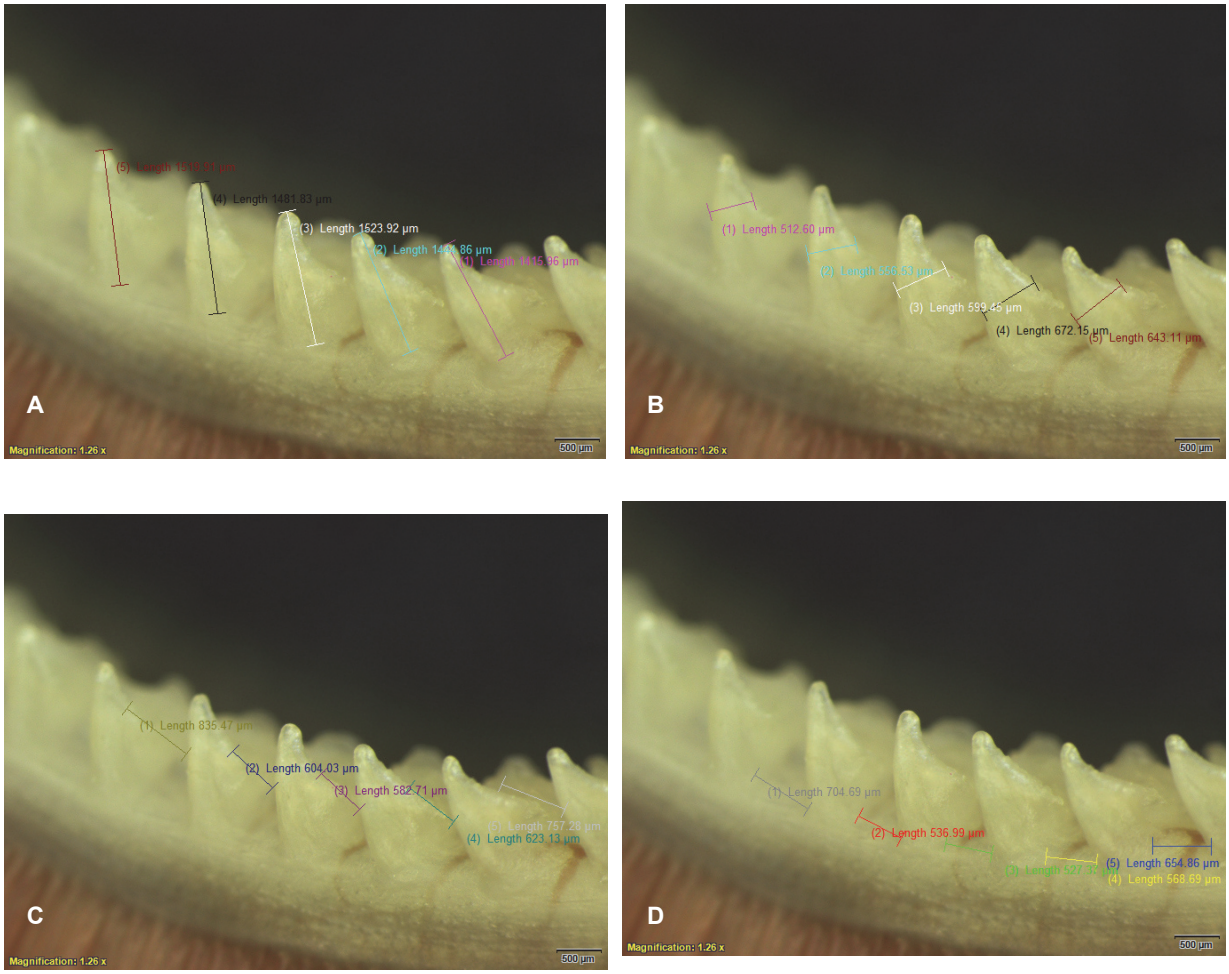

Figure 3.4 The length of the gill rakers (A), their width at the mid-point of the length (B), the inter-raker distance at mid-point of the length (C), and inter-raker distance at base of gill rakers (*Photograph by: Melissa Mayo*).

Fish that received microalgae as their sole source of nutrition did not grow. The mean weight of fish was influenced by the presence or absence of formulated fish feed over time ($F_{(1,\ 12)}$ = 26.67, $p < 0.001$, Figure 3.6). The weight of those in treatments supplemented with formulated fish feed increased from 34.61 ± 1.76 g at the start of the experiment to 57.38 ± 3.44 g in week 11, whereas there was no increase in those that received microalgae only (Figure 3.6). This relationship was similar for fish in the high and low algal concentration treatments since there was no interaction between factors (MF RM-ANOVA: $F_{(1,\ 12)}$ = 0.43, p = 0.52); the addition of microalgae at a high concentration did not have a significant effect on the weight of the fish ($F_{(1,\ 12)}$ = 0.03, p = 0.87).

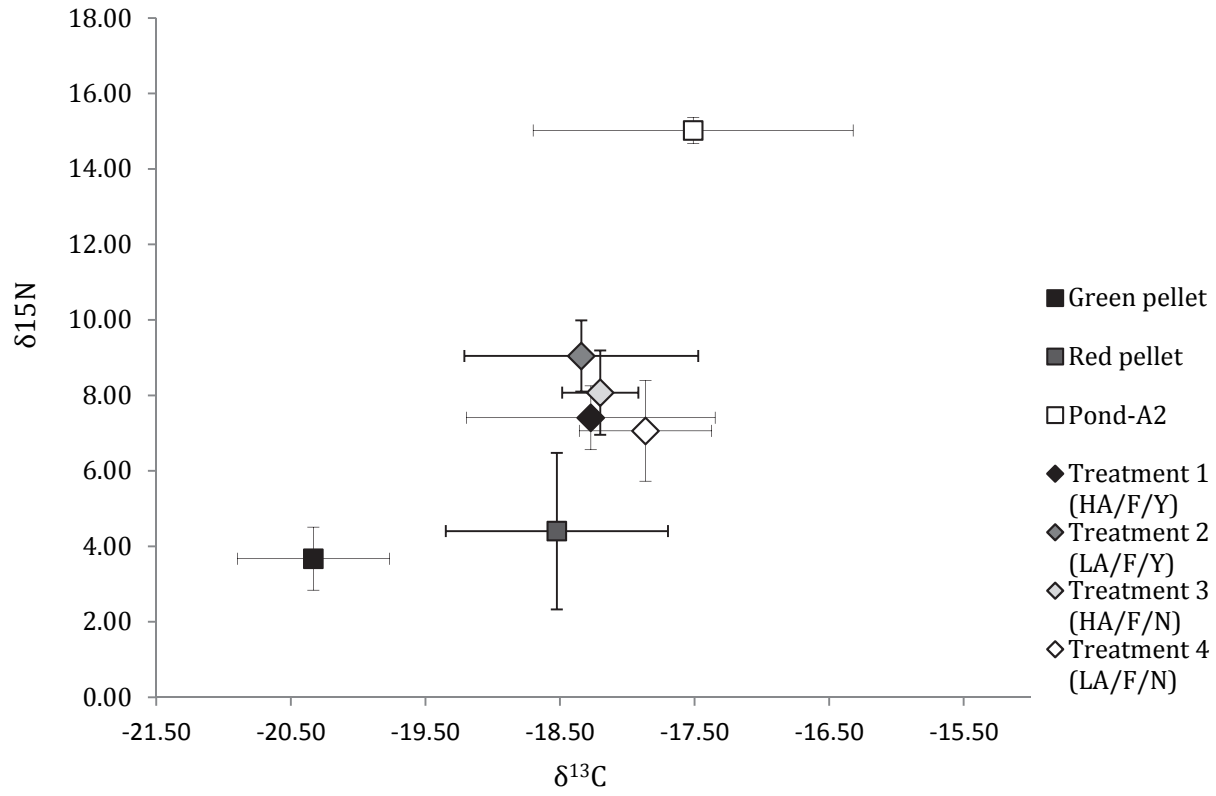

Figure 3.5 The mean carbon (δ13C) and nitrogen (δ15N) (± standard deviation) signatures of homogenous fish tissue samples (after lipid extraction) from Treatments 1 to 4, in relation to the microalgae from the high rate algal pond (HRAP-A2) and formulated fish feed diets (green and red pellet) where HA = high alga; LA = low alga; F = fish; Y = additional commercial fish food; N = no additional commercial fish food.

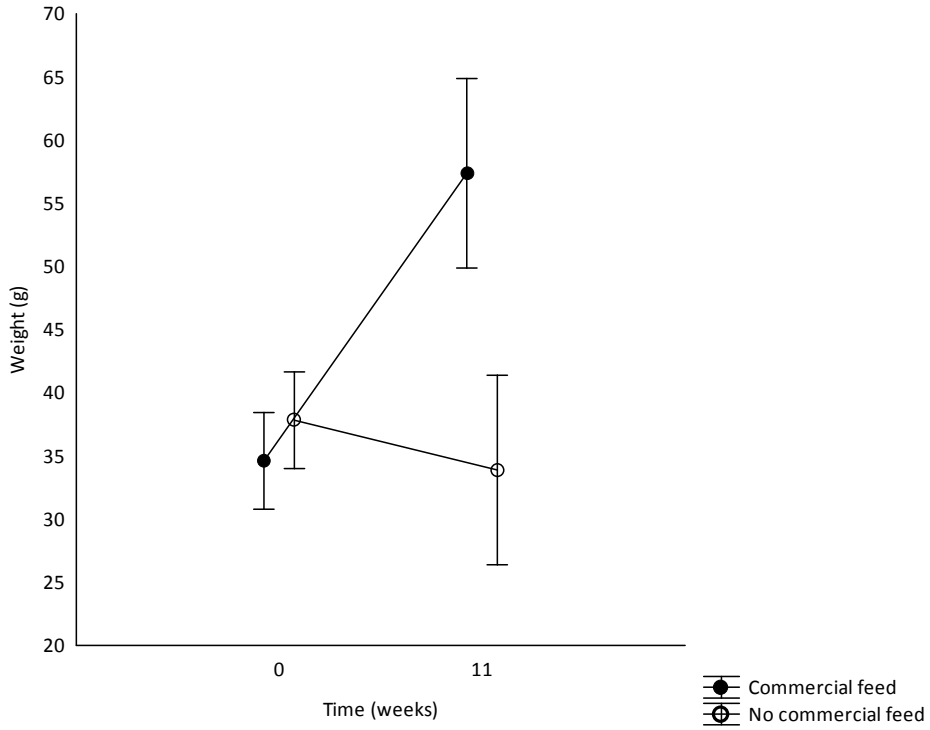

Figure 3.6 The mean (± 95% confidence interval) weight of *O. mossambicus* in treatment tanks that were or were not supplemented with formulated fish feed (MF-ANOVA: $F_{(1, 12)} = 26.67$, $p < 0.001$).

Fish energy reserves (measured as visceral fat weight and the liver weight relative to whole body weight) were significantly reduced in fish that were fed an alga-only diet. The mean hepatosomatic index (HIS) was influenced by the algal concentration ($F_{(1, 12)}$ = 9.00, p = 0.012, Table 3.2), where it decreased from a combined mean of 2.40 ± 0.04% when fish were subject to a highly concentrated algae to that of 2.25 ± 0.03% in the low algal concentration treatments (Table 3.2). The presence or absence of the commercial feed did not affect HIS ($F_{(1, 12)}$ = 0.02, p = 0.89) and there was no interaction between factors (MF ANOVA: $F_{(1, 12)}$ = 0.24, p = 0.63). Similarly, the mean visceral fat index of the fish was influenced by the algal concentration ($F_{(1, 12)}$ = 25.3, p < 0.001, Table 3.2), where it decreased from a combined mean of 0.09 ± 0.008 in animals that were subject to the highly concentrated algal HRAP effluent to 0.03 ± 0.009 in the low algal concentration treatments (Table 3.2). There was also no interaction between the main factors (p = 0.94) and, on its own, the presence or absence of commercial feed had no significant influence on the VFI ($F_{(1, 12)}$ = 0.36, p = 0.56).

Table 3.2 The mean (± standard error) body measurements of tilapia subject to high rate algal pond effluent with either a high or low concentration of alga, both with and without the addition of commercial feed. Means with a different superscript within a row were significantly different (MF ANOVA, p<0.05).

	Treatment 1 High algal conc. Commercial feed	n	Treatment 2 Low algal conc. Commercial feed	n	Treatment 3 High algal conc. No commercail feed	n	Treatment 4 Low algal conc. No commercial feed	n	p
Liver weight (g)	1.44 ± 0.22	4	1.52 ± 0.29	4	0.96 ± 0.12	4	1.25 ± 0.29	3	p = 0.65
Gut length (mm)	767.50 ± 34.25	4	812.50 ± 105.70	4	712.50 ± 62.77	4	720.00 ± 58.59	3	p = 0.80
Eviscerated weight (g)	52.93 ± 6.11	4	60.63 ± 11.93	4	35.18 ± 5.05	4	48.90 ± 11.83	3	p = 0.75
Visceral fat index	0.09 ± 0.01 [a]	4	0.03 ± 0.01 [b]	4	0.09 ± 0.01 [a]	4	0.02 ± 0.01 [b]	3	p < 0.001
Hepatosomatic index	2.41 ± 0.05 [a]	4	2.23 ± 0.05 [b]	4	2.39 ± 0.05 [a]	4	2.27 ± 0.06 [b]	3	p = 0.012

Commercial feed influenced the rate that algae were removed from HRAP effluent. When the fish's diet was supplemented with feed, the rate at which algae were removed from the effluent was significantly higher on numerous occasions over the course of the experiment, whereas the mean change in algal biomass was nearly always negative (i.e. there was an increase in algal biomass) when the fish did not receive commercial feed ($F_{(11, 121)}$ = 10.12, p < 0.001; Figure 3.7). The increase in algal biomass was not expected and was due to the production of algae in the fish tanks between the time that algae were placed in the fish tank and the end sample 24 h later. This resulted in an underestimation of the absolute rate at which the fish "removed" the algae reported here since algae were also produced; the relative differences discussed probably remain comparable between treatments.

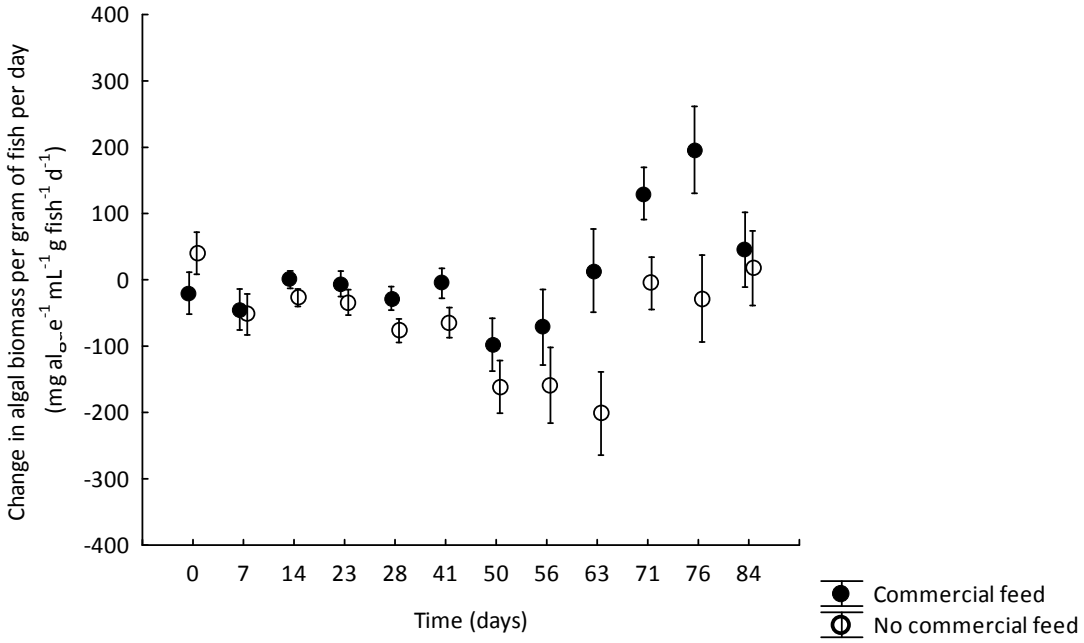

Figure 3.7 The mean change in algal biomass per gram of fish per day (± 95% confidence intervals) in tanks that contained fish and that either were or were not supplemented with additional formulated fish feed, from June to September 2014 (MF RM-ANOVA: $F_{(11, 121)} = 10.12$, $p < 0.001$).

3.3.2 Change in algal biomass with and without fish in the tank

A comparison of Treatments 3 and 4 (i.e. high and low algal concentration, both with fish) with Treatments 5 and 6 (i.e. a high and low algal concentration, but with no fish in the tanks) confirmed that algal productivity took place in the tanks between placing the HRAP effluent into the fish tanks and when the final algal biomass was recorded 24 hours later. When fish were present in the tank, algal biomass was nearly always removed by the fish (a positive value; Figure 3.8) and when fish were absent from the tank algal biomass usually increased (a negative value; Figure 3.8). This difference in the change in algal biomass was not significant between the fish present and fish absent treatments at a low algal concentration, whereas at a high algal concentration the drop in algal biomass when fish were present was often significantly different from the increase in algal biomass when fish were absent (MF RM ANOVA, $F_{(12, 72)} = 2.74$, $p = 0.004$; Figure 3.8).

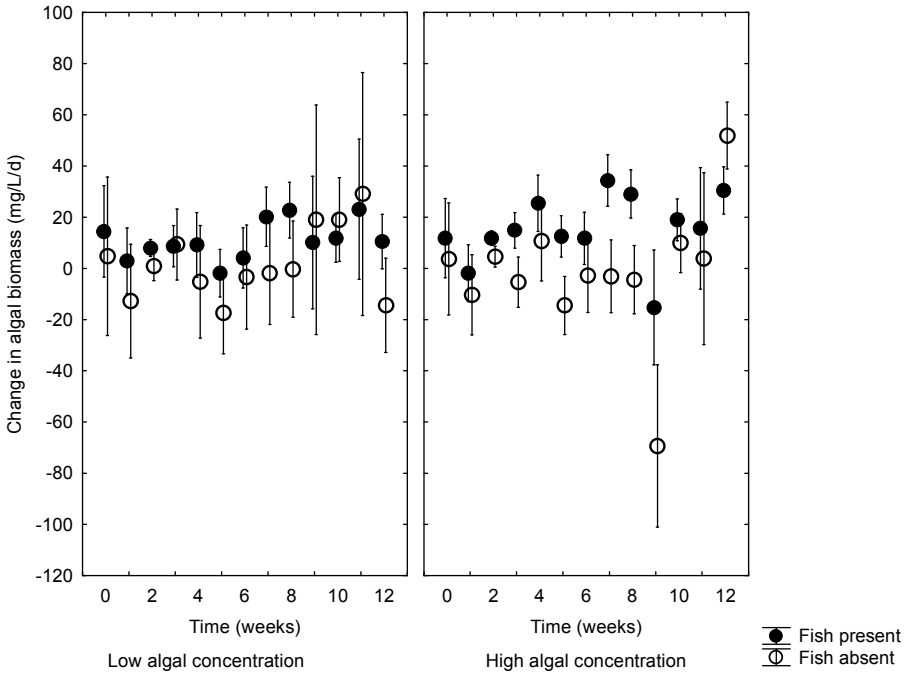

Figure 3.8 The mean change in algal biomass (± 95% confidence intervals) in tanks that contained high and low concentrations of alga, both with and without fish present in the tank (MF RM ANOVA, $F_{(12, 72)} = 2.74$, $p = 0.004$). The change in algal biomass was equal to the biomass at the end of a 24 h period, less the biomass at the start of that period; a positive number indicates a drop in biomass and a negative value indicates an increase in biomass.

The fish were eating algae off the bottom of the tank in the form of benthic algae and probably algae that had settled out of suspension. When fish were absent from the tank, significantly more algal biomass (31.78 ± 1.41 g/tank) was collected off the bottom of the tanks compared to those with fish (6.95 ± 0.87 g/tank; $F_{(1, 7)} = 300.30$, $p < 0.001$). There was no interaction between the main factors of the multifactor ANOVA for benthic biomass (MF ANOVA, $F_{(1, 7)} = 2.86$, $p = 0.13$), and the algal concentration of the HRAP effluent in the tanks also had no significant effect on the biomass of algal collected from the bottom of the tanks at the end of the experiment ($F_{(1, 7)} = 4.76$, $p = 0.07$).

3.3.3 Water quality in the fish culture tanks

The mean effluent characteristics of the two effluent sources used to fill up the low algal concentration treatments in these experiments (i.e. the “header tank” in which HRAP water was stored after algal cells had been allowed to settle out) and the high algal concentration treatments (i.e. a combination of effluent from the “header tank” and from effluent directly out of the HRAP with its full algal complement) are summarised in Table 3.3.

Table 3.3 The mean (± standard error) water temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), chemical oxygen demand (COD_F), ammonia, nitrite, nitrate, phosphate, and chloride concentrations of the header tank, and high rate algal pond (HRAP) used to for the low and high algal concentration treatments respectively.

Parameter	Header tank			HRAP			N
Temp (°C)	24.42	±	0.59	16.54	±	0.68	13
pH	9.12	±	0.17	9.28	±	0.19	13
EC (µs/cm)	3111.65	±	28.39	2937.29	±	38.41	13
DO (mg L^{-1})	4.92	±	0.39	9.85	±	0.43	13
COD (mg L^{-1})	66.60	±	11.02	69.20	±	11.44	5
NH_4^+-N (mg L^{-1})	0.48	±	0.09	0.68	±	0.23	9
NO_2^--N (mg L^{-1})	0.67	±	0.16	0.81	±	0.29	11
NO_3^--N (mg L^{-1})	78.31	±	14.80	73.09	±	16.74	10
PO_4^--P (mg L^{-1})	48.21	±	5.83	40.65	±	4.76	10
Cl^- (mg L^{-1})	208.25	±	9.86	198.40	±	10.47	10

The mean temperature of water in the culture tanks ranged from 24.5 to 26.6°C with an overall mean of 24.83 ± 0.15°C (Table 3.4). Dissolved oxygen was also similar among treatments with an overall mean of 7.33 ± 0.08 mg/L (Table 3.4). Ammonium and nitrite averaged 0.43 ± 0.02 and 0.54 ± 0.03 mg/L respectively, whereas nitrate had an overall average of 81.64 ± 3.10 mg/L with similar ranges in all treatments (Table 3.4)

Table 3.4 The mean (± standard error) temperature, dissolved oxygen (DO), ammonium, nitrite, nitrate, phosphate, electrical conductivity (EC), chemical oxygen demand (COD_F) and the pH (median) recorded in the six treatments that made up the two experiments. In the first experiment, Treatment 1 to 4 included either a high or low concentration of algal from the high rate algal pond; the fish in each of these treatments were either fed a commercial feed or they received no additional feed. In the second experiment, Treatments 3 to 4 included the high and low algal concentration treatments with fish present in the tank, whereas Treatments 5 and 6 included the same algal concentrations only with no fish in the tank.

	Treatment 1 **High algal conc.** **Commercial feed** **Fish present**	**n**	**Treatment 2** **Low algal conc.** **Commercial feed** **Fish present**	**n**	**Treatment 3** **High algal conc.** **No commercail feed** **Fish present**	**n**	**Treatment 4** **Low algal conc.** **No commercial feed** **Fish present**	**n**	**Treatment 5** **High algal conc.** **No commercial feed** **No fish**	**n**	**Treatment 6** **Low algal conc.** **No commercial feed** **No fish**	**n**
Temp. (°C)	25.03 ± 0.26	52	25.38 ± 0.24	52	25.58 ± 0.23	52	24.91 ± 0.29	52	25.32 ± 0.35	26	25.49 ± 0.35	26
DO (mg L^{-1})	7.07 ± 0.11	52	6.97 ± 0.10	52	7.34 ± 0.09	52	7.28 ± 0.09	52	8.08 ± 0.43	26	7.78 ± 0.14	26
NH_4-N (mg L^{-1})	0.43 ± 0.04	36	0.43 ± 0.04	36	0.44 ± 0.04	36	0.41 ± 0.04	36	0.36 ± 0.05	18	0.40 ± 0.05	18
NO_2-N (mg L^{-1})	0.50 ± 0.06	44	0.56 ± 0.07	44	0.57 ± 0.08	44	0.61 ± 0.06	44	0.40 ± 0.07	22	0.37 ± 0.05	22
NO_3-N (mg L^{-1})	82.40 ± 7.21	40	83.24 ± 6.89	40	84.10 ± 7.61	40	79.57 ± 7.28	40	83.26 ± 10.99	20	80.48 ± 10.61	20
PO_4-P (mg L^{-1})	50.55 ± 2.97	40	216.35 ± 4.35	40	49.49 ± 2.91	40	53.93 ± 3.38	40	48.06 ± 3.07	20	47.14 ± 3.48	20
EC (us cm^{-1})	3356.14 ± 15.40	52	3397.32 ± 14.31	52	3398.84 ± 15.15	52	3351.53 ± 22.18	52	3379.53 ± 21.92	26	3408.18 ± 16.26	26
Cl^- (mg L^{-1})	211.70 ± 4.38	40	216.35 ± 4.35	40	214.08 ± 4.25	40	213.55 ± 4.67	40	210.75 ± 6.02	20	213.75 ± 6.49	20
COD (mg L^{-1})	87.65 ± 11.71	20	73.65 ± 3.57	20	69.50 ± 5.21	20	90.70 ± 10.53	20	71.30 ± 6.29	10	68.70 ± 6.86	10
pH	8.97	52	8.97	52	9.13	52	9.13	52	9.24	26	9.32	26

3.4 Discussion

The fish in all the treatments ingested microalgae from the HRAP effluent. Algae were present in a large proportion of the intestine of all the fish in these experiments and green pigmentation was also observed in their faeces. Mozambique tilapia is omnivorous and, in addition to ingesting food such as invertebrates and macrophytes through its mouth, it is known to obtain plankton through filter feeding (Skelton 2001; Wanatabe *et al*. 2002).

The ingestion of microalgae from HRAP effluent was made possible due to Mozambique tilapia gill structure and because of the formation of algal flocs or clumps in HRAP effluent. Gill rakers are structures on the fish gill that are largely responsible for filtering microorganisms from the water that passes over the gills. Although most of the individual algal cells in the HRAP were an order of magnitude smaller than the spaces between the gill rakers measured here, a large portion of the microalgae in the HRAP effluent formed flocs or clumps (Figure 3.3). These flocs or clumps typically consist of a consortium of single cell microalgae, bacteria, microbial grazers and protein plasma. A pH increase from around 8 to above 10 results in this algal clumping (Granados *et al.* 2012; Wu *et al.* 2012) and the pH of the HRAP was often in excess of 10. The diameters of these clumps are frequently in excess of 500 µm (Valigore et al. 2012) and were observed here with a diameter larger than 1000 µm. The mean spaces between the gill rakers of the tilapia were ca. 600 µm, making these structures suitable for removing the algal colonies. The ingestion of algae in other tilapias has been shown to increase with an increase in particle size, primarily due to colonial aggregation of microorganisms (Drenner *et al.* 1987; Northcott *et al.* 1991). Furthermore, tilapias such as the Nile tilapia (*Oreochromis niloticus*) secrete a mucus layer over the gills which improves filter feeding efficiency (Northcott *et al*. 1991), and this mucus secretion probably also contributed to the ingestion of algae recorded here.

Although algal biomass was filtered from the HRAP effluent by the fish, it did not appear to contribute substantially to nutrient assimilation in fish tissue. The isotopic δ13C values in the fish tissue samples were similar to those of the red fish food pellets and they also overlapped with those of the microalgae harvested from the HRAP, whereas the δ15N values of the fish tissue differed from those of the algae by an estimate of two trophic levels. The separation between trophic levels is distinguished by an increase or decrease of approximately 3.0 ‰ (Vander Zanden & Rasmussen 2001), and the differences observed in the δ15N between the microalgae and fish tissue in this study was more than 6.0 ‰. Since the fish were ingesting the algae, it is possible that the fish were unable to digest and assimilate the nutritive value contained within the microalgal cells that were ingested.

The reason for the low rate of algal assimilation into fish tissue might have been due to reduced digestion efficiency as a result of environmental stress. Mozambique tilapia stomachs are designed to digest plant matter (de Moor *et al.* 1986). Microalgal cell walls are broken down by means of acid hydrolysis (Moriarty 1973) and the length of their gut relative to total body length is long, allowing for efficient digestion and absorption of plant material, which is consistent with the gut structure of fishes that eat plant material (de Moor *et al.* 1986). However, gastric secretion, which is necessary for the digestion of algal cell walls, can be compromised in stressed fish (Moriarty 1973) and this might negatively affect the digestion of plant material. All the fish were subject to extreme water quality conditions: The pH of the HRAP was at, and on occasions exceeded, the upper end of the pH tolerance range of for Mozambique tilapia, i.e. pH 10.3 (Webb & Maughan 2007). Similarly, fish are highly sensitive to elevated ammonium levels, but usually only at a high pH when a greater portion of ammonium exists as “free-ammonia” (i.e. NH_3), which is the unionised form of NH_4 (Wurts 2003). The free ammonia in the fish culture tanks of this experiment ranged from 0.013-0.325 mg/L; studies on

Nile tilapia demonstrate that free ammonia suppressed growth at levels that ranged from 0.07-0.14 mg/L (El-Shafai 2004). Also, the exposure of fish to high nitrate levels for an extended period has been found to compromise the immune system (Plumb 1999) and prolonged exposure to high nitrite, which is more toxic to fish than nitrate, negatively influences physiological functions such as dehydrogenase activity needed for cell respiration, for example (Sudharsan *et al.* 2000). The extreme conditions in the fish culture tanks were due to the nature of the HRAP water source which, in the current trials, remained unaltered. It is possible that these conditions placed the fish under physiological stress and this could have accounted for the low rate of nutrient assimilation recorded here.

Microalgae reproduced and grew in the fish culture tanks, and this, together with algal settlement and the presence of microbial grazers, probably confounded the results relating to the absolute rate at which algae were removed by the fish. The rate at which the fish removed algae from the HRAP effluent peaked at about 200 mg algae/mL effluent/g fish/day. Turker *et al.* (2003a) found that Nile tilapia removed about 702 mgC/kg/h; a direct comparison with these data is not possible since Turker *et al.* (2003a) did not provide the volume of water from which the carbon was collected. When fish were absent from the culture tanks, the biomass of microalgae tended to increase; and a similar increase in algal biomass most probably took place in the fish tanks too, but it was not possible to calculate that algal productivity. Although we were able to demonstrate that fish in the tank resulted in a net decrease in algal biomass, the absolute value in this drop in biomass that was attributed to filter feeding was probably under estimated here. This is because the algal biomass was also subject to algal productivity, i.e. the proliferation of algae in the fish tanks. Furthermore, algal floc also settled out of suspension and settled algae were probably removed when fish faeces were removed off the bottom of the tanks on a daily basis. In addition, there might have been a loss of algal biomass to microbial grazers. These changes in algal biomass also remained unaccounted for in the methods used to estimate the rate of filter feeding here. While it might not have been possible to determine the absolute rate of algal removal, it was possible to determine the relative rate of algal removal between treatments. To do this it was assumed that the confounding factors discussed here were similar in all treatments.

Fish fed the commercial feed removed more algae from the effluent than fish that had access to the microalgae only, and this was probably related to the behaviour and health of these fish. The growth and energy reserves of fish that were fed algae only were compromised, probably because of the lower nutritional value of the algae (28% protein and 0.5% lipid; Jones *et al.* 2014) relative to the commercial feed (32% protein; 7% lipid) and since the nutrients in the algae appeared to remain unavailable to the fish in this study. It has already been established that stress due to poor water quality probably had a negative effect on other physiological processes, such as digestion of the algal cell walls, for example (Moriarty 1973). Since nutritional deficiencies also result in physiological stress and the behaviour and normal functioning of animals can be compromised in unhealthy individuals, the nutritional deficiency alone might account for the reduced rate of filter feeding in the underfed fish. Furthermore, an unhealthy animal is often less active and, although the level of activity of the fish in this experiment was not measured, reduced swimming activity combined with the passive nature of filter feeding in tilapia, might also have contributed to a lower rate algal filtration.

3.5 Conclusion

Fish can be used to reduce the biomass of algae in HRAP effluent. However, the absolute rate at which algal biomass can be removed by the fish was not determined since the results were

confounded by the production of algae in the fish culture tanks. This was not initially expected and was confirmed in a second experiment. The relative rate of algal removal was used to demonstrate that withholding formulated feed does not improve the rate of filter feeding. On the contrary, fish fed a commercial formulated feed removed significantly more algal biomass from HRAP effluent than those that had access to microalgae only. Alone, the algae had insufficient nutrients and fish growth, energy reserves and general health were compromised when additional feed was withheld and this probably reduced their filter feeding efficiency.

This work has demonstrated the potential for using fish to remove algae from HRAP pond effluent. Future research needs to focus on developing methods/technologies aimed at mitigating the negative effects that HRAP water chemistry has on fish physiology; for example, adjusting pond effluent chemistry prior to exposing fish to the water or possibly using alternative fish species that are better adapted to withstand the extreme environmental conditions of HRAP effluent. There are species of fish that are morphologically more adapted to remove algae that have settled out of suspension; either way, future work should also focus on making sure that fish have greater access to algae by increasing the portion of the algal biomass that forms a biofloc that are either in suspension or settled. This work also needs to be designed to ensure that filtration rates of the fish can be adequately estimated, taking algal productivity and the effect that algal settlement and microalgal grazers have on algal biomass into account.

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4. Nutrient removal from brewery effluent using duckweed and its use as a tilapia feed supplement

The investigation into the use of duckweed to remove nutrients from brewery effluent and its use as a tilapia feed did not form part of the original proposal. However, this aspect of the work was added to the program since it contributes to the theme of finding alternative ways of removing nutrients from organic effluent, while generating a product with potential value. This work formed the basis of student Mr Richard Taylor's honours research project. He has completed the project and the report that is presented here is an abbreviated version of Mr Taylor's thesis (Taylor 2013).

Most of this report was prepared by Mr Taylor and parts of this report are taken directly from his honours thesis (Taylor 2013) and parts of this report will be published as it appears here.

4.1.1 Rationale

Algal ponding systems have been used to treat brewery effluent, but harvesting the algae remains a bottle-neck in the process. Duckweed could potentially be used as an alternative to algae in the treatment of post-anaerobically digested brewery effluent. It is a small free floating aquatic plant is a member of the Lemnaceae family (Culley *et al.* 1978). A lot of research has been done on the use of duckweed for renovation of wastewater containing high levels of nutrients (Culley *et al.* 1978, Chaiprapan *et al.* 2005, Gurion *et al.* 1994, Cheng & Stomp 2009). These studies concluded that duckweed can be used to renovate organic effluents. Duckweed is more efficient at assimilating nitrogen in the form of ammonia (Cheng & Stomp 2009). This makes duckweed suitable for organic effluent renovation as post anaerobic digester organic effluents are usually high in ammonia (Chaiprapan *et al*. 2005). It has high growth rates doubling its biomass every 1.5-3.0 days when grown on swine effluent at 20-26^{o}C (Culley *et al.* 1978). It is easy to harvest as it floats on the water surface (Culley *et al.* 1978, Chaiprapan *et al.* 2005, Gurion *et al.* 1994). On average duckweed has a dry weight protein content of 40% (Cheng & Stomp 2009, Chaiprapan *et al.* 2005, Gurion *et al.* 1994). Duckweed starch content ranges from 3-75% depending on the growing conditions but averages around 35% (Cheng & Stomp 2009, Iqbal 1999). Duckweed has a wide range of uses because of its high protein and carbohydrate content. Duckweed can be used as mulch, fertiliser, a source of biofuel and animal feed (Culley *et al.* 1978, Chaiprapan *et al.* 2005, Gurion *et al.* 1994). Duckweed cannot be used as a complete animal feed but rather as a supplement to commercial feed, primarily because it has a low lipid content (Iqbal 1999). Between 0-40% of cow, chicken, duck and pig feed can be supplemented with duckweed without compromising growth (Iqbal 1999). Similarly duckweed can be used to supplement commercial fish food but not entirely replace it, mainly due to its low lipid and energy content (Iqbal 1999).

4.1.2 Aims and objectives

This study used two experiments to evaluate the growth and nutrient removal efficiency of duckweed grown on brewery effluent and its quality as a tilapia (*Oreochromis mossambicus*) feed supplement. In the first experiment nutrient removal rates of duckweed grown in brewery effluent and river-like water where compared. The second experiment evaluated the quality of duckweed as a tilapia feed supplement by comparing the growth, feed conversion ratio and condition factor of tilapia fed either duckweed grown in brewery effluent or river-like water, with or without commercial tilapia feed and commercial tilapia feed only.

4.2 Methods and materials

In the first experiment duckweed (*Lemna minor*) was grown in twenty 40 L containers. Ten were filled with brewery effluent and 10 were filled with a nutrient solution that represented river water (control). The change in dissolved phosphorus (P) and dissolved nitrogen (N) in its various forms were recorded and duckweed growth and proximate composition were compared between treatments. In the second experiment tilapia growth was compared between five feeding treatments (Table 4.1). In Treatment 1 tilapia where fed excess brewery effluent grown duckweed. Tilapia in Treatment 2 where fed control grown duckweed in excess. In Treatment 5 tilapia where fed commercial feed slightly in excess. Tilapia in Treatment 3 and 4 where fed 50% of feed given in Treatment 5 and brewery effluent grown duckweed or control duckweed in excess. The growth, feed conversion ratio and health of the tilapia fed the different diet combinations were compared (Taylor 2013).

Table 4.1 Five treatments used to test the quality of brewery effluent grown duckweed as a tilapia feed supplement. The symbol "x" represents the volume of feed placed into each fish tank where fish were fed commercial tilapia feed to apparent satiation.

	Commercial tilapia feed	Effluent grown duckweed	Nutrient solution grown duckweed
Treatment 1	0	In excess	0
Treatment 2	0	0	In excess
Treatment 3	50% of x	In excess	0
Treatment 4	50% of x	0	In excess
Treatment 5	Apparent satiation (x)	0	0

4.3 Results

4.3.1 Nutrient removal from brewery effluent using duckweed

At the start of the trial, the mean total nitrogen concentrations in river-like water and brewery effluent treatments were 31.11 ± 1.67 and 51.79 ± 3.17 mg/L, while mean PO_4-P concentrations were 8.25 ± 1.43 and 26.08 ± 1.84 mg/L, respectively.

There was no difference in the N removal rate of duckweed and its associated microorganisms grown in brewery effluent and the river-like water (combined mean 3.47 ± 0.033 mg/L/d; Mann-Whitney U, Z = 0.64, p = 0.52; Table 4.2). Duckweed and its associated microorganisms, on average, lowered total dissolved N by 95.17 ± 0.60% with no difference being observed between the two treatments (Student t-test, T = 0.62, p = 0.54; Table 4.2). Dissolved P was removed at a faster rate from brewery effluent (1.54 ± 0.04 mg/L/d) than the river-like water (0.79 ± 0.02 mg/L/d) by duckweed and its associated microorganisms (Student t-test, T = 16.64, p < 0.0001; Table 4.2). Duckweed and its associated microorganisms resulted in a greater decrease in dissolved P in brewery effluent (90.80 ± 0.43%) than the river-like water 83.77 ± 0.64% (Student t-test, T = 9.12, p < 0.0001; Table 4.2). Water temperature in both treatments were similar with a combine mean of 14.19 ± 0.01^{o}C (Student t-test, T = 0.33, p = 0.74; Table 4.2). The pH ranged between (6.83-7.92) in brewery effluent containers and (6.12-7.70) in river-like water containers (Table 4.2).

The average wet weight (24.11 ± 0.05 g/d) and dry weight (1.25 ± 0.03 g/d) of duckweed removed from tanks in both treatments were similar (Student t-test, T = 1.65, p = 0.12; Table 4.2). There was

no difference in the moisture content of brewery effluent grown duckweed and river-like water grown duckweed (overall mean: 94.8 ±0.02; Student t-test, T = 0.21, p = 0.83; Table 4.2).

Table 4.2 The range in pH and average temperature, duckweed moisture content, duckweed biomass, total dissolved nitrogen (N), phosphorus (P) removed of brewery effluent and control tanks. Values in the same row represented by a different superscript symbol are significantly different (Student t-test/Mann-Whitney U, P < 0.05).

	Brewery effluent	Control	T/Z value	P value
N removal rate (mg/L/d)	3.4 ± 0.06	3.5 ± 0.03	Z = 0.64	0.52
P removal rate (mg/L/d)	1.54 ± 0.04[a]	0.79 ± 0.02[b]	T = -16.64	< 0.0001
N removal (%)	95.28 ± 0.25	95.07 ± 0.25	T = -0.62	0.54
P removal (%)	90.80 ± 0.43[a]	83.77 ± 0.64[b]	T = -9.12	< 0.0001
Wet weight (g/d)	24.20 ± 0.08	24.02 ± 0.06	T = -1.65	0.12
Dry weight (g/d)	1.25 ± 0.01	1.24 ± 0.01	T = -1.65	0.12
Moisture (%)	94.8 ± 0.20	94.7 ± 0.20	T = -0.21	0.83
Temperature (^{0}C)	14.2 ± 0.16	14.19 ± 0.22	T = -0.33	0.74
pH	6.83-7.92	6.12-7.70		

4.3.1 The use of duckweed as a tilapia feed supplement

The protein content of brewery effluent duckweed 28.48% and river-like duckweed 28.11% was similar to the protein content of commercial feed 31.61% (Table 4.3). Brewery effluent duckweed, river-like duckweed and commercial tilapia feed had similar fat, dry matter and energy levels (Table 4.3). Both duckweeds had a higher ash and moisture content than commercial tilapia feed (Table 4.3). Commercial tilapia feed had an energy content of 17.86 KJ/kg which was higher than the energy content of brewery effluent duckweed 14.11 MJ/kg and river-like duckweed 14.96 MJ/kg (Table 4.3).

Table 4.3 Proximal analysis of brewery effluent grown duckweed, river-like water grown duckweed and commercial feed fed to tilapia.

	Brewery effluent duckweed	River-like duckweed	Commercial tilapia feed
Protein (%)	28.48	28.11	31.61
Ash (%)	17.47	12.90	6.65
Fat (%)	2.55	3.17	3.71
Moisture (%)	9.13	8.65	5.73
Dry matter (%)	90.87	91.35	94.27
Energy MJ/kg	14.11	14.96	17.86

There was no difference in the condition factor (1.5 ± 0.1), weights (18.32 ± 1.94 g/fish) and lengths (119.50 ± 1.09 mm/fish) of tilapia in the different treatments at the start of the experiment (ANOVA, p > 0.05).

After 42 days, fish fed brewery effluent duckweed only and river-like duckweed only where significantly smaller (23.53 ± 1.15 g and 22.82 ± 3.88 g respectively) than fish fed commercial feed supplemented with brewery effluent duckweed (37.78 ± 2.00 g) or river-like duckweed (39.38 ± 2.94 g) and commercial feed only (41.46 ± 4.53 g); (ANOVA, $F_{(4,20)} = 41.48$ p < 0.00001; Figure 4.1). There was no difference in the weight of tilapia fed commercial feed only and tilapia fed commercial feed supplemented with brewery effluent grown duckweed and control duckweed (Figure 4.1).

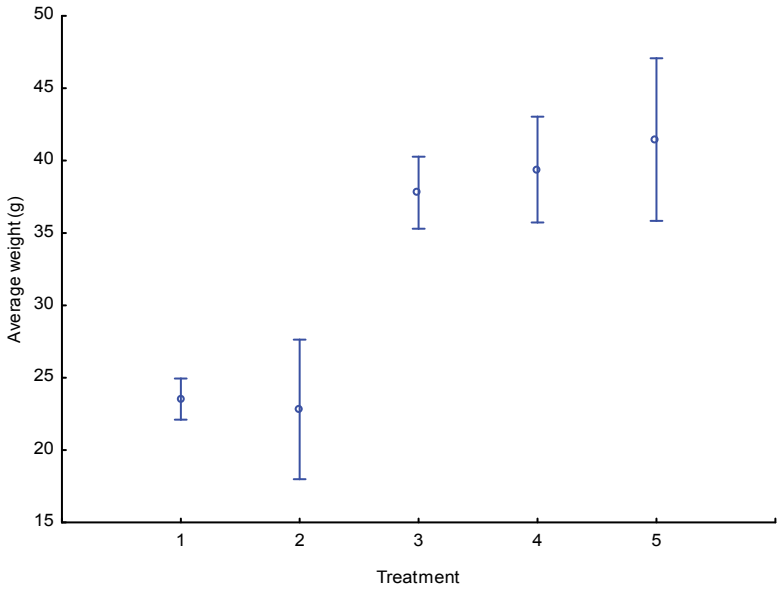

Figure 4.1 The weight of tilapia (mean ± 95% confidence interval) fed brewery effluent duckweed only (1), river-like duckweed only (2), commercial feed supplemented with brewery effluent duckweed (3), commercial feed supplemented with river-like duckweed (4) and commercial feed only (5) (ANOVA, $F_{(4,20)} = 41.48$, $p < 0.00001$).

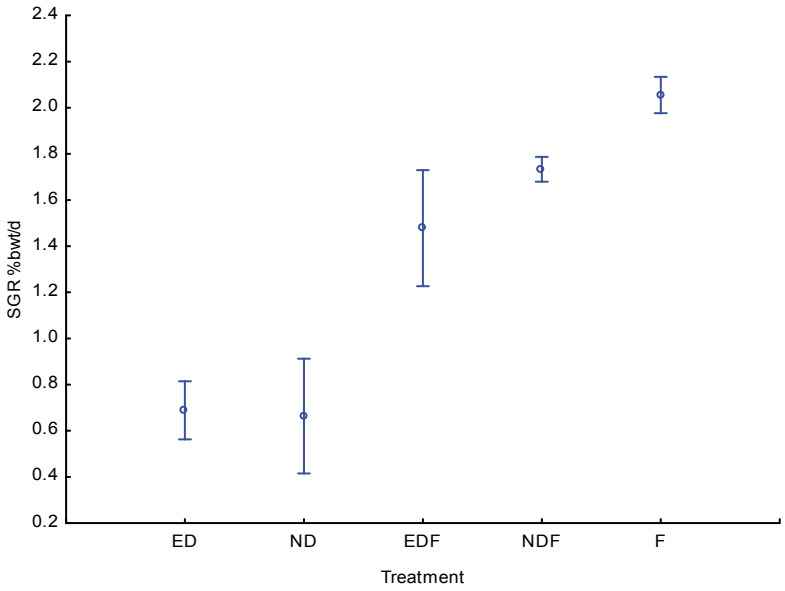

Figure 4.2 The mean ± 95% confidence interval specific growth rate (SGR) of tilapia fed brewery effluent duckweed only (1), river-like duckweed only (2), commercial feed supplemented with brewery effluent duckweed (3), commercial feed supplemented with river-like duckweed (4) and commercial feed only (5) (ANOVA, $F_{(4,20)} = 100.46$, $p < 0.00001$).

Tilapia fed duckweed only (Treatments 1 and 2) had the lowest specific growth rates (0.67 ± 0.06% bwt/d); (ANOVA, $F_{(4,20)} = 100.46$, $p < 0.00001$; Figure 4.2). Fish fed commercial feed only had significantly higher specific growth rates 2.06 ± 0.03%bwt/d than fish fed commercial feed supplemented with brewery effluent grown duckweed or river-like duckweed 1.6 ± 0.05%bwt/d (ANOVA, $F_{(4,20)} = 100.46$, $p < 0.00001$; Figure 4.2). Fish fed commercial feed supplemented with

brewery effluent duckweed or river-like duckweed has similar specific growth rates (ANOVA, P > 0.05; Figure 4.2).

Fish fed duckweed only (Treatments 1 and 2) consumed significantly less food (0.21 ± 0.01 g/fish/d) than fish with commercial feed in their duet (ANOVA, $F_{(4,20)}$ = 884.72, p < 0.00001; Figure 4.3). Furthermore fish fed commercial feed only (0.85 ± 0.01 g/fish/d) consumed significantly more feed that fish fed commercial feed supplemented with duckweed (0.54 ± 0.02 g/fish/d), (ANOVA, $F_{(4,20)}$ = 884.72, p < 0.0001; Figure 4.3).

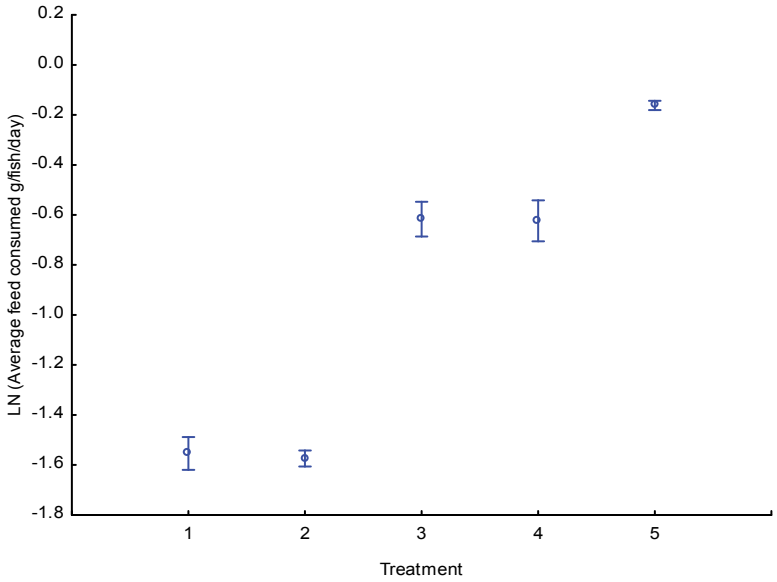

Figure 4.3: The average feed consumed (mean ± 95% confidence interval) by tilapia (dry weight) fed brewery effluent duckweed only (1), river-like duckweed only (2), commercial feed supplemented with brewery effluent duckweed (3), commercial feed supplemented with river-like duckweed (4) and commercial feed only (5) (ANOVA, $F_{(4,20)}$ = 884.72, p < 0.00001).

Percentage weight gain after 42 days was significantly less for tilapia fed brewery effluent grown duckweed only 33.64 ± 2.56% and river-like duckweed only 32.53 ± 5.01% than tilapia fed commercial feed only or commercial feed supplemented with duckweed (ANOVA, $F_{(4,20)}$ = 231.68, p < 0.0001; Table 4.4). Tilapia fed commercial feed only had significantly higher percentage weight gain (137.14 ± 2.84%) than tilapia fed commercial feed supplemented with duckweed (96.85 ± 4.42%) (ANOVA, $F_{(4,20)}$ = 231.68, p < 0.0001; Table 4.4). Tilapia fed duckweed only were significantly shorter (114 ± 2.06 mm) than tilapia fed commercial feed only (132 ± 3.65 mm) and tilapia fed commercial feed supplemented with duckweed (132 ± 1.99 mm) (ANOVA, p = 0.0001; Table 4.4). The length of tilapia fed commercial feed only (132 ± 3.65 mm) and tilapia fed commercial feed supplemented with duckweed (132 ± 2.34 mm) were similar after 42 days (ANOVA, p = 0.42; Table 4.4). After 42 days tilapia fed commercial feed only had higher condition factor values (1.8 ± 0.1) than tilapia with duckweed in their diet (1.6 ± 0.1) (ANOVA, p = 0.04; Table 4.4). There was no difference in condition factor between tilapia receiving duckweed in their diet, with an overall mean of 1.6 ± 0.1 (ANOVA, p = 0.5; Table 4.4). There was no difference in feed conversion ratio of tilapia between all treatments with an overall mean of 1.45 ± 0.13 (ANOVA, p = 0.15; Table 4.4).

There was no difference in the survival rate of tilapia between treatments 84 ± 2.89% (Kruskal Wallis, p = 0.89; Table 4.4).

In fish tanks where tilapia where fed duckweed only there was significantly less dissolved ammonia (0.4 ± 0.04 mg/L) than tanks in all other treatments (ANOVA, p < 0.0001; Table 4.5). There was no significant difference in dissolved ammonia levels in fish tanks where fish were fed commercial feed only and commercial feed supplemented with duckweed (0.6 ± 0.02 mg/L) (Kruskal Wallis, p = 0.4; Table 4.5). Nitrite concentrations ranged between 0.05-0.11 mg/L (Table 4.4). The nitrate concentration in fish tanks where fish were fed commercial feed only (73.79 ± 1.22mg/L) were higher than nitrate concentrations in all other tanks (ANOVA, p = 0.004; Table 4.5). Nitrate concentrations in fish tanks were fish were fed duckweed only (Treatment 1 and 2) and commercial feed with duckweed (Treatment 3 and 4) were similar (67.25 ± 1.31 mg/L), (ANOVA, p = 0.75; Table 4.5). The temperature in all culture tanks over the whole experiment ranged from 22.2-26.1 with no difference between treatments and an overall average of 23.9 ± 0.1 (ANOVA, p = 0.77; Table 4.5). The pH in all the culture tanks over the whole experiment ranged from 7.11-8.35^{o}C (Table 4.5).

Table 4.4 Average performance indicators of tilapia fed brewery effluent grown duckweed (1), river-like duckweed (2), brewery effluent grown duckweed and fish feed (3), river-like grown duckweed and fish feed (4) and commercial fish feed only (5). Values in the same row represented by a different superscript symbol are significantly different (ANOVA/Kruskal Wallis, p < 0.05).

	Treatment														
	1		2		3		4		5		F/H value		P value		
Weight gain (%)*	33.64	±	32.53	±	86.57	±	107.12	±	137.14	±	F	=	<		
	2.56^{a}		5.01^{a}		7.18^{b}		1.66^{b}		2.84^{c}		90.68		0.0001		
Length (mm)	114	±	114	±	131	±	132	±	132	±	F	=	<		
	1.01^{a}		3.11^{a}		11.71^{b}		2.26^{b}		3.65^{b}		14.54		0.0001		
Condition factor	1.6	±	1.5	±	01.7	±	1.7	±	1.8	±	F	=	0.0044		
	0.13^{a}		0.12^{a}		0.16^{a}		0.15^{a}		0.18^{b}		5.31				
Feed conversion ratio	1.53	±	1.77	±	1.31	±	1.11	±	1.51	±	F	=	0.15		
	0.09		0.39		0.05		0.03		0.09		1.87				
Survival (%)	88	±	84	±	80	±	84	±	88	±	H	=	0.89		
	4.52		7.53		6.31		7.54		8.01		1.16				

Note * data in this row were square-root transformed to generate F and P values.

Table 4.5 Average water quality parameters and pH ranges of fish tanks where tilapia were fed brewery effluent duckweed only (1), river-like duckweed only (2), commercial feed supplemented with brewery effluent duckweed (3), commercial feed supplemented with river-like duckweed (4) and commercial feed only (5). Values in the same row represented by a different superscript symbol are significantly different (ANOVA, $p < 0.05$).

	Treatment						
	1	2	3	4	5	F value	P value
NH_4-N (mg/L)	0.4 ± 0.027^a	0.41 ± 0.041^a	0.55 ± 0.027^b	0.6 ± 0.020^b	0.64 ± 0.02^b	15.42	< 0.0001
NO_2-N (mg/L)	0.05 ± 0.007	0.05 ± 0.001	0.07 ± 0.003	0.09 ± 0.001	0.11 ± 0.002		
NO_3-N (mg/L)	14.96 ± 0.255^a	14.41 ± 0.284^a	15.07 ± 0.281^a	14.98 ± 0.346^a	16.22 ± 0.269^b	5.33	0.004
Temperature (^oC)	23.80 ± 0.105	23.86 ± 0.096	23.88 ± 0.106	23.94 ± 0.091	23.76 ± 0.141	0.44	0.77
pH	7.87-8.32	7.11-8.25	7.75-8.30	7.27-8.35	7.71-8.34		

4.4 Discussion

Nutrient removal from brewery effluent using duckweed

Duckweed and its associated microorganisms had similar nitrogen removal rates when grown on brewery effluent and the river-like water. They removed on average 3.47 mg/L/d of dissolved nitrogen, lowering the dissolved nitrogen concentration by an average of 95.17% over 12 days. The decrease in dissolved nitrogen is influenced by retention time (Gurion *et al.* 1994, El-Shafari 2004, Iqbal 1999). Our results are comparable with (Gurion *et al.* 1994) who grew duckweed (*Lemna gibba*) in anaerobically treated sewage where 50% of nitrogen was removed in 4 days and 98% of nitrogen was removed after 20 days. The main factor that influences the removal rate of duckweed is its growth (Boniardi *et al.* 1994, Iqbal 1999, Cheng and Stomp 2009). Since the main factors that influence duckweed growth (pH, temperature, harvesting regime and tank design, season) where similar between both treatments the nitrogen removal rates by duckweed and its associated microorganisms were similar when grown on brewery effluent and river-like water (Boniardi *et al.* 1994, Cheng & Stomp 2009, Iqball 1999).

Nitrogen removal rate observed by duckweed and its microorganisms in this experiment was slower than the reported rates in the literature. On average 3.47 mg/L/d of dissolved nitrogen was removed from brewery effluent and river-like water. Removal rates of dissolved nitrogen between 20-30 mg/L/d are not uncommon (Cheng *et al.* 2002, Cheng & Stomp 2009, Gidgoen *et al.* 2004, Hillman 1961). These authors grew duckweed at 28°C in summer months when duckweed growth is at its maximum. Cheng & Stomp (2009) and Culley *et al.* (1981) found that duckweed growth and nutrient removal rates are heavily reduced at temperatures below 20°C. Since this experiment was carried out in the middle of winter (July-September) with an average water temperature of 14°C the slow removal rates are comparable. Algal ponds are popular wastewater treatment systems used to remove dissolved nutrients from organic effluent. They have nitrogen removal rates of 20-40 mg/L/d when managed at optimal conditions (Garcia *et al.* 2000, Aslan & Kapdan 2006). Their nitrogen removal rates decrease to 2-15 mg/L/d when operated at temperatures below 20°C (Garcia *et al.* 2000, Aslan & Kapdan 2006). Algal systems are slightly faster at removing dissolved nitrogen from nutrient rich waste when compared to duckweed systems run at similar temperatures (Al Saied *et al.* 2000, Brenner *et al.* 1998). Duckweed is easy to harvest and can be fed directly to animals, this makes duckweed wastewater treatment systems more efficient than algal wastewater treatment systems (Chaiprapan *et al.* 2005, Culley *et al.*1981).

Duckweed and its associated microorganisms where efficient at dissolved phosphorus removal. Removing phosphorus faster in brewery effluent (1.54 mg/L/d) than river-like water (0.79 mg/L/d). In duckweed systems phosphorus removal is done by plant uptake, adsorption to clay particles and organic matter and chemical precipitation (Agami *et al*. 2004, Iqball 1999). Brewery effluent has more organic matter than the river-like water which would have resulted in more phosphorus being removed by absorption. Algae was noticed in both the brewery effluent containers and river-like water containers. The biomass of algae in the containers was not measured and it may have been higher in the brewery effluent which may have resulted in the faster phosphorus removal rate observed in brewery effluent. Cheng *et al.* (2009) compared the growth and removal rates of duckweed grown at different nutrient concentrations and different seasons. He found that the same mass of duckweed had a faster phosphorus removal rate when grown on a higher concentration of phosphorus. Brewery effluent had a higher, initial phosphorus concentration of 28 mg/L when compared the river-like water 8 mg/L. The faster removal rate of phosphorus in brewery effluent cannot be clearly explained and is most likely due to its higher concentration in brewery effluent and a combination of algal growth and phosphate absorption to organic particles. The underlying

mechanisms of differential phosphorus removal rates in the duckweed ponds requires further investigation.

Duckweed wastewater treatment systems are efficient at removing dissolved phosphorus from nutrient rich wastewater. In this experiment duckweed and its associated microorganisms removed 1.57 mg/L/d of phosphorus from brewery effluent. This is significantly slower than the rates reported in the literature of 5-15 mg/L/d of phosphorus for duckweed grown under optimal conditions (Cheng and stomp 2009, Culley *et al.*1994, Gidgoen *et al.* 2004). Nutrient removal is heavily reduced at temperatures below 20°C (Cheng & stomp 2009, Gidgoen *et al.* 2004, Culley *et al.* 1981). Since this experiment was carried out in the middle of winter (July-September) with an average water temperature of 14°C the slow removal rates are comparable. Algal ponds show wide variation in their ability to remove dissolved phosphate. Awuah *et al.* (2004), Blanco *et al.* (2009), Cilliers (2012) and Garcia *et al.* (2000) found that algal ponds are unreliable in phosphorus removal as in some months they remove phosphorus and in others they do not. Overall duckweed systems are more reliable, and more efficient at removing dissolved phosphorus from nutrient rich effluents than algal ponds (Awuah *et al.* 2004, Culley *et al.* 1981).

Effect of feed and duckweed on fish tank water quality

Commercial feed in tilapia diets affected the ammonia concentration in the water. Ammonia concentrations were lower in fish tanks where fish were fed duckweed only when compared to fish tanks were fish were fed commercial feed. Duckweed assimilates ammonia and probably would have removed ammonia from fish tanks where fish were fed duckweed (Hillman 1961). This was not significant enough to reduce the ammonia levels in fish tanks where fish were fed commercial feed and duckweed as these tanks had similar ammonia levels to tanks where fish were fed commercial feed only. The quantity of ammonia excreted by fish is positively related to the quantity of nitrogen supplied by the protein in the feed (Baird *et al.* 1997). The amount of protein in duckweed (28. 30%) and commercial feed (31.61%) was similar. Tilapia fed duckweed only consumed two times less food on a dry weight basis than tilapia that had commercial feed in their diet. Tilapia fed duckweed only consumed less protein which resulted in less ammonia production and lower ammonia concentrations their respective tanks.

The inclusion of duckweed in fish tanks probably contributed to improved fish tank water quality. Fish tanks where fish were fed commercial feed only had higher concentrations of nitrate than fish tanks where fish had duckweed in their diet. Firstly, tilapia fed commercial feed only, consumed the most amount of food on a dry weight basis. They would have therefore excreted more ammonia, which would have been oxidised by nitrifying bacteria in the filter into nitrate (Baird *et al.* 1997, Vymzal 2007). This would have resulted in higher concentrations of nitrate in fish tanks where fish were fed commercial feed only. To add to this, in all other treatments fish tanks had duckweed in the tanks. Since duckweed readily assimilates dissolved nitrate it would have further lowered the nitrate levels of fish tanks were tilapia had duckweed in their diet (Hillman 1961, Sutton *et al.* 1975).

Total dissolved ammonia, nitrite and nitrate varied between treatments but were kept within the acceptable range for tilapia culture (Balarin & Halton 1979). Even though there was significant differences in water quality parameters between treatments it is not likely that it affected tilapia growth (Balarin & Halton 1979). Tilapia fed commercial feed only were exposed to the highest ammonia, nitrite and nitrate concentrations while having the highest specific growth rate.

Quality of duckweed as a tilapia feed supplement

Fresh duckweed does not affect the survival rate of tilapia when included in their diet. There was no difference in the survival rate of tilapia fed the five different treatments. Survival was not affected by

water quality, as water quality was kept within the acceptable limits for tilapia culture in all treatment tanks. Balogun *et al.* (1999) concluded that duckweed (*L. minor*) has no effect on the survival of tilapia (*Oreochromis niloticus)* when included in their diet. Mbangwa & Okoye (1984) compared the growth of tilapia (*Sarotherodon galilaeus*) fed duckweed and commercial feed and observed no difference in survival rates with an overall average survival of 85%. Hassan & Edwards (1992) found lower survival rates of tilapia (*O. niloticus*) fed *L. gibba* at 100% (wet weight) of their body weight per day when compared to tilapia fed commercial feed. This was because duckweed decreased the oxygen concentration to below 0.5 mg/L in fish tanks. The inclusion of fresh duckweed in tilapia diets does not affect the survival of tilapia provided its presence in the tank does not compromise the water quality the tilapia are grown in.

Duckweed resulted in a poorer condition factor, indicating that these fish were probably less healthy than those fed the commercial feed. Tilapia fed commercial feed only had a higher condition factor than tilapia fed commercial feed supplemented with duckweed and tilapia fed duckweed only. Gaigher *et al.* (1984) and Balogun *et al.* (1999) observed a decrease in the fat content of tilapia *O. niloticus* fed duckweed. Tilapia with duckweed in their diet consumed less feed than tilapia fed commercial feed only. Duckweed is deficient in methionine which in needed for healthy tilapia growth (Blakeney *et al.* 1980, Yan 1992). Duckweed had a lower energy content (14.58 MJ/kg) than commercial feed (17.56 MJ/kg). The decrease in condition factor could be due to: duckweed is an inferior food quality for tilapia when compared to commercial feed, tilapia that fed on duckweed consumed less feed or the high moisture content of fresh duckweed decreases its digestibility (El-Shafai 2004). Condition factor, used in this experiment, was calculated using weight and length and is not a complete indicator of fish health (Adams *et al.* 1993, Froese 2006). Future studies should look at the fat and water content of tilapia muscle tissue as well as a thorough histological examination of the gut, liver, gill tissue and blood. This will enable a better understanding of how dietary inclusion of duckweed in tilapia affects their health.

Duckweed inclusion in tilapia diets did not influence feed conversion ratio. Tilapia subjected to the five different feeding treatments had similar feed conversion ratio's ranging between 1.2 and 1.7. There was a wide variation in feed conversion ratio of tilapia in each treatment which resulted in there being no difference between treatments. These results are comparable with the reported ranges of FCR (1.2-1.6) of tilapia ranging from 15-68 g receiving fish meal based diets combined with plant ingredients (El-Sayed 1992, Fontainhas *et al.* 1999, Papoutsoglau & Tziha 1996, Mbahinzireki *et al*. 2001, Middleton *et al.* 2001). Tavares *et al.* (2011) reported larger FCR's for tilapia fed duckweed when compared to tilapia fed commercial feed. The results obtained in this experiment are comparable with Gaigher *et al.* (1984) who found that tilapia fed duckweed only and a combination of duckweed and fish feed had FCR's between 1.2-2.0. The FCR of fish is negatively affected by the decrease in dietary protein and energy levels (Lee & Kim 2001). Duckweed and commercial had similar protein levels and different energy levels, however this did not appear to effect the feed conversion ratios of tilapia.

There was no difference in the quality of brewery effluent or river-like water as a nutrient solution to grow duckweed for tilapia feed. The specific growth rate of tilapia fed brewery effluent duckweed and tilapia fed river-like duckweed was similar. To add to this tilapia fed commercial feed supplemented with brewery effluent grown duckweed and tilapia fed commercial feed supplemented with river-like water grown duckweed had similar specific growth rates. The growth of tilapia is influenced by the protein and energy content of their diet (Stickney & Winfree 1981). Since the protein and energy contents of brewery effluent duckweed and river-like duckweed are similar and they are both plants of the same species one would expect them to be of similar quality as a tilapia feed.

Tilapia fed duckweed only had reduced growth rates which was probably due to the quality, digestibility and decreased feed consumption of duckweed. The average length, weight and specific growth rate of tilapia fed duckweed only was smaller/slower than tilapia with commercial feed in their diet. Tilapia fed duckweed only, consumed two times less food on a dry weight basis than tilapia fed commercial feed supplemented with duckweed and commercial feed only. Adeniji *et al.* (1992) compared the growth of tilapia (*S. galilaeus)* fed duckweed only and commercial feed and recorded low SGR of 0.2-0.5 when tilapia were fed duckweed only. This was attributed to the bulk effect created by the excess water in duckweed which restricted the amount of food they consumed. El-Shafai (2004) reported reduced digestibility of fresh duckweed when compared to commercial tilapia feed due to the high moisture content of duckweed which is thought to dilute the enzyme concentration in the gut. The reduced growth of tilapia fed duckweed only is partly due to the high moisture content of duckweed which causes reduced food consumption and decreased digestibility (Gaigher *et al.* 1984, Edwards & Hassan 1992).

The reduced growth of tilapia fed duckweed only could also be due to the shortage of essential amino acids and lower energy levels of duckweed. The amino acid content of duckweed exceeds the levels set by the Food and Agriculture Organisation for animal feed with the exception of methionine and lysine (Blakeney *et al.* 1980, Adeniji & Mbagwu 1988, Culley *et al.* 1981). El-Dahhar & El-Shazly (1993) and Yamamoto *et al.* (2001) demonstrated the role of essential amino acids lysine, methionine and cystine in enhancing the growth performance of fish. El-Gohary *et al.* (2004) and Tavares *et al.* (2010) found that tilapia fed duckweed had significantly lower growth performance indicators than tilapia fed commercial feed and this was attributed low protein content and shortage of methionine in duckweed. Fresh duckweed cannot be used to entirely supplement commercial tilapia feed without decreasing growth because it is deficient in amino acids (methionine and lysine), has lower energy levels and a high moisture content.

Duckweed can be used to supplement commercial tilapia feed at levels up to 50% supplementation without compromising the weight of the fish. The weight of tilapia fed commercial feed only and commercial feed supplemented with either brewery effluent duckweed or river-like duckweed was similar. Balogun *et al.* (1999), El-Shafari (2004), Adeniji *et al.* (1992) and Gaiger *et al.* (1984) concluded that you can use duckweed to supplement commercial fish feed at levels between 10 to 30%, without compromising growth. Tavares *et al.* 2010 found similar growth of red tilapia (*O. mossambicus*) fed commercial feed only and commercial feed supplemented at 50% by dry duckweed.

Tilapia fed commercial feed only had significantly higher specific growth rates than tilapia fed commercial feed supplemented with brewery effluent grown duckweed or river-like duckweed. This could be due to commercial feed (17.56 MJ/kg) having a higher energy content than duckweed (14.58 MJ/kg). Since tilapia fed commercial feed consumed significantly more food than fish in all other treatments one would expect then to have a higher specific growth rate. Fresh duckweed tends to "fill" the digestive system in tilapia due to its high moisture content and air pockets in the leaves (Hassan & Edwards 1992, Gaigher *et al*. 1984). When tilapia eat fresh duckweed it fills their stomach and reduces the amount of commercial feed they will eat. This resulted in an overall decrease in food consumption by tilapia fed duckweed and commercial feed which in turn resulted in a decrease specific growth rate when compared to tilapia fed commercial feed only. Fresh duckweed can be used to supplement commercial feed at levels which do not inhibit the consumption of commercial feed.

4.5 Conclusions

Duckweed is efficient at removing dissolved nitrogen and phosphorus from brewery effluent by lowering the concentrations by 95. 28% and 90.80%, respectively, over 12 days at 14^{o}C. There was no difference in the quality of brewery effluent grown duckweed and river-like water grown duckweed as a tilapia feed supplement. Brewery effluent grown duckweed and river-like water grown duckweed had a protein content of 28% and an energy content of 14 MJ/kg which was comparable commercial tilapia feed with a protein content of 31% and an energy content on 17 MJ/kg. The final weight and length of tilapia fed commercial feed supplemented at 50% with fresh brewery effluent grown duckweed or river-like duckweed were similar to tilapia fed commercial feed. Tilapia fed commercial feed supplemented at 50% with fresh brewery effluent grown duckweed had lower specific growth rate and condition factor than tilapia fed commercial feed only and was probably to increased feed consumption.

Future research should focus on at the digestibility of fresh and dry duckweed as well as refining duckweed which is partially dry because research has shown that commercial fish feed has the highest digestibility at 39% moisture and this work suggests that inclusion levels are limited due to the high moisture content of duckweed. Research also needs to be carried out on the effect of dietary duckweed on the general health of tilapia and should include a histological examination of the gut, liver, gill tissue and blood chemistry.

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5. Use of brewery effluent as a hydroponic plant nutrient source

This section of the report was prepared by Mr Sean Power originally and was taken from a chapter in his MSc thesis (Power 2014). It is a follow on to work that was presented by Jones *et al.* (2014). This deliverable did not form part of the project's original proposal, but it contributes to the greater program and was generated using the resources (running costs and student bursary) made available by the WRC. The SAB Ltd made the research site and utilities available for the work.

This report has been submitted for publication as it appears here in *Cleaner Production*, with some minor additions here.

5.1.1 Rationale

Water scarcity and resource competition are ever-growing challenges, particularly in developing countries or water stressed areas, including the Eastern Cape Province, South Africa (Arnell 2004; Department of Water Affairs 2012; WWF-SA 2013). This research, conducted at a brewery in Port Elizabeth, South Africa, describes the potential for an alternative use for brewery effluent that could contribute to industrial resource efficiency and cost reduction among similar industries that produce an organic effluent stream. Extracting the maximum value from a given unit of water is a possible strategy to mitigate water and resource stress. This value maximisation principle applies in any region of the world, to industry or any resource experiencing a stress, scarcity, or pursuing economic and environmental efficiency.

Industrial breweries around the world are significant water consumers, producing between 4-6 L of wastewater per litre of beer produced, with a global production of roughly 18,000,000 m^3 in 2010 (Ascher 2012; Brito *et al.* 2007; Fillaudeau *et al.* 2006). This effluent requires treatment before disposal into the environment, contributing to the financial and energy operating costs of the brewery. The effluent at this brewery, like many food and beverage processing facilities around the world, is treated in an onsite anaerobic digester (AD); however, at this facility a small part of the AD effluent stream is drawn into an experimental treatment system consisting of a primary facultative pond (PFP) and a high-rate algal pond (HRAP) system as part of an alternative wastewater treatment experiment. This particular brewery currently discharges around 150 m^3, roughly 65% of the total volume of bought-in water, of anaerobically digested brewery effluent per day to the municipal treatment works, at a cost of US$ 0.85 per kl (Mabuza pers. comm. 2012), in keeping with the discharge of breweries around the world. This is the volume of water potentially available for hydroponic production from just one facility. Developing an alternative use for this effluent stream could save the brewery up to US$ 1000.00 per day in municipal water discharge costs alone as well as provide numerous benefits for society and the environment through crop production and improved water and nutrient management.

Anaerobic digestion is a cascade of biological conversion processes, which break down biodegradable organic matter into its most oxidised or reduced forms (Angelidaki & Sanders 2004; Batstone *et al.* 2002; Speece 1983). Organic carbon is converted into methane (CH_4) and carbon dioxide (CO_2), and other mineralised or constituent elements are released including ammonia (NH_3) and phosphate (PO_4) (Angelidaki & Sanders 2004; Batstone *et al.* 2002; Sötemann *et al.* 2005; Speece 1983). Anaerobic digestion is a popular treatment technology because of its low energy inputs, low sludge production, and the opportunity to recover energy from the produced biogas (Tauseef *et al.* 2013). The residual nitrogen (N) and phosphorus (P) nutrient load in the AD effluent stream can pose a threat to the environment and usually requires further treatment, typically through energy intensive

or financially expensive processes with their own cost-efficiencies to consider (Samuelsson *et al.* 2007).

Much attention has been directed towards the use of plants and wetlands for effluent management or nutrient removal from municipal and industrial wastewaters (Calheiros *et al*. 2012; Kivaisi 2001; Konnerup *et al*. 2009; Melián *et al*. 2010; Zurita *et al*. 2009). Effluent management and treatment objectives can also be combined with a process to derive some value from the effluent through, for example, cut flower or biomass production (Kivaisi 2001; Konnerup *et al*. 2009; Zurita *et al*. 2009). The authors are not aware of previous studies applying these techniques through tomato production in brewery effluent. This brewery discharges its sewage through a separate system because water recovery is practised and they cannot allow for sewage to come into contact with water that is reused on site; i.e. there is complete physical segregation of the brewery effluent from the sewage system at this brewery. This removes the risk of faecal or pathogenic contamination of crops grown in the digested brewing and cleaning wastewater. Developing a productive stage for the brewery effluent system could provide benefits to a number of parties, particularly in developing and water stressed areas.

Generally, higher vascular plants require 17 'essential elements' to grow and reproduce (Epstein & Bloom 2004; Freeman 2005). Previous work used the post-HRAP treated effluent to produce hydroponic lettuce (Jones *et al*. 2011; 2014). The observed growth deficiencies, nutrient deficiency symptoms and specimen mortalities showed that the effluent in its unaltered form was not an ideal nutrient source for these lettuce plants (Jones *et al*. 2011; 2014). The high pH of the effluent as it moves through the treatment system (±8.5 before the HRAP, up to ±9.5 after the HRAP) was noted as a potentially significant influence on nutrient bioavailability and plant performance. Solution pH is known to affect the form and bioavailability of various nutrients and consequently plant development (Bar-Yosef *et al*. 2009; Lucas & Davis 1961; Tyson *et al*. 2007 Zhao *et al*. 2013). Plants have been shown to have cultivar specific preferences and tolerances to stresses or nutrient forms (Cuartero & Fernández-Muñoz 1999; Lastra *et al*. 2009). Therefore the results of the lettuce trial do not necessarily inform the results of experiments with different crops or cultivars. A longer-term objective of this or similar work could attempt to find the most suitable plant for a particular effluent as well as adjusting the effluent characteristics to improve plant performance.

The brewery effluent system offers an opportunity to positively benefit each of the factors in the water-food-energy nexus in a region at risk of water stress with a great need for socio-economic development (Department of Water Affairs 2012). This study presents some primary work on the feasibility on developing a productive wetland or hydroponic facility that will exploit the water and nutrient value available in the brewery effluent.

5.1.2 Aims and objectives

The objectives of this work were to compare the vegetative growth rate of tomato plants grown in brewery effluent drawn from different points in the algal ponding treatments system, with and without pH adjustment to plants grown using a conventional inorganic hydroponic solution. There was no literature available, which considered the use of brewery effluent as a hydroponic nutrient solution for tomato production, so this trial was designed as a preliminary study into the relationship between hydroponically-grown tomato plants and brewery effluent. It would have been premature to attempt to produce a fruit crop without first assessing the fundamental relationship between the plant and the alternative nutrient source.

5.2 Methods and materials

A multifactor experiment was designed where nutrient solution (factor 1) was tested in conjunction with pH adjustment (factor 2). Brewery effluent, as the nutrient solution, was drawn either (a) after the effluent had undergone treatment in the AD and PFP (post-PFP) or (b) after it had undergone treatment in the AD, PFP and the high rate algal ponds (post-HRAP). The effluent systems were also compared to (c) control treatments comprised of commercially available hydroponic inorganic-fertilizer (Hygrotech®; Registration number K5709; Act 36 of 1947, South Africa), and calcium nitrate (11.7% nitrogen and 16.6% calcium), mixed in a ratio of 1:0.8 and dissolved in municipal tap water to achieve an EC of 2000 µS/cm (Hygrotech (Pty) Ltd., South Africa). Each of these three nutrient solutions were either subject to (a) pH adjustment to between 5.8 and 6.5 with 80% phosphoric acid (Protea Chemicals (Pty) Ltd., South Africa) or (b) their pH was left unaltered and ranged from pH 8.3 to 9.9 (i.e. factor 2), which resulted in a total of six treatments.

Each treatment was replicated five times, with a total of 30 independent recirculating hydroponic systems making up the full experiment (Figure 5.1). Each system consisted of one 1500 mm long tubular polyvinylchloride (PVC) growth channel with a diameter of 160 mm. This channel supported five 120 mm diameter common plastic garden pots (Figure 5.2). Each pot was perforated with 5.0 mm holes and filled with 10 mm diameter quartz gravel (Figure 5.2). The growth channels that supported the pots were placed on a table in a greenhouse tunnel (Figure 5.1). The nutrient solution for each channel was contained in a 30 l plastic sump placed on the ground at the foot of each channel (Figure 5.1). This solution was pumped from the sump to each pot, using an 18 watt submersible aquarium pump (Resun®, Model: SP-2500, China) and a 15 mm delivery line. A micro-valve at each pot was used to ensure an even irrigation rate into each pot. The nutrient solution drained through the gravel of the pot by gravity into the growth channel, which drained back to the sump. The drain in the growth channel was adjusted to create a submerged zone for the root system that was approximately 50 mm deep.

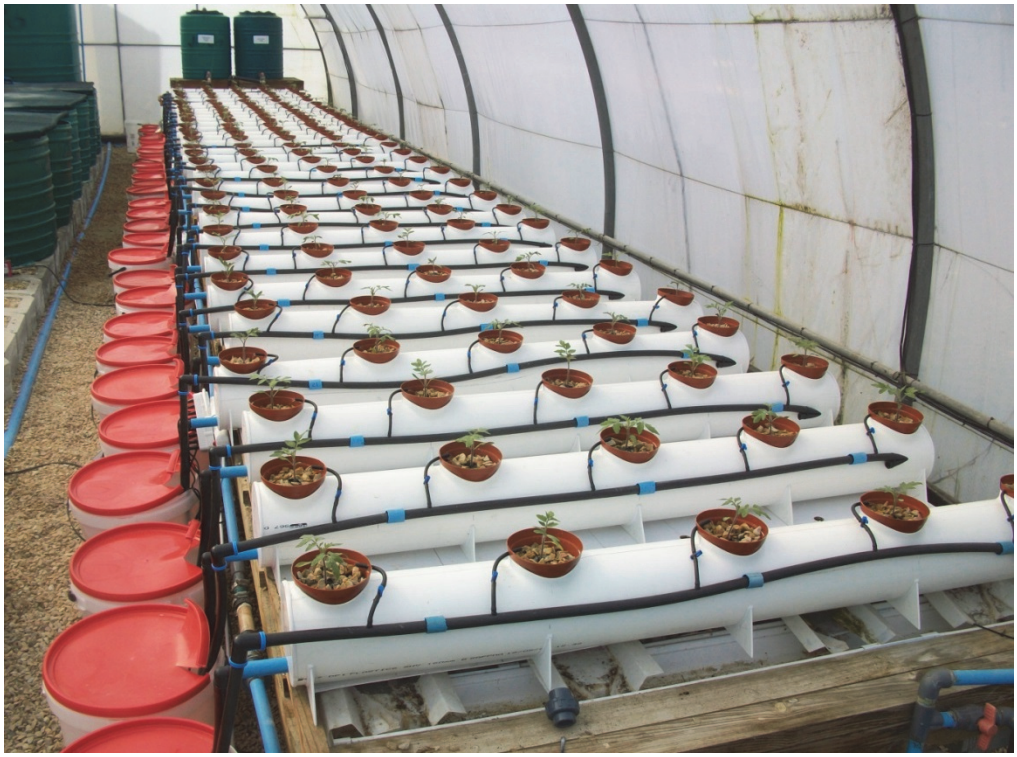

Figure 5.1 The complete 30 channel experimental system with plants at the start of the first trial.

Figure 5.2 Views of the completed growth channels including reservoir, pump, irrigation connections, gravel-filled pots, and drain.

There was no climate, photoperiod or light intensity control available in the tunnel. The tunnel was located at -33.835594 °S, 25.541070 °E. The mean time of sunrise and sunset over the trial (26 September 2012-14 November 2012) was 05:28 AM and 6:36 PM respectively.

Solanum lycopersicon tomato plants were germinated from commercially available seed ("Moneymaker" Starke Ayres, South Africa). At least two seeds were sown in 36 mm diameter, peat pellets (Jiffy-7, Jiffy®, Canada) with at least 400 seeds sown across 200 pellets. The pellets were distributed evenly between three miniature plastic greenhouses (Jiffy®, JiffyPro 70 Self-Watering Greenhouse, Canada). The pellets were soaked in municipal water before the seeds were sown and irrigated when necessary. Two weeks after sowing, the smaller seedling was cut to allow the larger seedling to thrive. Four weeks after germination the seedlings were transplanted into the 120 mm pots and surrounded with gravel. The 150 plants were randomly allocated to the various treatments and exposed to their particular nutrient solutions for the first time. The plants were grown solely on their treatment's nutrient solution for the next 49 days.

The NH_4^+-N, NO_3^--N and PO_4-P concentration in the nutrient solution of each replicate was recorded before and after the solution was replaced on each occasion. This was carried out on filtered samples (8.0 µm), using a spectrophotometer (Merck Spectroquant Pharo 100, product number 100706, Darmstadt, Germany) and commercial test kits (Section 2.2.4). It was not possible to determine dilution ratios for each of the parameters and each of the treatments when the tested parameter exceeded the range of the test because of the time required for each test and the number of samples that needed testing. Values that were above the maximum range of the test were recorded as the maximum.

Electrical conductivity (EC) and pH were measured with a pH/EC/total dissolved solids probe (Hanna, HI 991300, United Kingdom). Readings were taken on fresh and discarded solutions when the solutions were replaced.

The average pH values were calculated by converting the pH readings to H+ concentrations with the formula below where x is the recorded pH value (Equation 5.1).

$$\mathrm{H}^{+} = 10^{-x} \tag{5.1}$$

The H+ concentration values were then averaged for each treatment and the mean H+ value was converted back to a pH value using the following formula (Equation 5.2):

$$\mathrm{pH} = -\log(\mathrm{H}^{+}) \tag{5.2}$$

Plant height and basal stem diameter were measured at the start, once a week during, and at the end of the trial, to the nearest millimetre with a tape measure and digital Vernier callipers respectively. All the plants were measured in a single session when measurements were recorded. The chlorophyll concentration index (CCI) was calculated on days 2, 6, 8, 14, 20, 22 , 26, 34, 35, 41 and 42 of the experiment, by recording the chlorophyll content of the uppermost fully expanded leaf of each plant using a chlorophyll meter (CCM-200 Plus Chlorophyll Content Meter, Opti-Sciences Inc., USA). At the end of the trial the number of fruit that were present on each of the plants was recorded. At this time the plants were separated into root biomass and aboveground biomass, the samples were oven dried (Scientific, Series 9000) at 80°C for 72 h (Borgognone *et al*. 2012) and the dried mass was weighed on a four-digit analytical balance.

Individual plant data were averaged among the five plants in each of the 30 hydroponic systems, and the mean value for each hydroponic system was used in all further analyses (n=30). These raw data were tested for homogeneity of variance (Levene's test; $p<0.05$) and normality of the residuals (Shapiro-Wilk W-test; $p<0.05$). A multifactor analysis ANOVA of the growth indices mentioned above was used to establish if there were interactions between factors (factors: water source and pH regime), at $p<0.05$. If there were no interactions, each factor was analysed separately with a one-way ANOVA, and Tukeys multiple range analysis was used to compare means among the treatments within each factor, at $p<0.05$. Data that were collected from the same treatments over the course of the trial were compared using repeated measures ANOVA, at $p<0.05$. All statistical analyses used StatSoft Inc., Tulsa, United States of America).

5.3 Results

There were no plant fatalities among the effluent-irrigated plants indicating that this brewery effluent contains the essential elements needed for Moneymaker tomatoes to survive.

Plant height was affected by a significant interaction between nutrient solution and pH adjustment (Multifactor ANOVA, $F_{(2,24)}=47.78$, $p<0.00001$; Figure 5.3; Figure 5.4). There was a significant difference in the mean plant height of the pH corrected effluent systems (T3: 443 ± 34 mm versus T4: 890 ± 15 mm, and T5: 378 ± 14 mm versus T6: 773 ± 8 mm; Figure 5.3; Figure 5.4), whereas the addition of acid did not have a significant effect on the mean plant height of the inorganic-fertilizer control treatments. There was no significant difference between the plant height of the pH uncorrected effluent treatments. The pH adjusted post-PFP plants grew significantly taller than the plants grown in pH adjusted post-HRAP effluent (T4: 890 ± 15 mm versus T6: 773 ± 8 mm; Figure 5.4). Similar interactions and trends were found for both basal stem diameter and the accumulation of leaf and shoot biomass (Multifactor ANOVA, $F_{(2,24)}=10.59$, $p=0.0005$ and $_{(2,24)}=11.06$, $p=0.00039$, respectively; Table 5.1). However, there was no interaction between pH adjustment and nutrient

solution in the accumulation of dry root biomass (Multifactor ANOVA, $F_{(2,18)}$=2.36, p=0.12). The grouped mean of the control systems developed significantly more root biomass than the effluent sources (ANOVA, $F_{(2,18)}$=186.05, p<0.00001; Table 5.1). As with the other parameters, the addition of phosphoric acid was also a significant factor with the pH adjusted treatments developing significantly more root biomass than the pH unaltered systems (pH adjusted 54.54 ± 9.14 g/system, pH unaltered 36.64 ± 9.77 g/system; ANOVA, $F_{(1,18)}$=32.14, p=0.00002; Table 5.1).

Figure 5.3 A visual comparison of plant development from the beginning of the trial and the same systems on the 31st of October 2012, after 35 days. In these images, the treatments are arranged randomly from left to right: T3, T6, T4, T2, T1 and T5. The treatment solutions indicated are as follows: T1 and T2 – municipal water and fertilizer, T3 and T4 – Post-Primary Facultative Pond Effluent (Post-PFP), and T5 and T6 – Post-High Rate Algal Ponds Effluent (Post-HRAP). Treatments T2, T4 and T6 received pH adjustment with phosphoric acid.

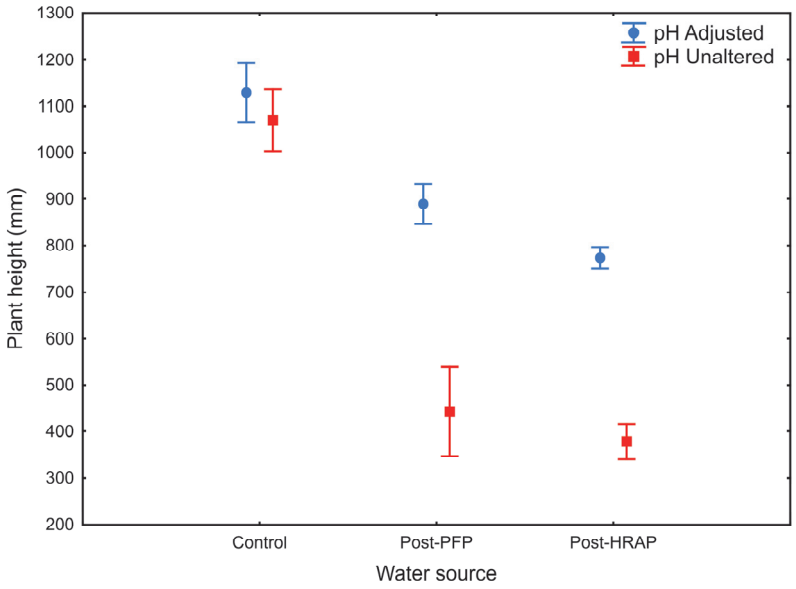

Figure 5.4 The mean (±95% confidence interval) height of *Moneymaker* tomato plants subject to treatments of municipal water and fertilizer (Control), or brewery effluent drawn after treatment in the experimental treatment system's primary facultative pond (Post-PFP) or high rate algal pond (Post-HRAP), each of which were subject to pH correction with phosphoric acid or the pH was left unaltered, for 49 days (Multifactor ANOVA, $F_{(2,24)}$=47.78, p<0.00001).

Table 5.1 The mean (± standard deviation) stem diameter, leaf and shoot dry weight, and root dry weight from *Moneymaker* tomato plants grown for 49 days in various nutrient sources and pH adjustment treatments. Mean values were calculated from four replicates, each containing five plants (n=20) for weights, and five replicates for height and diameter (n=25). Means within each column with a different superscript were significantly different (Multifactor ANOVA, $p<0.05$).

	Nutrient solution	Stem diameter (mm)		Leaf & shoot dry weight ($g.plant^{-1}$)		Root dry weight ($g.plant^{-1}$)	
T1	Fertilizer	11.2 ± 0.5	a	112.7 ± 16.0	a	16.3 ± 2.3	a
T2	Fertilizer + P acid	11.4 ± 0.8	a	113.5 ± 10.0	a	18.9 ± 1.8	a
T3	Post-PFP	5.6 ± 1.2	b	5.2 ± 1.8	b	3.3 ± 0.7	b
T4	Post-PFP + P acid	8.5 ± 0.2	c	38.6 ± 4.7	c	8.8 ± 2.2	c
T5	Post-HRAP	6.0 ± 0.5	b	4.4 ± 0.8	b	2.4 ± 0.3	b
T6	Post-HRAP + P acid	8.7 ± 0.6	c	32.3 ± 5.5	c	5.0 ± 0.7	c
	Overall	8.6 ± 2.4		51.1 ± 47.0		9.1 ± 6.7	

Chlorophyll concentration index was influenced by a significant interaction between nutrient solution and pH adjustment. The CCl increased over the period of the experiment in all treatments, with the exception of plants grown in post-HRAP with no pH adjustment, where CCl decreased over the period of the trial (Repeated measures ANOVA, $F_{(20,240)}=9.36$, $p<0.00001$; Figure 5.5). There was no significant difference in mean CCI recorded at the end of the trial between the two inorganic-fertilizer treatments (T1 and T2) and the pH corrected post-PFP effluent treatment (T4), and these were all significantly higher than the other treatments (Figure 5.5). The pH correction in the effluent treatments resulted in a significantly higher CCI compared to plants grown in the same effluent, but without pH correction (Figure 5.5).

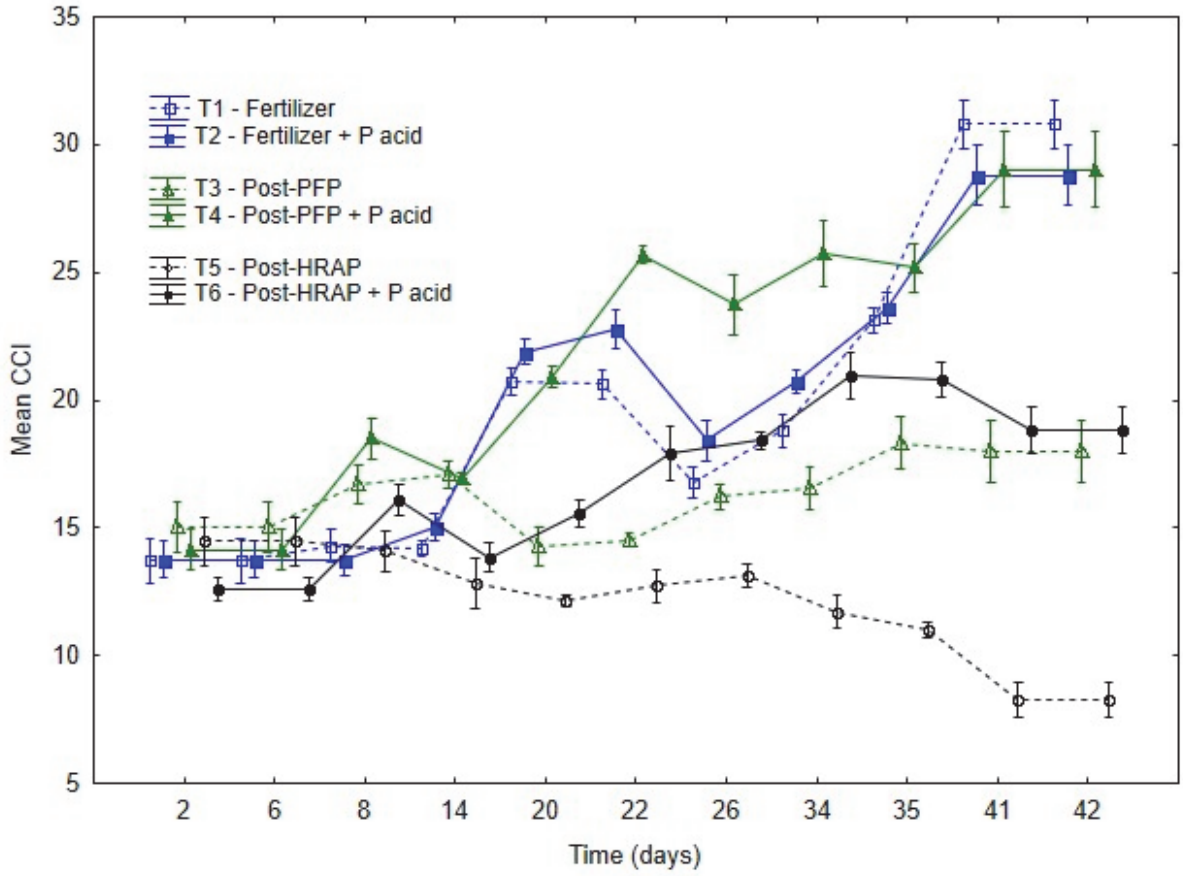

Figure 5.5 The mean (± standard error) chlorophyll concentration index (CCI) of *Moneymaker* tomato plants grown in municipal water and inorganic-fertilizer (Control; T1 and T2), post-primary facultative pond effluent (PFP; T3 and T4), or post-high rate algal pond effluent (HRAP; T5 and T6). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. (Repeated measures ANOVA, $F_{(20,240)}$=9.36, p<0.00001).

The mean number of fruit produced per plant at the end of the trial was not influenced by a significant interaction between nutrient solution and pH adjustment (Multifactor ANOVA, $F_{(2,24)}$=2.51, p=0.127; Figure 5.6). Nutrient solution on its own, however, had a significant effect on fruit production, where plants subjected to inorganic fertiliser produced significantly more fruit (3.6±0.5 to 3.8±0.9 fruit/plant) compared to those grown in treated brewery effluent (ANOVA, $F_{(2,24)}$=21.00, p=0.0001). Plants grown in effluent without pH adjustment produced an average of 0.6 fruit/plant (i.e., a range from 0 to 1 fruit/plant) whereas those grown in effluent where the pH was maintained between 6.0 and 6.5 produced an average that ranged between 1.2 and 3.2 fruit/plant within the period of the experiment (Figure 5.6).

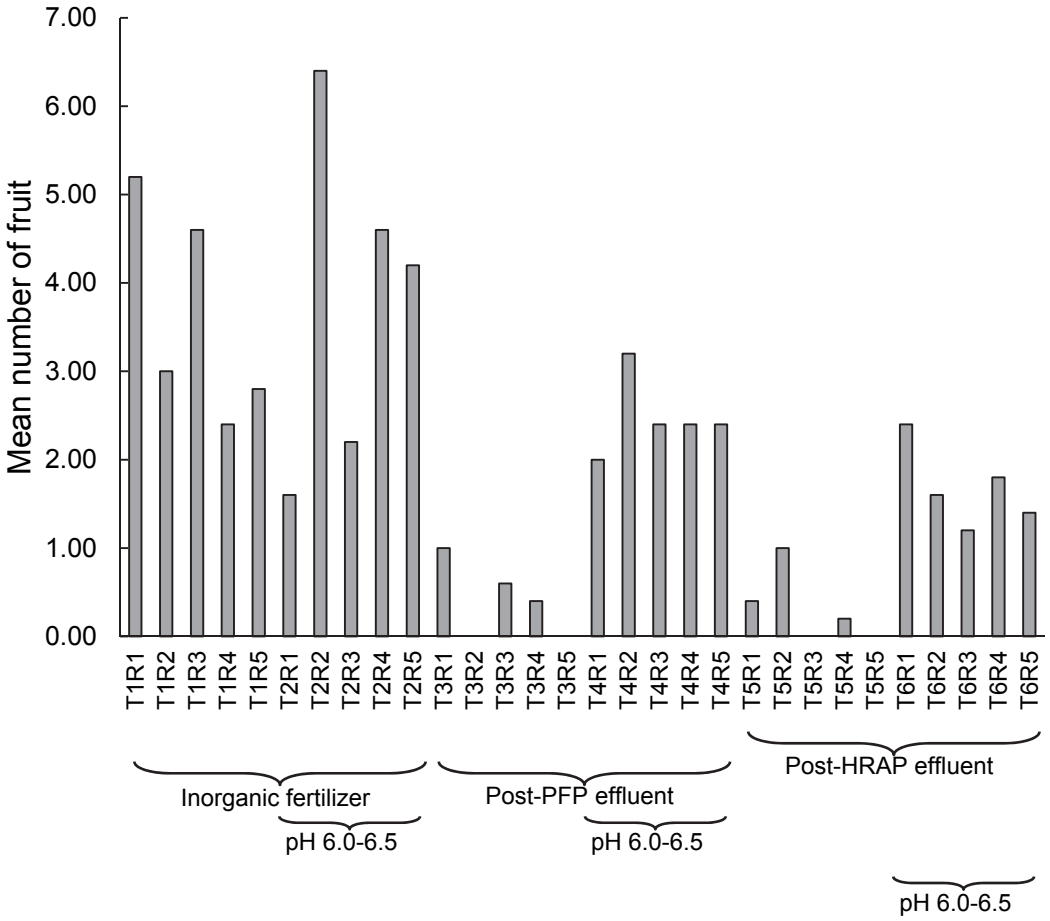

Figure 5.6 The mean number of tomato fruits produced per plant in each of the thirty systems after 49 days. The plants were grown in either municipal water and inorganic-fertilizer (T1 and T2), post-primary facultative pond effluent (post-PFP; T3 and T4), or post-high rate algal pond effluent (post-HRAP; T5 and T6); Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. The R-numbers in the X-axis labels refer to the replicate number of each treatment and these data are the average number of fruit on the five plants in each replicate.

There were differences between the water quality in the nutrient solutions between water source and pH adjustment factors. The post-HRAP solutions had the highest EC values, and T6 had the highest mean pH. The effluent treatments without phosphoric acid had higher mean pH values than the effluent treatments which received phosphoric acid (Table 5.2).

Table 5.2 The mean, maximum and minimum pH values and the mean (± standard deviation) electrical conductivity for the individual treatments. The treatment solutions included: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid (P acid).

	Nutrient solution	pH			Electrical conductivity ($\mu S.cm^{-1}$)
		Mean	Max	Min	
T1	Fertilizer	7.38	8.05	6.90	1974 ± 93
T2	Fertilizer + P acid	5.97	6.98	5.50	1982 ± 88
T3	Post-PFP	8.91	9.43	8.71	2135 ± 84
T4	Post-PFP + P acid	6.16	7.03	5.71	2169 ± 184
T5	Post-HRAP	9.35	9.86	8.33	2451 ± 162
T6	Post-HRAP + P acid	6.16	6.98	5.65	2405 ± 283

Post-PFP solutions had higher ammonium-nitrogen levels while the post-HRAP and fertilizer treatments had higher nitrate-nitrogen levels (Figure 5.7). The acid-corrected effluent treatments had final ammonium-nitrogen concentrations below 5.0 mg/L and nitrate-nitrogen concentrations below 6.0 mg/L in the waste solutions, apart from the reading in T4 taken from the first solution replacement (Figure 5.7). The brewery effluent was highly alkaline, requiring around 25 mL of 80% phosphoric acid to achieve a pH reduction into the range 5.8-6.5 for 25 L of effluent while the municipal systems required only 2.5-3.0 mL of acid per 25 L. The phosphate-phosphorus levels were not reduced to low concentrations in any of the treatments, suggesting the effluent as a fertilizer was not phosphorus limited. The acid corrected effluent treatments consistently had readings above the range of the test.

Fresh solutions: Replacement solutions:

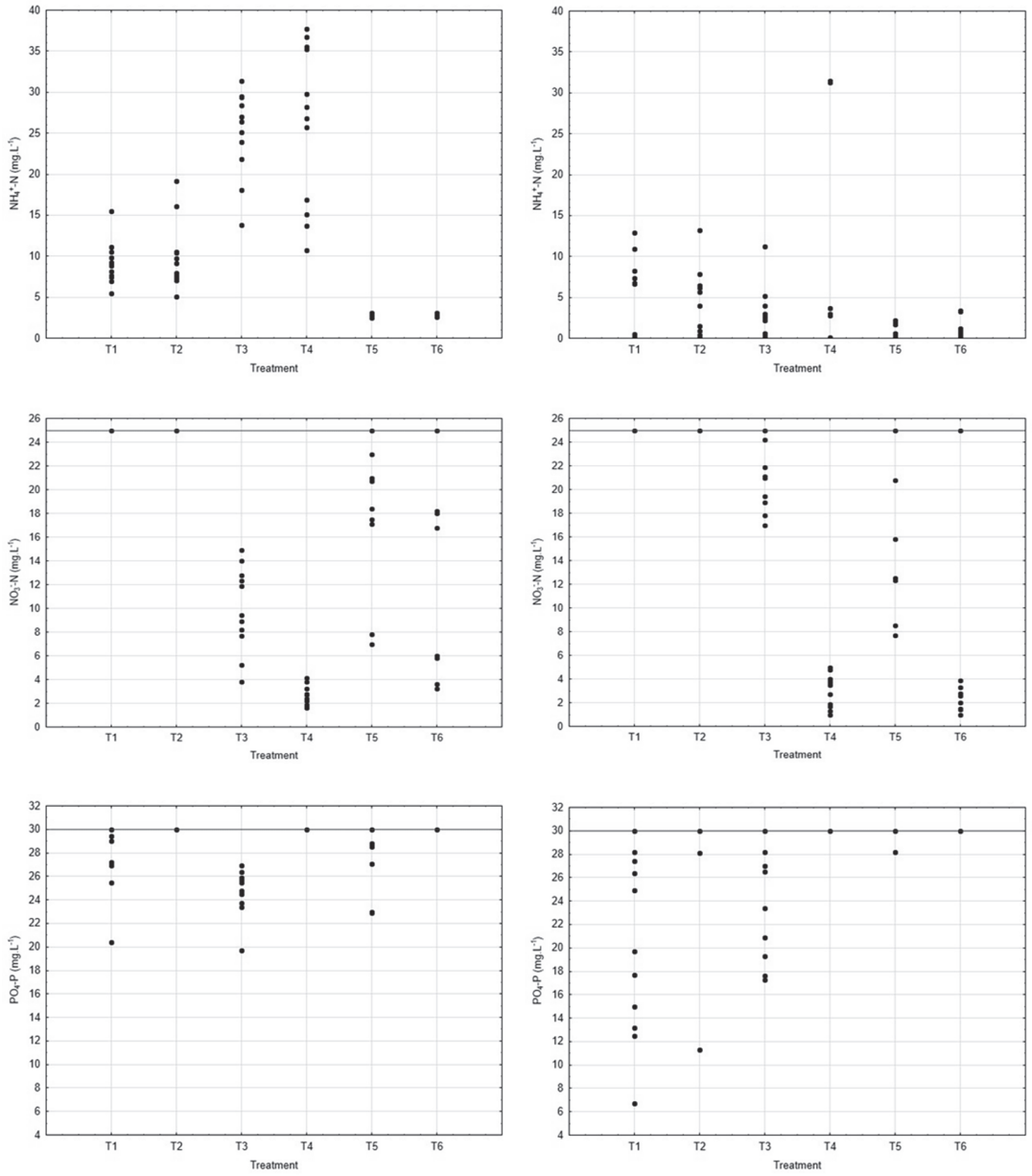

Figure 5.7 Ammonium-nitrogen (NH_4^+-N mg/L), nitrate-nitrogen (NO_3^--N mg/L) and phosphate-phosphorus (PO_4-P mg/L) levels from fresh samples (left) and samples from irrigation solutions just prior to being replaced (right) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. Horizontal lines on graphs indicate the upper concentration limit of the test.

5.4 Discussion

The results confirmed that brewery effluent can support the vegetative growth of *Moneymaker* tomatoes and the early development of fruit. The manipulation of the effluent pH with phosphoric acid significantly improved the vegetative growth and development of the plants in all the parameters measured (stem diameter, plant height, dry biomass and CCI) and increased the number of fruit produced per plant in the period of the trial. The improvement in biomass accumulation was notable with the pH adjusted effluent plants accumulating well over 200% more dry mass than the pH unaltered effluent plants, and pH adjustment also doubled the number of fruit that appeared on the plants within the 49 days of the experiment. The effluent plants did not grow as well as the fertilizer plants which suggests that the effluent system contained nutrient deficiencies, toxicities or plant stresses inhibiting the growth of the plants.

The acid corrected effluent plants were consuming nearly all of the available nitrogen (as nitrate and ammonium) in the effluent however phosphate levels remained high, which was to be expected given the addition of phosphoric acid. The plants irrigated with effluent drawn from after the PFP received nearly all the available nitrogen as ammonium due to the anaerobic digestion process (Angelidaki & Sanders 2004; Batstone *et al*. 2002; Sötemann *et al*. 2005). Despite being a primary source of nitrogen, ammonium-rich or exclusive nutrition has been shown to negatively influence the development of some plants through ion competition and exclusion or stress of key micronutrients (Borgognone *et al*. 2012; Britto & Kronzucker 2002; Horchani *et al*. 2010). Ammonium induced cation deficiency has been suggested to present secondary stresses in, among others, three essential elements; potassium, calcium, and magnesium in numerous studies and plants (Borgognone *et al*. 2012; Britto & Kronzucker 2002; Gloser & Gloser 2000; Horchani *et al*. 2010; Salsac *et al*. 1987). These nutrient stresses, along with the nitrogen limitation suggested by the near complete removal of nitrogen from the pH adjusted effluent systems and their restricted physical development, may have inhibited the development of the plants compared to those grown in fertilizer. Optimizing the nutrition of the crop needs to be addressed in further work.

The presence of ammonium in the effluent may have increased the CCI of the post-PFP irrigated plants. The readings over the course of the trial showed that the ammonium-rich post-PFP effluent plants had an equal or higher CCI than the other treatments. A positive correlation has been demonstrated between external ammonium concentration and the chlorophyll concentration in tomato plants (Horchani *et al*. 2010). The effluent contains less nitrogen than the inorganic-fertilizer mixture, which would explain the lower CCI for post- post-HRAP effluent plants than those in the control systems. The slightly elevated CCI in the unaltered pH post-PFP treatment (T3) are likely due to the uptake of ammonium, however the high pH stress on nutrient uptake meant that these plants could not assimilate nitrogen as was seen in the wastewater quality results, and they were probably experiencing other pH-induced nutrient stresses (Lucas & Davis 1961).

The high alkalinity of the effluent is probably due to upstream injections of sodium hydroxide (NaOH) as a pH buffer during the neutralisation of the raw brewery effluent prior to anaerobic digestion and the AD itself, which is a source of carbonate alkalinity. The CO_2 generated in the AD partially dissolves in the digester liquor, generating carbonate alkalinity and carbonic acid (Batstone *et al*. 2002; van Rensburg *et al.* 2003). When the digested effluent is exposed to normal atmospheric partial pressure after leaving the AD, the volatile carbonic acid is stripped and the effluent loses acidity but the carbonate alkalinity remains (Musvoto *et al*. 2000; van Rensburg *et al.* 2003). This residual alkalinity contributes to the high pH and the volume of acid needed to reduce to pH to optimum levels. The influence of pH adjustment on plant growth is clearly shown in the results. Optimising the pH of the

effluent will be a key step in developing a practical hydroponic system and understanding the sources of alkalinity will be an important factor in addressing the pH adjustment.

Water used in the production of crops for human consumptions needs to be pathogen free. The brewery practices complete physical segregation of beer manufacturing effluent from the sewage system, because water recovery is practiced at this facility and recovered water cannot come into contact with sewage or any other potentially contaminated effluent. As such, it is unlikely that a hydroponic vegetable crop grown in this effluent would be exposed to pathogenic bacteria that would render them unsafe for human consumption. Furthermore, HRAP systems are efficient at removing pathogens from sewage (Craggs *et al.* 2004; El Hamouari *et al.* 1994; Gaigher *et al.* 1985). This is probably due to a combination of the high pH and high dissolved oxygen concentrations in the algal ponds and due to exposure to sunlight (Oswald 2003). Algal ponding systems have been successful at the complete removal of nematodes and *Salmonella* sp. from human sewage (El Hamouari *et al.* 1994) and were more efficient at reducing ammonia, phosphate and *Escherichia coli* than that of conventional two-stage oxidation ponds in the treatment of dairy effluent (Craggs *et al.* 2004). If this technology were used in industry where there was a chance of contamination of the effluent prior to its use a hydroponic nutrient and water source, the HRAP system could be used as a step in the treatment process to counter this concern without compromising downstream hydroponic crop yields, provided the pH of the post-HRAP nutrient solution was adjusted to pH 6.0-6.5.

5.5 Conclusions

Tomato plants can be grown in anaerobically digested brewery effluent, and the adjustment of the effluent pH significantly improved their vegetative growth and it also improved early fruit production. However, effluent grown tomatoes did not grow as well as those produced using inorganic fertiliser and there are broader nutrient deficiencies or stresses that were probably responsible for this, which should be addressed in future work.

The brewery effluent system is nitrogen limited and must be considered holistically if effective solutions to maximise the potential of the final effluent are to be found. The cleaning-in-place procedures in the brewery utilize phosphoric acid and not nitric acid, which may be contributing to the final effluent N:P ratio; also, proteinaceous rich trub wastes are disposed separately, which is an additional loss of nitrogen. Ideally, brewery in-house practices and effluent treatment could be altered to contribute to produce a final effluent with the best possible chemical composition for hydroponic production. This would add considerable value to this effluent. Interventions to improve nutrient supply must be considered while being mindful of the limits on downstream nutrient release and bearing in mind the restrictions of the core business processes. It would be unreasonable to suggest a change in brewing practice that will alter product quality or increase operational expenses but, where there are options, the down-stream use of the effluent should be considered.

The treated brewery effluent is highly alkaline, which is a consequence of numerous upstream factors including the anaerobic digestion of the raw effluent. Managing the effluent alkalinity, nutrient deficiencies and limiting downstream nutrient releases are the key challenges to using brewery effluent as a hydroponic water resource.

The experimental HRAP system did not improve the suitability or nutritional potential of the brewery effluent as a nutrient source for tomato production and therefore it is suggested that the HRAP system is not needed if the effluent is to be used as a hydroponic nutrient solution, provided the effluent is free of pathogenic microbes. Future research should focus on effluent drawn directly from the PFP or the AD.

Brewery and other organic effluent streams could represent an alternative nutrient and/or water resource for communal or commercial hydroponic vegetable production. This work demonstrates the potential value of what was considered a costly liability to the primary water user.

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6. The use of brewery effluent as a water and nutrient source in irrigated crop production and its effect on soil

The work presented here did not form part of the original work plan and objectives of the Water Research Commission (WRC) project K5/2284. However, K5/2284 made this work possible by involving MSc student Richard Taylor as a technical assistant in the other WRC student projects and Taylor managed the WRC research site at Ibhayi Brewery while collecting these data. His involvement and this project were made possible through a student bursary (Ada & Bertie Levenstein Bursary, Rhodes University), financial contributions made by SAB Ltd and the Rhodes University Research Committee. The SAB Ltd also made the research site and utilities available for this project.

This research is reported here because of the role that the WRC played in making it possible and since it contributes to the overall aim of our WRC research program. This report was prepared by Mr Richard Taylor and is based on a chapter in his MSc thesis and it will be published as it appears here.

6.1.1 Rationale

Brewery effluent (BE) is an organic effluent that contains the plant nutrients nitrogen and phosphorus, and a range of organic and inorganic compounds (Senthilraja *et al.* 2013, Power 2014). Nitrogen and phosphorus are essential for good plant growth and health (Epstein & Bloom 2005). Farmers normally have to buy inorganic nitrogen and phosphorus fertilisers. Brewery effluent has the potential be used as a source of water and nutrients in irrigated crop production (Muyen *et al.* 2011, Senthilraja *et al.* 2013, Power 2014). However, BE also has properties that may inhibit the growth of plants or even diminish the fertility of soils when used to irrigate crops (Kaushik *et al.* 2005, Senthilraja *et al.* 2013, Power 2014). Various authors have found that the irrigation of soils with brewery wastewaters have led to a deterioration of the physical profile of the soils and diminishing soil fertility (Ajmal & Khan 1984, Kaushik *et al.* 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). The effect that BE has on crop production rates, plant health and soil structure is not clear, as well as what pre-treatment methods would make the effluent most suitable for crop irrigation.

Brewery effluent at Ibhayi Brewery (SAB Ltd, Port Elizabeth) is treated in an anaerobic digester (AD) and activated sludge system (AS) before being either piped to a municipal sewer or it is channelled back to the factory for re-use (Naiker, pers. comm., Senior Engineer, Ibhayi Brewery, SAB Ltd., July 2015). A small stream of post-AD BE is fed into an experimental treatment facility, run by Rhodes University, which uses various alternative, sustainable methods of BE treatment (Jones *et al.* 2014). This treatment facility includes bioremediation facilities such as a primary facultative pond (PFP), a high rate algal pond (HRAP) and a constructed wetland (CW). Each treatment process results in BE having different water quality parameters such as pH, form and concentration of nitrogen, and the concentration of phosphorus, sodium and other dissolved salts (Cilliers 2012, Jones *et al.* 2014). These parameters been have shown to directly and indirectly affect plant growth and soil fertility (Lucas & Davis 1961, Lieth & Al-Masoom 1993, Garcia *et al.* 1994).

Different methods of BE pre-treatment have been found to influence nutrient availability and downstream crop productivity (Power 2014, Power & Jones 2015). Dakoure *et al.* (2013) found that the sodium content of BE negatively affected the physical properties of the irrigated soil. It is therefore essential that the most suitable pre-treatment method of BE is found so that the nutrients in the effluent are made accessible to the plants while minimising any negative impacts BE may have on the soil.

6.1.2 Aims and objectives

The aim of this study was to determine the best pre-treatment method or combination of pre-treatment methods to make BE suitable for crop irrigation, and to evaluate the potential of BE as an irrigation water source. This was done by comparing the change in soil characteristics and growth of crops irrigated with treated BE to crops irrigated with a conventional irrigation solution. Cabbage plants were grown in the soil and irrigated with BE after treatment using AD, PFP, HRAP, CW and a commercial fertigation solution. All the irrigation treatments were subject to both pH adjustment and no pH adjustment. The mass, height, weight, diameter and health of the plants under each irrigation treatment were determined and compared between treatments. Physical, chemical and biological properties of the soil were also recorded to determine the effect that each irrigation water had on soil fertility.

6.2 Methods and materials

6.2.1 Experimental species and system

Two hundred cabbage seedlings (*Brassica oleracea* cv. Star 3301; Starke Ayres Pty Ltd, South Africa) were purchased from a commercial nursery (Moorland Seedlings Pty Ltd, Humansdorp). Of these 120 similar size seedlings were used in this experiment.

Cabbage plants were grown out doors in 23 l pots. These pots were filled with top soil (oxidic sandy loam, 5-10% silt, 20-25% clay, 65-70% sand) obtained from a commercial supplier (Habata farm Pty Ltd, Sundays River Valley, South Africa). One cabbage plant was planted in each pot.

6.2.2 Treatments

Six irrigation solutions were applied to the cabbages which included post-AD, post-PFP, post-HRAP, post-CW, a commercial irrigation solution and municipal water (Table 6.1). The pH of each irrigation treatment was either adjusted to 6.5 with 98% sulphuric acid (Protea Chemicals Pty Ltd, South Africa) or left unadjusted (Table 6.1). This resulted in a total of 12 irrigation treatments being tested.

The plants irrigated with municipal water served as the control. The nutrient solution (NS) was comprised of a commercially available inorganic-fertilizer (Hygrotech Pty Ltd, South Africa; Registration number K5709; Act 36 of 1947), and calcium nitrate with a composition of 11.7% nitrogen and 16.6% calcium, mixed in a ratio of 1:0.8 and dissolved in municipal water to achieve an EC of 1800 µm (Hygrotech Pty Ltd, South Africa). Each treatment was replicated ten times with a replicate consisting of a single plant in a pot.

Table 6.1 Irrigation treatments (T1-T12) that were used to irrigate cabbage plants, and typical characteristics of the nutrient solutions.

Irrigation solution	pH not adjusted	pH adjusted to 6.5
AD effluent	T1	T7
PFP effluent	T2	T8
HRAP effluent	T3	T9
CW effluent	T4	T10
Municipal water	T5	T11
Municipal water with inorganic fertiliser	T6	T12

	pH	COD (mg/L)	NH_4-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)	Chloride (mg/L)
AD effluent	8.0	183.0	33.7	0.9	23.0	27.1	151.9
PFP effluent	8.2	164.3	35.8	0.9	18.8	25.6	153.1
HRAP effluent	9.2	135.0	2.5	0.4	9.2	16.1	166.9
CW effluent	8.3	141.3	2.1	0.3	7.9	17.3	189.1
Municipal water	7.5	16.0	0.7	0.1	5.1	6.1	80.7
Municipal water with inorganic fertiliser	6.7	18.9	17.0	0.1	25.0	29.9	81.8

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), chemical oxygen demand (COD_F).

6.2.3 Irrigation regime

Cabbages were irrigated with one litre two to three times a week, depending on the moisture content of the soil. Every day a 10 cm long stick was pushed into the soil. If the stick came out dry, then the cabbage plants were irrigated. If the stick came out muddy, then the plants were not irrigated. If cabbage plants showed signs of wilting they were also irrigated. During irrigation care was taken not to wet the cabbage leaves.

The maximum amount of water irrigated at one time was one litre. This was done to ensure that leaching did not occur. Water was not observed draining out the bottom of the pots. In total each cabbage plant received 198.1 mm of treatment irrigation water and 91 mm of rain during the twelve week growth trial.

One month after planting, diamond back moth larvae were noticed on some of the cabbages. Cabbages plants were sprayed with Malasol (active ingredient: Mercaptothion, Efekto Agro-serve Pty Ltd) to kill the larvae. When a spraying occurred every plant was sprayed, an event that occurred five times during the trial. As a result no plants suffered severe damage from the diamond back moth larvae.

6.2.4 Data collection

Irrigation water chemistry

The pH, temperature and electrical conductivity (EC) of the water used in each treatment was recorded before each irrigation using an electronic probe (Hanna, HI 991300, United Kingdom). Chemical oxygen demand (COD_F), ammonia, nitrite, nitrate and phosphate of each irrigation solution was recorded weekly, using a spectrophotometer (Merck Spectroquant Pharo 100 spectrophotometer, product number 100706, Darmstadt, Germany) and commercially available test kits, using standard methods (Merck Pty Ltd; Section 2.2.4). Each sample was filtered through an eight micron filter paper prior to analysis.

Plant productivity

The height and width of each cabbage plant was recorded (1 mm accuracy) at the start of the experiment and every four weeks until the end of the experiment. At the end of the trial the root mass, stem mass, and leaf mass of each cabbage plant was recorded.

The chlorophyll concentration index (CCI) of cabbage plant leaves was recorded using a chlorophyll content meter (CCM-200 Plus Chlorophyll Content Meter, Opti-Sciences Inc., USA). Readings were recorded at the start of the trial and every four weeks until the end of the experiment, on the uppermost fully expanded leaf of each plant.

At the beginning of the trial 12 plants were randomly chosen and used for leaf chemical analysis. These plants were not used in the experiment due to the destructive nature of the sampling. At the end of the trial three plants were randomly selected from each treatment and used for leaf chemical testing. All samples were analysed for N, P, Na, Cl, K, Al, Ca, Cu, Fe, Mn, Mg and Zn content at a commercial analytical laboratory (BemLab Pty Ltd, Strand, South Africa).

Photographs of the plants and stress symptoms of the plants were described and recorded to determine if the plants were experiencing any nutrient deficiencies or diseases. Daily temperature and rainfall data were recorded using a rainfall gauge situated next to the experiment and a thermometer (Hanna, HI 991300, United Kingdom).

Soil monitoring

The physical properties of the soil that were measured included infiltration rate, moisture content, porosity, aggregate stability, bulk density, compaction and water potential. Infiltration rates were determined, every four weeks, by pouring one litre of irrigation treatment water into each pot in twenty seconds and recording the time it took for the water to drain into the soil. Timing was only started once all the water had been poured into the pot. Infiltration rate was then calculated using Equation 6.1:

Infiltration rate = (volume of water added/surface area of pot)/time [6.1]

Soil aggregate stability was measured at the beginning and end of the experiment (Le Bissonnais 1996). At the beginning of the trial 10 samples were taken from the soil used in the trial. At the end of the trial five composite samples were taken from each treatment. A composite sample consisted of soil obtained from two pots in each treatment. Five grams of soil sample was placed in distilled water and allowed to stand for ten minutes. The sample was then passed through a 0.05 mm sieve and aggregates >0.05 mm were collected and transferred onto a 0.50 mm sieve previously immersed in ethanol, and shaken five times with a gentle regular helical rotation movement. The >0.5 mm aggregates on the sieve were collected, dried at 40°C, and then gently dry sieved using a column of six sieves: 2.00, 1.00, 0.50, 0.20, 0.10, and 0.05 mm. The aggregate stability was represented by the mean weight diameter (mm) of aggregates and was calculated using Equation 6.2:

$$\text{Mean weight diameter} = \sum (d \times m) / 100 \quad [6.2]$$

where d was the mean diameter between the two sieves (mm) and m was the weight fraction of aggregates remaining on the sieve (%).

Air filled porosity (AFP), bulk density and moisture content were measured, in each pot at the beginning and end of the trial, according to the Australian standard for potting mixes (Handreck & Black 1994). The apparatus used was a 110 mm plastic pipe with an end cap that had four 3.0 mm holes drilled into it. The pipe was bored into the soil to get an undisturbed soil sample. The soil was then run through a series of wetting cycles as follows: 30 min wetting and five min draining, 10 min

wetting and five min draining, and 10 min wetting and five min draining. A gauze was placed over the top of the vessel and submerged in water to just above the surface of the soil. The holes in the bottom were then sealed and the vessel was moved into a tray, where the holes were unblocked. The vessel was left to drain for 30 min and the amount of water collected was measured. Air filled porosity was calculated using Equation 6.3. Directly after the AFP test the vessel was placed in a drying oven at 105^{o}C and allowed to dry for a minimum of 24 h, until a constant mass was achieved. Water holding capacity was calculated using Equation 6.4, and bulk density was then calculated using Equation 6.5.

Air filled porosity (%) = (volume drained/volume of soil) × 100 [6.3]

Water holding capacity (%) = ((wet weight – dry weight)/volume) x 100 [6.4]

Bulk density = dry weight/volume 6.5]

Soil compaction was measured at the start and end of the experiment in every pot using a pocket penetrometer (Szkurlat Pty Ltd, Poland).

A psychrometer (PST-55-15 thermocouple psychrometer/hygrometer, Psypro, Wescor Inc., Logan, UT, USA) was used to construct a soil suction test, which related gravimetric soil water content to soil water potential. At the beginning of the trial 10 samples were taken from the soil used in the trial. At the end of the trial four composite samples were taken from each treatment. A composite sample consisted of soil taken from two pots from each treatment. Each sample was oven dried at 40^{o}C for 48 h, until a constant mass was achieved. Each sample was then wet with distilled water to 30% moisture content, by weight, and placed in a 30 mL air tight glass vial. Glass vials were then placed in a fridge for 48 h to allow the water to become evenly distributed throughout the soil. To determine the water potential of the soil, soil psychrometers were sealed into the vials and calibrated against standard solutions of 0.1 to 1.0 molar NaCl. Once analysed, samples were placed in an oven at 36^{o}C, in their vials with the lids off and allowed to dry until they reached 20% moisture content by weight. This was done by monitoring the weight of the samples every 30 min. Once samples reached 20% moisture content the lids were put back on the vials (making them air tight) and put into a fridge for 48 h to ensure the water become evenly distributed throughout the soil. After 48 h the samples were re-weighed to calculate their exact moisture content and then analysed. Once analysed the samples were then dried to 10% moisture content by weight and analysed. The psychrometer was calibrated before water potential readings were taken at each of the soil moisture contents described above.

The chemical properties of the soil that were recorded included pH, EC, cation exchange capacity (CEC), C, NH_4, P, Na, Cl, K, Ca, Cu, Mn and Mg. Electrical conductivity and pH were measured in every pot at the start and end of the experiment using a pH and conductivity meter (Hanna, HI 991300, United Kingdom) where the soil sample was mixed with distilled water at a ratio of 1:2.5 (Hati *et al.* 2007). At the beginning of the trial 10 samples were taken from the soil used in the trial and used for soil chemical analysis. At the end of the experiment three composite soil samples from each treatment were used for soil chemical analysis. A composite sample consisted of soil taken from three pots in each treatment. These samples were sent to a commercial analytical laboratory and analysed for CEC, C, NH_4, PO_4, Na, K, Ca, Cu, Mn and Mg (BemLab Pty Ltd, Strand, South Africa). The sodium adsorption ration of the soil was also calculated using Equation 6.6, where Na, Ca, Mg and K are expressed in milliequivalents per litre, (meq/L) obtained from a saturated paste soil extract (Sumner *et al.* 1995, Qadir & Schubert 2002).

Sodium adsorption ratio = $\mathrm{Na} \div \sqrt{\frac{\mathrm{Ca}+\mathrm{Mg}}{2}}$ [6.6]

Community level physiological profiling was used to describe the biological health of the soil. This was done by direct inoculation of soil samples into single carbon source wells of microtitre plates (Eco Microplates BL1506, Biolog Inc, USA), followed by incubation and spectrometric detection of heterotrophic activity (Garland & Mills 1991). At the beginning of the trial 12 samples were randomly taken from the soil before it was placed into the pots. At the end of the trial three composite samples were taken from each treatment, where the composite samples consisted of soil taken from three pots in each treatment. Samples were analysed at the Department of Biochemistry and Microbiology, Rhodes University. One gram of soil sample was placed in 99 mL of sterile saline solution (0.2% NaCl) and allowed to settle. A further 10 x dilution was made by dispensing two millilitres into 18 mL sterile saline (10^{-3} dilution). After mixing, 150 µL was pipetted into each of the wells in the microtitre plates. The plate was then incubated at 25^{o}C and readings were taken every 24 h, for five days, using a microplate reader (PowerWave HT Microplate Spectrophotometer, Biotek, USA) at a wavelength of 590 nm.

Microbial activity in each plate was expressed as average well colour development (AWCD) and was determined using Equation 6.7 (Garland & Millis 1991, Gomez *et al.* 2004):

$$\text{Average well colour development} = \sum OD_i/31 \quad [6.7]$$

where OD_i was the optical density value from each well, corrected by subtracting the blank well (inoculated, but without a carbon source) values from each plate well (Garland & Millis 1991, Weber & Legge 2009). Richness (R) values were calculated as the number wells with a positive optical density (the number of oxidised carbon substrates, Magdalena *et al.* 2012). Shannon-Weaver index (H) values were calculated using Equation 6.8:

$$\text{Shannon Weaver index} = -\sum p_i(\ln p_i) \quad [6.8]$$

where p_i was the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum OD_i$; Garland & Millis 1991, Magdalena *et al.* 2012). Plate reading at 119 h of incubation were used to calculate AWCD, R and H. The carbon substrates on each plate were grouped into the following five categories: (1) carbohydrates; (2) carboxylic and acetic acids; (3) amino acids; (4) polymers; and (5) amines and amides (Weber & Legge 2009). Each category was expressed as a percentage of total absorbance value of the plate corresponding to a particular treatment (Weber & Legge 2009).

Microbial counts were also performed by direct inoculation of soil suspension onto sterile nutrient agar plates. Five composite samples per treatment were analysed with a composite sample containing soil from two replicates. Dilutions were prepared as described above and 100 µL of the 10^{-3} dilution was pipetted onto a sterile nutrient agar plate. Then solution was spread across the surface of the nutrient agar plate using a sterile bent glass rod. Plates were then incubated at 25^{o}C for 24 h and the number of colonies on each plate was counted and colony forming units (CFU) were calculated using Equation 6.9.

$$\text{CFU} = \text{number of colonies} \times 10^4 \quad [6.9]$$

6.2.5 Statistical analysis

Treatment means were compared using a one-way and multi-factor analysis of variance (ANOVA) and a Tukey multiple range analysis at $p<0.05$. Data collected over the course of the trial were compared using a one-way and multifactor repeated measures ANOVA ($p<0.05$). All data were checked for equality of variance and for the normal distribution of the residuals using Levene's test and a Shapiro-Wilk plot of the residuals, respectively. If the assumptions were not met then the data were

log or square-root transformed and checked for equal variance and normal distribution of residuals. If the assumptions were still not met, a non-parametric Mann-Whitney U test or a Kruskal Wallis ANOVA was used to compare the data between treatments. All analyses were performed using the Statistica (version 10) software package (StatSoft Inc, Tulsa, USA).

Statistical analysis of the data obtained from the Biolog plates was performed on the actual OD density of the wells and on transformed data where wells with an OD lower than 0.1 was set to zero. When this was done no differences was found in the conclusions from the analysis and therefore the data presented from Biolog plates were obtained from the actual OD of the wells.

6.3 Results

6.3.1 Irrigation water chemistry

The conductivity of the BE irrigation treatments (3301.85 ± 34.46 µs/cm^2) was significantly higher than the nutrient solution (1904.91 ± 31.10 µs/cm^2) and water-only (595.86 ± 17.466 µs/cm^2) treatments (Kruskal Wallis, $H_{(11,264)}$=239.57, p<0.0001; Figure 6.1). The conductivity increased for all irrigation treatments when the pH was adjusted to 6.5 with sulphuric acid (Figure 6.1).

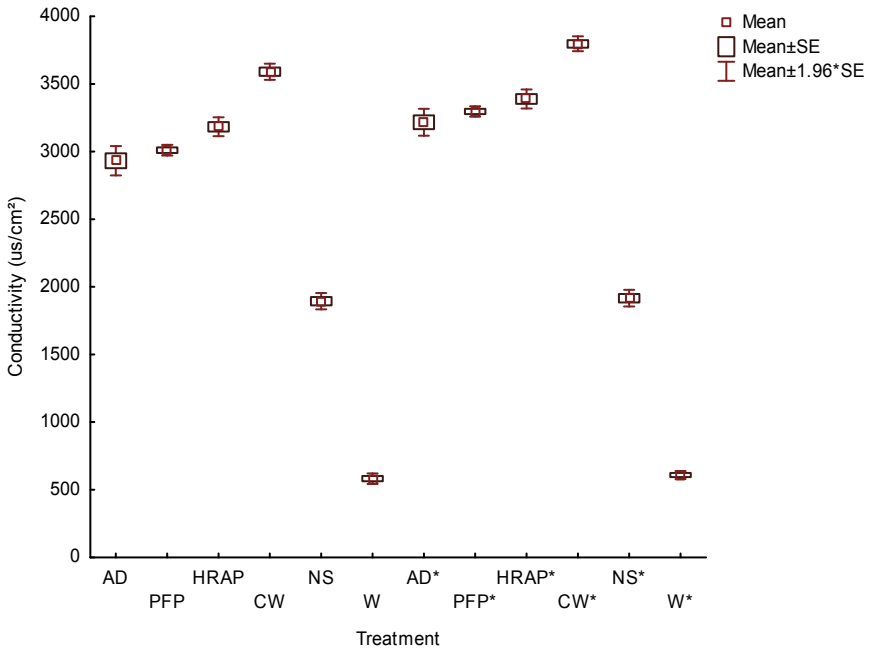

Figure 6.1 The mean (± standard error) conductivity irrigation treatments including: brewery effluent subject to anaerobic digestion (AD), primary facultative pondinging (PFP), high rate algal pondinging (HRAP) or a constructed wetland (CW), and nutrient solution (NS) and water (W) (Kruskal Wallis, $H_{(11,264)}$=239.57 p<0.0001). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

Brewery effluent irrigation treatments had a higher pH than the nutrient solution (7.56 ± 0.02) and water (6.2 ± 0.05) treatments (Figure 6.2). High rate algal pond water had the highest pH (9.17 ± 0.14) while the other BE irrigation waters had a mean pH of 8.14 ± 0.03 (Figure 6.2). The ammonia-nitrogen concentration of the nutrient solution (17.64 ± 0.69) was lower than the AD and PFP (34.74 ± 2.18) irrigation treatments but higher than the water after the HRAP and CW (2.34 ± 0.27) irrigation treatments (Kruskal Wallis, $H_{(5,162)}$=141.30, p<0.0001; Table 6.2). The nitrate and phosphate concentration was highest in the nutrient solution, AD and PFP treatment water (Kruskal Wallis, p<0.05; Table 6.2). High rate algal pond, CW and water irrigation treatments had the lowest nitrate and phosphate concentration. The chloride concentration was highest in CW and HRAP irrigation treatments, followed by AD and PFP treatments (Kruskal Wallis, $H_{(5,162)}$=119.30, p<0.0001; Table 6.2). The nutrient solution and water irrigation treatments had the lowest chloride concentration (Table 6.2).

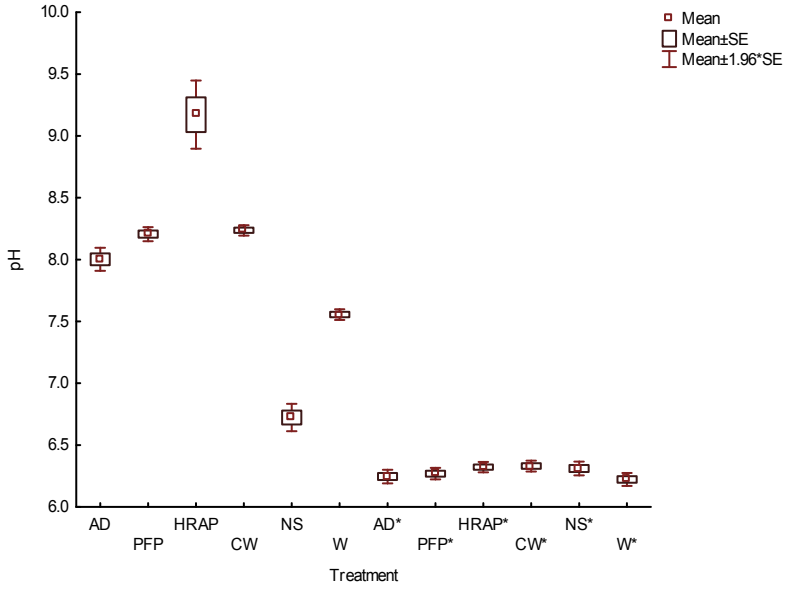

Figure 6.2 The mean pH of the various irrigation treatments, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

Table 6.2 The mean (± standard error) water quality parameters of the various irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different means (Kruskal Wallis, P<0.05).

Property	Treatment							
	AD	PFP	HRAP	CW	NS	Water	H	P
COD_F (mg/L)	183.1±9.49 [a]	164.3±7.42 [a]	135.0±8.52 [a]	141.3±5.38 [a]	18.9±1.09 [b]	16.0±0.32 [b]	114.27	0.0001
NH_4-N (mg/L)	33.7±2.58 [a]	35.8± 1.78 [a]	2.5±0.19 [b]	2.1±0.34 [b]	17.0±0.69 [c]	0.7±0.05 [b]	141.30	0.0001
NO_2-N (mg/L)	0.9±0.02 [a]	0.9± 0.04 [a]	0.4±0.03 [b]	0.3±0.02 [b]	0.1±0.01 [c]	0.1±0.04 [c]	133.26	0.0001
NO_3-N (mg/L)	23.0±0.51 [a]	18.8±1.29 [a]	9.2±0.69 [b]	7.9±0.53 [b]	25.0±0.87 [a]	5.1±0.45 [b]	124.34	0.0001
PO_4-P (mg/L)	27.1±0.97 [a]	25.6± 1.05 [a]	16.1±0.95 [b]	17.3±0.93 [b]	29.9±0.12 [a]	6.1±0.45 [c]	124.51	0.0001
Chloride (mg/L)	151.9±4.82 [a]	153.1±4.75 [a]	166.9±5.13 [ab]	189.1±4.69 [b]	81.8±1.77 [c]	80.7±1.67 [c]	119.30	0.0001

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS). Filtered chemical oxygen demand (COD_F).

6.3.2 Plant productivity

The final mass of cabbages was not influenced by an interaction between pH regime and irrigation water source (Multifactor ANOVA, $F_{(5,108)}$=0.93, p=0.46; Figure 6.3). The pH adjustment of the irrigation waters did not affect the final mass of the cabbage plants (Multifactor ANOVA, $F_{(1,108)}$=2.83, p=0.10), whereas there was a significant difference in the final weight of crops irrigated with water from different sources (Multifactor ANOVA, $F_{(1,108)}$=446.12, p<0.0001). Cabbage plants irrigated with NS had the greatest mass (1223.32 ± 40.98 g) followed by cabbage plants irrigated with AD and PFP BE (478.13 ± 17.39 g; Figure 6.3). The AD, PFP and HRAP treatments resulted in cabbages that were significantly larger than those grown using water-only (Figure 6.3). The mass of cabbaged plants irrigated with CW effluent and water-only were the smallest, with no significant difference in plant weight between them (Figure 6.3). The CCI of cabbage plants was not influenced by an interaction between pH regime and the irrigation water source (Multifactor repeated measures ANOVA, $F_{(15,321)}$=0.63, p=0.85). The pH adjustment of the irrigation water sources had no effect on the CCI of cabbage plants (Multifactor repeated measures ANOVA, $F_{(5,107)}$=1.15, p=0.34). There was a significant difference in the CCI of cabbages irrigated with the various irrigation water sources, with the NS irrigated cabbages having the highest CCI, followed by AD and PFP irrigated cabbages (Multifactor repeated measures ANOVA, $F_{(15,321)}$=25.41, p<0.0001; Figure 6.4). Cabbages irrigated with water, and HRAP and CW effluent had the lowest CCI values over the course of the trial (Figure 6.4).

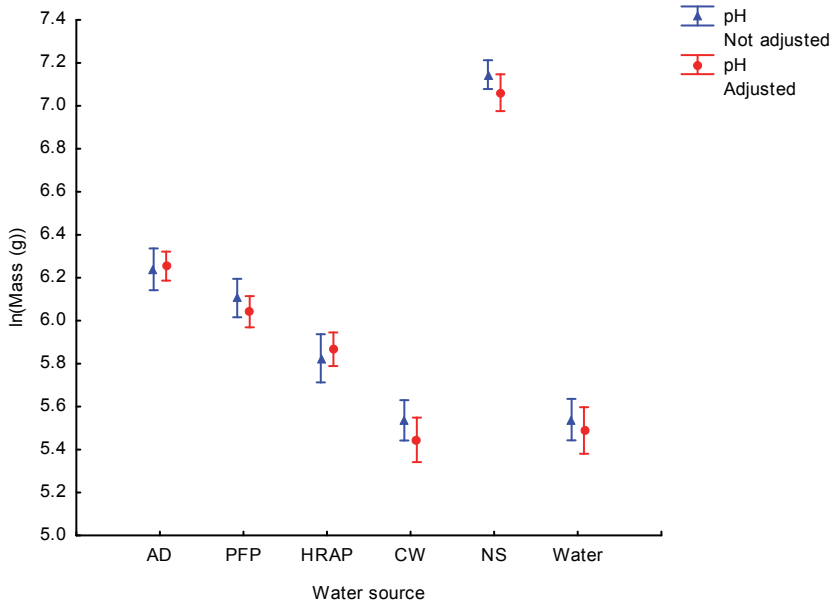

Figure 6.3 The mean (± 95% confidence interval) log transformed mass of cabbages subject to various irrigation treatments after 12 weeks, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (Multifactor ANOVA, $F_{(5,108)}$=0.93, p=0.46). The pH of each irrigation treatment was adjusted to 6.5 with sulphuric acid and was not adjusted.

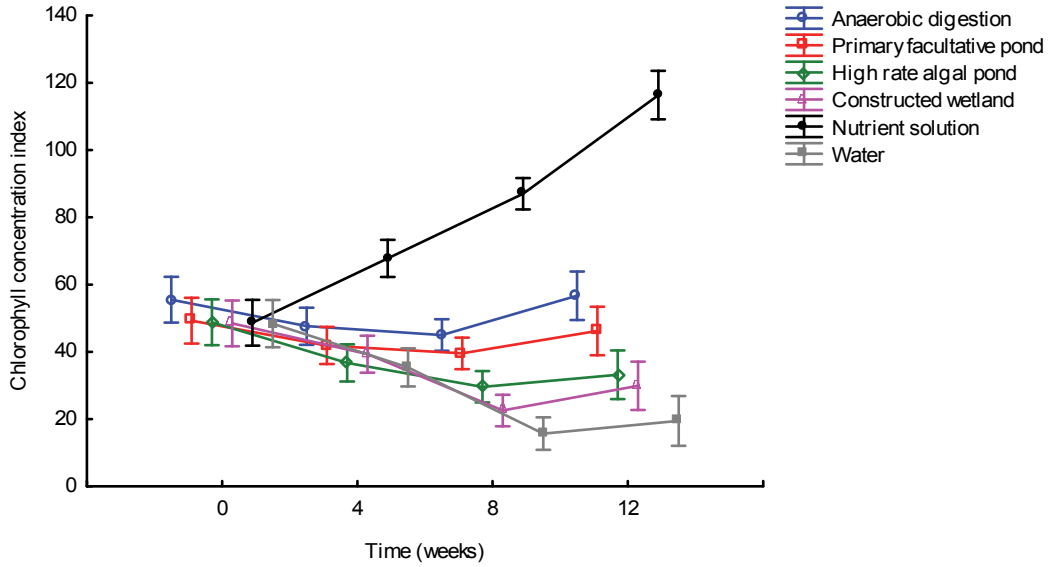

Figure 6.4 The mean (± 95% confidence interval) chlorophyll concentration index of cabbages subject to various irrigation treatments over the 12 week trial (Multifactor repeated measures ANOVA, $F_{(15,321)}$=25.41, p<0.0001).

6.3.3 Chemical characteristics of plants

Cabbage plants irrigated with NS had significantly higher concentrations of N, P and K in their leaf tissue than cabbages subject to any of the BE irrigation treatments (Kruskal Wallis, p<0.05; Figure 6.5 and Table 6.3). The leaf concentration of N, P and K was similar for cabbages irrigated under all the BE and water irrigation treatments (Figure 6.5 and Table 6.3). The pH adjustment of the irrigation treatments had no effect on the N, P and K content of cabbage leaves (Figure 6.5 and Table 6.3).

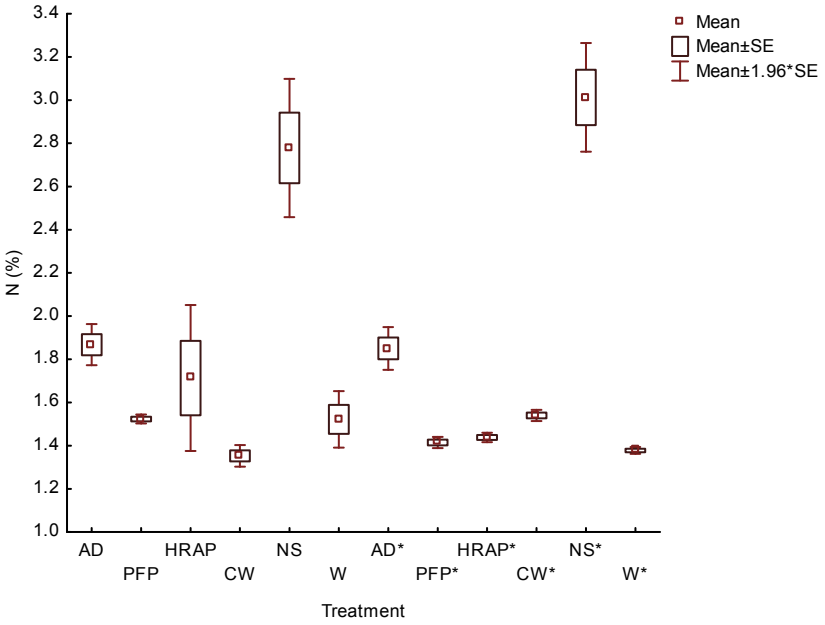

Figure 6.5 The mean nitrogen leaf content of cabbage plants irrigated under the different irrigation treatments anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W) (Kruskal Wallis, $H_{(11,36)}$=32.18 p=0.0007). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

The irrigation of cabbages with BE treatment waters did not increase the sodium content of cabbage leaves when compared the sodium content of the cabbage seedlings (Table 6.3 & 6.4). The Na content of cabbage leaves was not influenced by pH, and the interaction between pH regime and water source (Multifactor ANOVA, $F_{(5,24)}$=0.85, p=0.53; Table 6.4). Cabbages plants irrigated with water had the lowest Na leaf content while cabbage plants subject to the rest of the irrigation treatments had similar Na leaf contents (Table 6.4). The pH adjustment of the irrigation solutions decreased the Na leaf concentration of cabbage plants (Multifactor ANOVA, $F_{(1,24)}$=17.48, p=0.0003). There was no difference in the Cl, Cu, Fe, Mn and Zn leaf concentration of cabbage plants subject to all twelve experimental irrigation treatments (Multifactor ANOVA/Kruskal Wallis, p>0.05; Table 6.4).

Table 6.3 The mean (± standard error) chemical composition of cabbage seedlings prior to planting.

Calcium (%)	Chloride (%)	Copper (mg/kg)	Iron (mg/kg)	Potassium (%)	Phosphorus (%)	Magnesium (%)	Manganese (mg/kg)	Nitrogen (%)	Sodium (mg/kg)	Zinc (mg/kg)
1.57 ± 0.14	1.59 ± 0.01	11.79 ± 0.23	232.37 ± 13.36	5.62 ± 0.54	0.62 ± 0.01	0.37 ± 0.01	218.97 ± 1.99	3.04 ± 0.05	6408 ± 249	31.33 ± 0.19

Table 6.4 The mean (± standard error) leaf chemical concentration for cabbage plants subject to the different irrigation treatments, after 12 weeks. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA/Kruskal Wallis, P<0.05).

	Treatment													
Element	AD	PFP	HRAP	CW	NS	Water	AD*	PFP*	HRAP*	CW*	NS*	Water*	F/H	P
Calcium	0.27	0.30	0.29	0.28	0.34	0.26	0.23	0.28	0.23	0.23	0.35	0.29	H=30.06	0.0015
(%)	± 0.01^{a}	± 0.01^{a}	± 0.01^{a}	± 0.01^{a}	± 0.01^{b}	± 0.01^{a}	± 0.01^{a}	± 0.02^{a}	± 0.02^{a}	± 0.01^{a}	± 0.01^{b}	± 0.04^{a}		
Chloride	0.20	0.19	0.22	0.15	0.14	0.21	0.13	0.24	0.10	0.12	0.13	0.22	F=2.07	0.1041
(%)	± 0.04	± 0.05	± 0.02	± 0.04	± 0.03	± 0.02	± 0.03	± 0.04	± 0.01	± 0.01	± 0.01	± 0.02		
Copper	2.46	3.03	2.00	2.96	1.88	3.29	3.01	1.88	3.74	2.91	2.05	2.03	F=2.57	0.0538
(mg/kg)	± 0.48	± 0.16	± 0.71	± 0.55	± 0.42	± 0.46	± 0.16	± 0.14	± 0.89	± 0.71	± 0.46	± 0.40		
Iron	155.02	160.35	100.26	91.96	83.01	90.21	99.62	143.96	135.51	144.67	121.85	89.58	H=19.13	0.0588
(mg/kg)	± 9.10	± 17.33	± 31.68	± 19.67	± 10.21	± 9.75	± 11.49	± 20.07	± 15.93	± 15.26	± 20.56	± 9.26		
Phosphorus	0.21	0.19	0.24	0.28	0.36	0.29	0.28	0.25	0.24	0.21	0.39	0.27	H=33.29	0.0005
(%)	± 0.01^{a}	± 0.01^{a}	± 0.02^{a}	± 0.01^{a}	± 0.02^{b}	± 0.01^{a}	± 0.01^{a}	± 0.01^{a}	± 0.01^{a}	± 0.01^{a}	± 0.02^{b}	± 0.01^{a}		
Potassium	2.81	2.51	2.83	2.61	3.45	2.72	2.87	2.57	2.60	2.59	3.65	2.68	H=33.10	0.0005
(%)	± 0.02^{a}	± 0.01^{a}	± 0.10^{a}	± 0.01^{a}	± 0.09^{b}	± 0.03^{a}	± 0.04^{a}	± 0.01^{a}	± 0.01^{a}	± 0.02^{a}	± 0.11^{b}	± 0.02^{a}		
Magnesium	0.17	0.17	0.16	0.17	0.19	0.16	0.15	0.16	0.15	0.15	0.20	0.17	F=1.63	0.1891
(%)	± 0.01^{ab}	± 0.01^{ab}	± 0.01^{ab}	± 0.01^{ab}	± 0.01^{ab}	± 0.01^{a}	± 0.01^{a}	± 0.01^{ab}	± 0.01^{a}	± 0.01^{a}	± 0.01^{b}	± 0.01^{ab}		
Manganese	25.89	27.08	25.98	24.91	28.46	24.19	24.40	25.04	28.85	25.13	27.85	24.72	H=17.10	0.1049
(mg/kg)	± 0.37	± 0.73	± 0.07	± 1.56	± 0.99	± 0.57	± 0.71	± 0.79	± 2.12	± 1.03	± 1.50	± 1.32		
Sodium	6130.15	5971.81	6086.81	5829.67	5786.04	5355.56	5465.53	5555.03	5366.19	5368.12	5748.60	5013.75	F=0.85	0.5302
(mg/kg)	± 253.52^{a}	± 149.29^{a}	±227.77^{a}	± 311.30^{ab}	± 115.34^{ab}	± 101.01^{ab}	±140.24^{ab}	±125.92^{ab}	±177.03^{ab}	± 176.10^{ab}	± 173.00^{ab}	± 139.94^{b}		
Zinc	18.27	15.92	15.75	17.12	17.07	17.25	18.98	16.43	19.49	15.71	19.67	16.37	H=18.44	0.0719
(mg/kg)	± 1.55	± 0.76	± 0.81	± 0.78	± 0.11	± 0.85	± 0.06	± 0.03	± 3.15	± 0.76	± 0.13	± 0.10		

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS. Treatments marked with * were subject pH adjustment using sulphuric acid.

The Al leaf content of cabbages plants was generally higher for BE irrigated plants, with the exception of plants irrigated with the acid adjusted NS treatment (Table 6.4). The Mg leaf content of cabbage plants was not influenced by pH, and the interaction between pH regime and water source (Multifactor ANOVA, $F_{(5,24)}$=1.63, p=0.19; Table 6.4). The Mg leaf content of cabbages subject to the NS irrigation treatments was significantly higher than cabbages subject to all the other irrigation treatments (Multifactor ANOVA, $F_{(1,108)}$=5.66, p=0.0014). The pH adjustment of irrigation solutions had no influence on the Mg content of cabbage leaves treatments (Multifactor ANOVA, $F_{(1,24)}$=1.68, p=0.21).

6.3.4 Soil physical characteristics

The water potential of soils was not influenced by an interaction between pH regime and water source (Multifactor repeated measures ANOVA, $F_{(10,72)}$=0.24, p=0.99). The pH of irrigation treatments did not influence the water potential of soils (Multifactor repeated measures ANOVA $F_{(2,72)}$=1.06 p=0.35). The water potential of soils receiving HRAP and CW irrigation treatments was consistently lower than soils subject to the other irrigation treatments (Multifactor repeated measures ANOVA $F_{(10,72)}$=45.64, p<0.0001; Figure 6.6). The difference in soil water potential of soils receiving the experimental irrigation treatments became more pronounced as the soil moisture content decreased (Figure 6.6). Soils receiving AD, PFP, NS and water irrigation treatments had similar water potentials at all soil moisture contents (Figure 6.6).

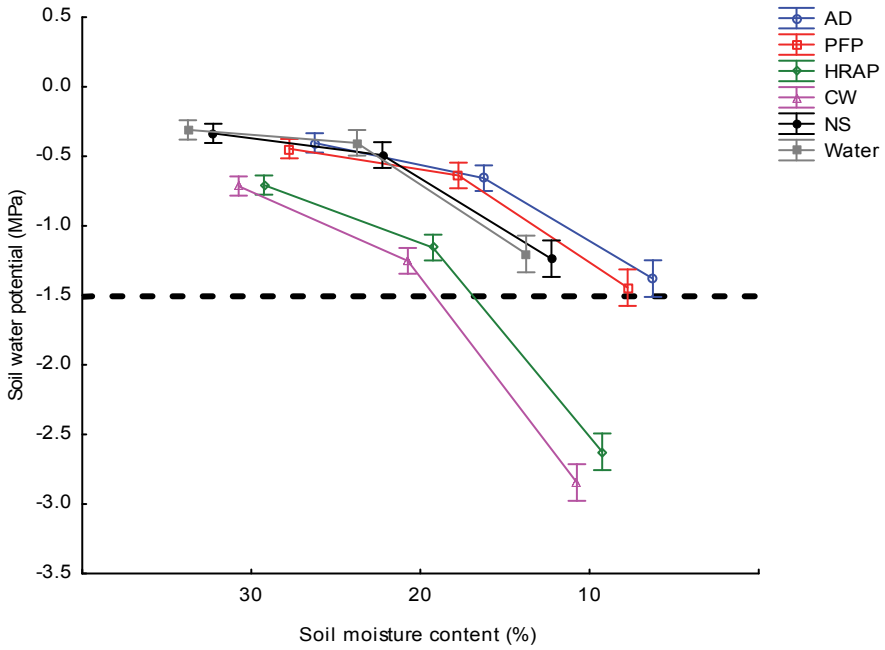

Figure 6.6 The mean (± 95% confidence interval) water potential of soil irrigated under the different irrigation treatments, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (Multifactor repeated measures ANOVA $F_{(10, 72)}$=45.64, p<0.0001). The dashed black line represents permanent wilting point, cabbages cannot access water from the soil below this line.

Mean weight diameter was not influenced by an interaction between pH regime and irrigation treatment (Multifactor ANOVA, $F_{(5,36)}$=0.65, p=0.66; Table 6.5). The pH adjustment of the irrigation treatments had no effect on the mean diameter of the soil particles (Multifactor ANOVA, $F_{(5,36)}$=0.26, p=0.61). Soils irrigated with AD, NS and water treatments had a higher mean diameter than soils irrigated with BE after PFP, HRAP and CW treatments (Multifactor ANOVA $F_{(5,36)}$=26.22, p<0.0001). The interaction between pH regime and irrigation treatment significantly influenced the infiltration rate of the soil (Multifactor ANOVA, $F_{(5,108)}$=4.10, p=0.002; Table 6.5). The infiltration rate of soils receiving AD, PFP and NS irrigation treatments was higher than soils subject to HRAP, CW and water irrigation treatments (Table 6.5). The pH of irrigation treatments did not influence the infiltration rate of the soil, with the exception of the PFP irrigation treatments (Table 6.5). There was no difference in the air filled porosity, moisture content, bulk density and compaction between soils subject the irrigation treatments (Multifactor ANOVA, p>0.05; Table 6.5).

Table 6.5 The mean (± standard error) starting and final physical characteristics of soils subject to the different irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA, P<0.05). Values from the starting column were not included in the statistical analysis.

		Treatment													
Property	Start	AD	PFP	HRAP	CW	NS	Water	AD*	PFP*	HRAP*	CW*	NS*	Water*	$F_{(5,108)}$	P
MWD (mm)	1.48 ± 0.03	1.48 ± 0.01 [a]	1.17 ± 0.04 [b]	1.21 ± 0.05 [b]	1.24 ± 0.05 [b]	1.45 ± 0.03 [a]	1.49 ± 0.05 [a]	1.45 ± 0.03 [a]	1.22 ± 0.03 [b]	1.27 ± 0.01 [b]	1.24 ± 0.03 [b]	1.50 ± 0.03 [a]	1.44 ± 0.06 [a]	1.28	0.29
Infiltration (cm/min)	2.13 ± 0.14	2.15 ± 0.26 [a]	1.95 ± 0.23 [a]	1.02 ± 0.15 [b]	0.94 ± 0.19 [b]	2.37 ± 0.26 [a]	0.47 ± 0.05 [b]	1.38 ± 0.25 [a]	1.60 ± 0.11 [a]	0.79 ± 0.10 [b]	0.84 ± 0.24 [b]	1.80 ± 0.16 [a]	0.68 0.07 [b]	4.10	0.0019
Air filled porosity (%)	13.45 ± 0.19	8.53 ± 0.24	8.31 ± 0.27	7.60 ± 0.17	8.34 ± 0.22	7.77 ± 0.48	7.70 ± 0.29	7.71 ± 0.34	7.87 ± 0.43	7.80 ± 0.39	8.13 ± 0.55	7.97 ± 0.26	7.35 ± 0.56	0.56	0.7283
Moisture content (%)	27.31 ± 0.93	27.78 ± 0.85	29.21 ± 1.20	30.82 ± 0.81	30.95 ± 1.04	28.18 ± 1.26	32.27 ± 1.18	30.96 ± 0.53	31.37 ± 1.03	29.69 ± 0.71	61.54 ± 1.03	29.52 ± 1.17	29.86 ± 1.03	2.12	0.0679
Bulk density (g/cm^3)	0.99 ± 0.01	1.06 ± 0.03	1.12 ± 0.04	1.10 ± 0.03	1.10 ± 0.04	1.10 ± 0.03	1.08 ± 0.03	1.07 ± 0.02	1.13 ± 0.03	1.05 ± 0.03	1.08 ± 0.03	1.11 ± 0.03	1.10 ± 0.04	0.31	0.9044
Compaction (kg/cm^2)	4.83 ± 0.86	14.18 ± 1.12	13.08 ± 0.91	13.27 ± 1.01	7.71 ± 0.89	17.94 ± 1.66	15.62 ± 1.01	14.24 ± 0.49	16.53 ± 1.35	11.68 ± 1.08	11.71 ± 1.12	19.49 ± 1.24	17.22 ± 0.85	0.82	0.5410

Treatments marked with * were subject to pH adjustment using sulphuric acid. Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS), mean weight diameter (MWD).

6.3.5 Soil chemical characteristics

Brewery effluent irrigation treatments increased the pH, conductivity and sodium content of the soil while NS and water treatments did not (Table 6.7, Figure 6.7 & 6.8). Soils irrigated with BE irrigation treatments had a higher pH (9.49 ± 0.07) than soils irrigated with NS or water (8.49 ± 0.06) treatments (Figure 6.7). The pH adjustment of HRAP and CW irrigation treatments decreased the soils pH when compared to unadjusted HRAP and CW irrigation treatments (Figure 6.7). The conductivity of the soil was not influenced by an interaction between pH regime and irrigation treatment (Multifactor ANOVA, $F_{(5,108)}$=2.05, p=0.08; Table 6.7). Irrigation treatments significantly affected the conductivity of the soil where soils subject to HRAP and CW irrigation treatments had the highest conductivity, followed by soils irrigated with AD and PFP BE (Multifactor ANOVA, F=131.92, p<0.0001). Soils irrigated with NS and water had the lowest conductivity with a combined mean of 1025.86 ± 50.11 µs/cm^2 (Table 6.7). Soils subject to BE irrigation treatments had significantly higher concentrations of sodium (3919 ± 94.77 mg/kg) than soils irrigated with NS or water (920.58 ± 27.46 mg/kg, Kruskal Wallis, $H_{(11,36)}$=32.62, p=0.0006; Table 6.7). After 12 weeks of irrigation, soils subject to BE irrigation treatments had a significantly higher SAR (8.18 ± 0.17) than soils irrigated with NS or water (2.20 ± 0.05, Kruskal Wallis, $H_{(11,36)}$=33.25, p=0.0005; Figure 6.8).

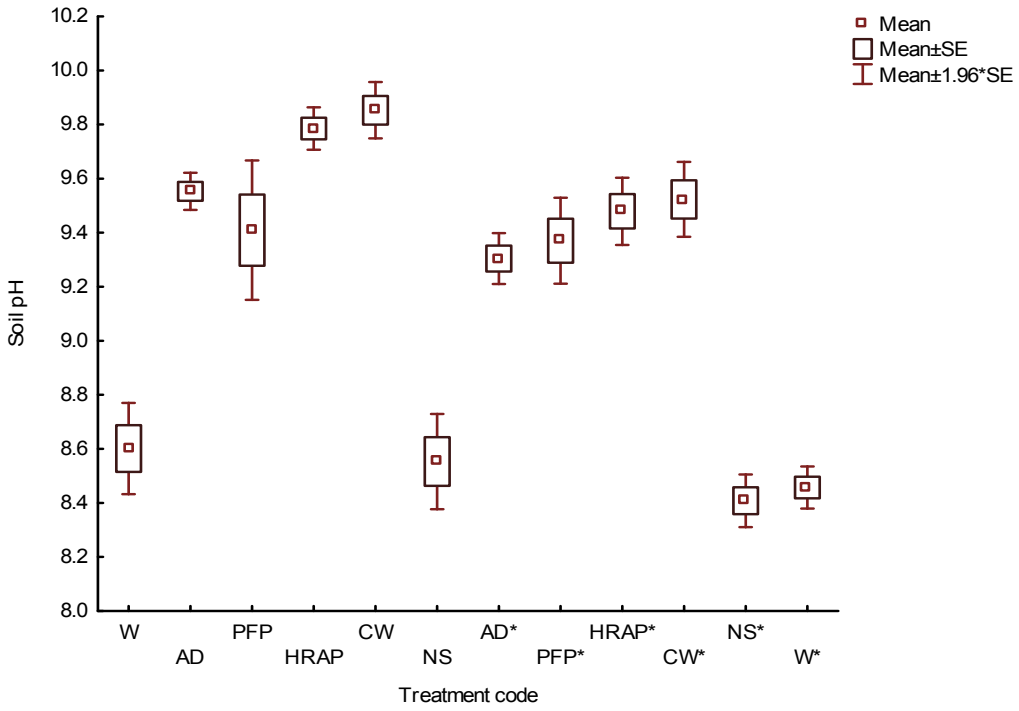

Figure 6.7 The mean pH of soil irrigated under the different irrigation treatments after 12 weeks, anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

The CEC of the soil did not change during the trial and no difference was observed between soils subject to the different irrigation treatments (Kruskal Wallis, $H_{(11,36)}$=11.74, p=0.09; Table 6.6 and 6.7). Soils irrigated with CW effluent and water unadjusted for pH had significantly higher Cl concentrations than soils irrigated with the other irrigation treatments (Kruskal Wallis, $H_{(11,36)}$=31.44, p=0.0009; Table 6.7). There was no difference in the concentration of C, Ca, Cu, Mg and Mn between soils subject to the different irrigation treatments (Multifactor ANOVA/Kruskal Wallis, p>0.05; Table 6.7). After 12 weeks of irrigation the NH_4 concentration of the soil was influenced by an interaction between pH regime and water source (Multifactor ANOVA $F_{(5,24)}$=4.10, p=0.008; Table 6.7)

Table 6.6 The mean (± standard error) chemical characteristics of the soil at the start of the experiment.

pH	Conductivity (µS/cm)	CEC (cmol(+)/kg)	Ammonia (mg(N)/kg)	Carbon (%)	Calcium (cmol(+)/kg)	Chloride (mg/kg)	Copper (mg/kg)	Magnesium (cmol(+)/kg)	Manganese (mg/kg)	Sodium (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)
8.52	840.17	13.60	0.08	0.79	56.35	574.26	1.71	13.63	29.47	946.80	13.42	444.20
±0.79	± 73.64	± 0.79	± 0.01	± 0.02	± 2.52	± 83.18	± 0.01	± 0.05	± 0.64	± 5.98	± 0.25	± 2.73

Sodium adsorption ration (SAR).

Soils irrigated with AD, PFP and NS treatments had higher ammonia concentrations than soils irrigated with water from the HRAP, CW or water treatments. The pH adjustment of AD and PFP irrigation solutions increased the ammonia concentration of soils whereas pH adjustment of HRAP, CW, NS and water treatments did not influence the ammonia concentration of the soil (Table 6.7).

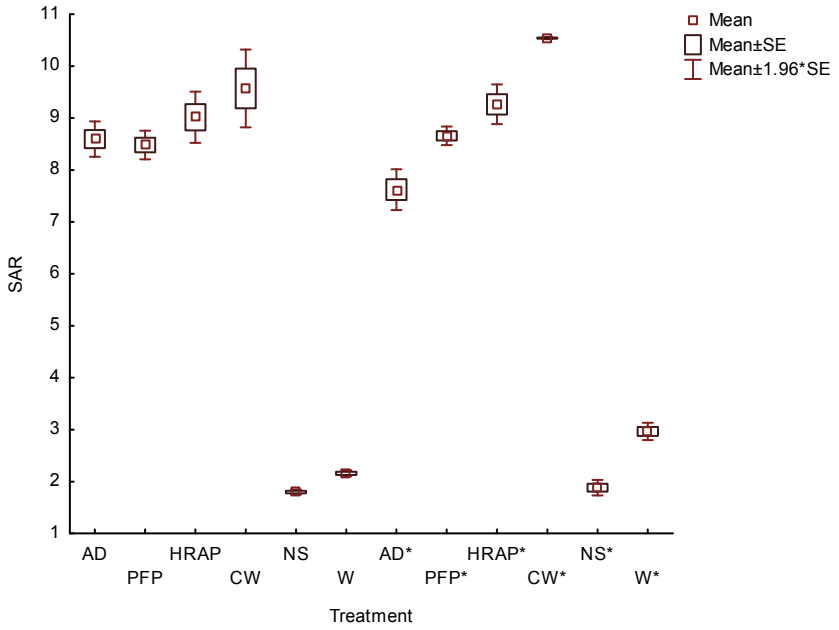

Figure 6.8 The mean sodium adsorption ratio (SAR) of soil irrigated under the different irrigation treatments after 12 weeks (Kruskal Wallis, $H_{(11,36)}$=33.25, p=0.0005). Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS) and water (W). The irrigation water of treatments marked with * were subject pH adjustment using sulphuric acid.

6.3.6 Soil biological characteristics

No significant difference was observed in carbon source utilisation of soils subject to the experimental irrigation treatments (Kruskal Wallis, p>0.05). On average soils contained 36.02% carbohydrate, 19.31% carboxylic and acetic acid, 22.11% amino acid, 18.45% polymer and 4.09% amine utilising bacteria (Figure 6.9). The interaction between pH regime and irrigation treatment had no influence on all the recorded soil biological indices (Multifactor ANOVA, P>0.05). No difference was observed for all soil biological indices recorded between pH adjusted and pH unadjusted irrigation treatments (Multifactor ANOVA, p>0.05). Therefore the data presented in Table 6.8 represents combined pH adjusted and pH unadjusted irrigation treatments. The AWCD was significantly higher for soils irrigated with AD, PFP and HRAP irrigation treatments than soil irrigated with CW and NS irrigation treatments (ANOVA $F_{(5,24)}$=11.21, p<0.0001; Table 6.8). Soils irrigated with water had significantly lower AWCD, colony forming units, Shannon Weaver index and richness compared to soils subjected to the other irrigation treatments (ANOVA, P<0.05; Table 6.8). Soils subject to AD, PFP, HRAP, CW and NS irrigation treatments had similar colony forming units, Shannon Weaver index and richness (Table 6.8).

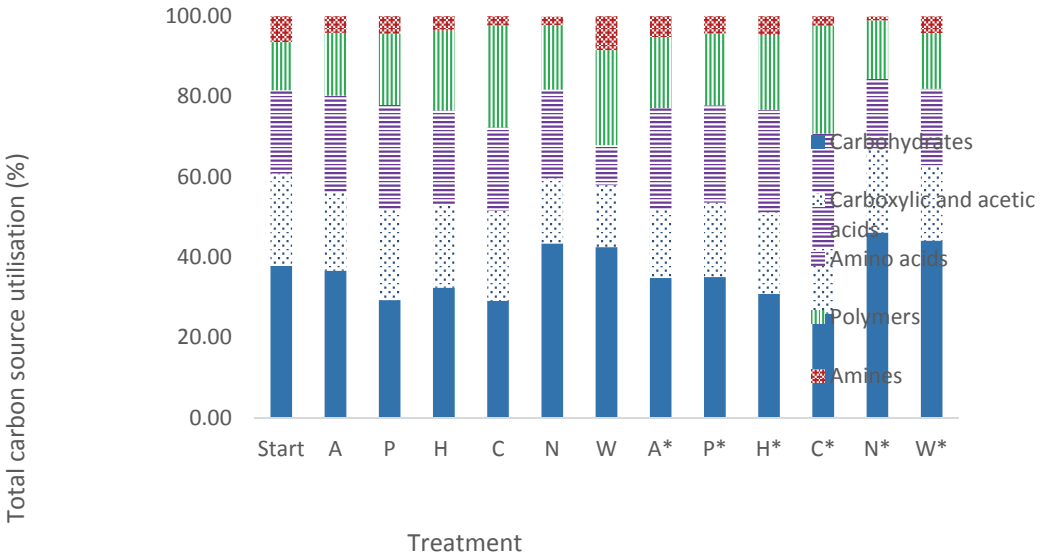

Figure 6.9 The mean carbon source utilisation (%) of soil irrigated under the different irrigation treatments after 12 weeks, treatments marked with * were subject pH adjustment using sulphuric acid. Anaerobic digestion (A), primary facultative pond (P), high rate algal pond (H), constructed wetland (C), nutrient solution (N) and water (W).

Table 6.7 The mean (± standard error) chemical characteristics of soils subject to the different irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means (Multifactor ANOVA/Kruskal Wallis, P<0.05).

Element	AD	PFP	HRAP	CW	NS	Water	AD*	PFP*	HRAP*	CW*	NS*	Water*	F/H	P
Conductivity	1484.60	1535.60	2037.00 ±	2145.40 ±	1049.20 ±	982.80	1663.40 ±	1824.40 ±	2026.60	2258.60 ±	1074.40 ±	949.30	F=2.05	0.0774
(µs/cm^2)	± 62.86^{a}	± 66.20^{a}	42.95^{b}	71.72^{b}	70.47^{c}	± 71.77^{c}	56.10^{a}	65.88^{ab}	± 46.51^{b}	85.07^{b}	31.66^{c}	± 44.98^{c}		
CEC	15.55	15.36	16.36	15.46	14.14	14.39	13.13	17.14	16.89	13.45	15.36	14.16	H=17.47	0.0948
(cmol(+)/kg)	± 2.76	± 1.23	± 2.31	± 2 .50	± 1.08	± 0.47	± 0.94	± 0.89	± 1.92	± 1.12	± 0.79	± 1.02		
Ammonia	9.38	7.67	5.24	4.94	12.71	5.10	14.38	12.27	5.37	6.61	14.05	4.33	F=4.10	0.0079
(mg(N)/kg)	± 1.33^{a}	± 0.52^{ab}	± 0.75^{b}	± 0.52^{b}	± 1.34^{a}	± 0.28^{b}	± 0.50^{a}	± 0.60^{a}	± 0.44^{b}	± 0.11^{b}	± 0.28^{a}	± 0.54^{b}		
Carbon	0.72	0.71	0.68	0.67	0.71	0.69	0.71	0.70	0.76	0.69	0.70	0.74	F=1.36	0.2758
(%)	± 0.01	± 0.02	± 0.01	± 0.02	± 0.02	± 0.02	± 0.02	± 0.02	± 0.01	± 0.02	± 0.03	± 0.03		
Calcium	61.40	60.82	61.50	65.65	63.72	60.05	63.90	59.07	61.47	61.75	61.18	67.58	H=15.33	0.1679
(cmol(+)/kg)	± 1.05	± 0.44	± 2.13	± 2.89	± 1.34	± 1.13	± 2.37	± 0.70	± 0.37	± 1.59	± 3.29	± 3.71		
Chloride	315.23	205.71	180.15	447.94	176.55	362.60	139.98	304.27	261.70	324.90	192.32	325.00	H=31.44	0.0009
(mg/kg)	± 11.60^{a}	± 57.36^{b}	± 10.61^{b}	± 9.96^{c}	± 10.38^{b}	± 27.19^{c}	± 15.54^{b}	± 9.46^{a}	± 14.35^{a}	± 10.54^{a}	± 21.64^{b}	± 9.13^{a}		
Copper	1.59	1.56	1.54	1.52	1.60	1.56	1.57	1.53	1.56	1.55	1.52	1.53	F=1.43	0.2497
(mg/kg)	± 0.02	± 0.02	± 0.02	± 0.02	± 0.01	± 0.02	± 0.03	± 0.03	± 0.02	± 0.02	± 0.02	± 0.00		
Magnesium	12.59	12.47	12.61	12.48	13.11	12.82	12.64	12.59	12.78	12.75	12.93	13.78	F=0.53	0.7462
(cmol(+)/kg)	± 0.04	± 0.08	± 0.17	± 0.08	± 0.18	± 0.11	± 0.16	± 0.05	± 0.13	± 0.14	± 0.06	± 0.19		
Manganese	37.18	35.17	35.52	37.77	39.79	37.19	40.05	39.67	41.63	39.40	41.75	35.86	H=16.61	0.1200
(mg/kg)	± 1.02	± 0.40	± 0.40	± 0.58	± 0.41	± 0.16	± 0.35	± 0.63	± 0.40	± 0.92	± 0.53	± 0.18		
Sodium	3797.67	3712.67	3967.00	4334.67	813.00	947.67	3423.00	3764.33 ±	4099.67	4675.00 ±	853.67	1374.00	H=32.62	0.0006
(mg/Kg)	±102.89^{a}	± 72.12^{a}	±166.00^{a}	±107.64^{a}	± 4.93^{b}	± 12.47^{b}	±140.53^{a}	56.70^{a}	± 79.39^{a}	46.52^{a}	± 21.18^{b}	± 71.24^{b}		
Phosphorus	24.00	28.00	25.33	40.67	79.33	25.67	34.33	32.67	35.33	38.67	83.33	16.67	H=34.02	0.0004
(mg/kg)	± 3.51^{a}	± 2.87^{a}	± 3.33^{a}	± 3.88^{a}	± 5.92^{b}	± 2.33^{a}	± 3.33^{a}	± 4.33^{a}	± 4.33^{a}	± 3.33^{a}	± 8.25^{b}	± 2.33^{a}		
Potassium	411.67	410.33	435.00	455.00	725.33	437.33	411.67	418.67	441.33	442.33	811.00	427.33	H=32.91	0.0005
(mg/kg)	± 1.76^{a}	± 0.88^{a}	± 2.52^{a}	± 1.53^{a}	± 1.76^{b}	± 3.38^{a}	± 2.73^{a}	± 1.76^{a}	± 5.70^{a}	± 2.91^{a}	± 12.66^{b}	± 3.38^{a}		

Treatments marked with * were subject to pH adjustment using sulphuric acid. Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS).

Table 6.8 The mean (± standard error) biological characteristics of soils subject to the different irrigation treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means (ANOVA, P<0.05). Values in the start column were not included in the statistical analysis.

	Treatment								
	Start	AD	PFP	HRAP	CW	NS	Water	F	P
Average well colour development	1.74 ± 0.22	1.61 ± 0.08 ^{a}	1.81 ± 0.19 ^{a}	1.75 ± 0.15 ^{a}	1.21 ± 0.21 ^{b}	1.08 ± 0.22 ^{b}	0.38 ± 0.09 ^{c}	11.21	0.0001
Shannon weaver index	3.97 ± 0.34	3.85 ± 0.24 ^{a}	3.65 ± 0.11 ^{a}	3.82 ± 0.25 ^{a}	2.94 ± 0.36 ^{a}	3.75 ± 0.08 ^{a}	2.37 ± 0.50 ^{b}	5.72	0.0013
Richness	57.33 ± 3.17	56.33 ± 2.08 ^{a}	53.50 ± 2.81 ^{a}	55.50 ± 2.28 ^{a}	27.67 ± 7.00^{b}	50.67 ± 3.56 ^{a}	18.33 ± 5.42 ^{b}	16.87	0.0001
Colony forming units/ gram soil	$7.94 \times 10^{5} \pm 2.15 \times 10^{4}$	$7.78 \times 10^{5} \pm 2.73 \times 10^{4}$ ^{a}	$8.17 \times 10^{5} \pm 2.94 \times 10^{4}$ ^{a}	$5.83 \times 10^{5} \pm 2.77 \times 10^{4}$ ^{a}	$5.22 \times 10^{5} \pm 2.64 \times 10^{4}$ ^{a}	$4.98 \times 10^{5} \pm 5.11 \times 10^{4}$ ^{a}	$2.95 \times 10^{5} \pm 2.31 \times 10^{4}$ ^{b}	16.35	0.0001

Anaerobic digestion (AD), primary facultative pond (PFP), high rate algal pond (HRAP), constructed wetland (CW), nutrient solution (NS).

6.3.7 Visual indicators

Cabbage plants irrigated with NS, AD and PFP treatments had green and healthy leaves (Figure 6.10). Cabbage plants subject to HRAP, CW and water irrigation treatments showed signs of nutrient deficiency with their outer leaves becoming purple and orange in colour (Figure 6.11). After 12 weeks of irrigation soils subject to AD, PFP, HRAP, CW and water irrigation treatments had surface cracking. Only soils subject to the NS irrigation treatments had little to no surface cracking. Four and three out of the ten replicates irrigated with HRAP and CW effluent, respectively, had a visual build-up of sodium on the surface of the soil.

Figure 6.10 Cabbage plants ten weeks after planting. From left to right the cabbages were irrigated with nutrient solution, anaerobically digested effluent and primary facultative pond effluent.

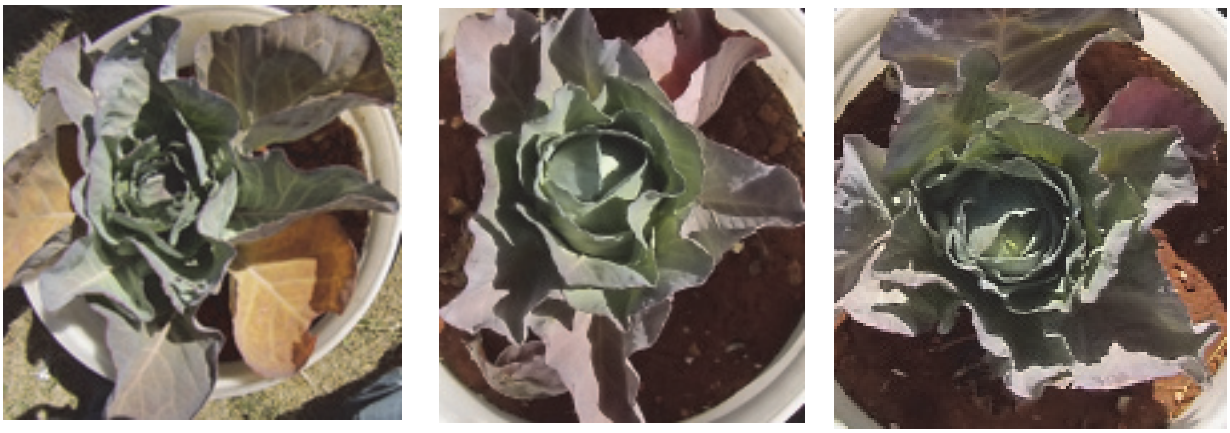

Figure 6.11 Cabbage plants ten weeks after planting. From left to right the cabbages were irrigated with high rate algal pond effluent, constructed wetland effluent and water-only.

6.4 Discussion

6.4.1 Plant growth and health

Each of the experimental irrigation treatments contained different concentrations of plant nutrients (Table 6.2), which should affect the growth and health of plants they are used to irrigate. Cabbages

irrigated with NS, AD and PFP irrigation treatments were significantly bigger than plants irrigated with HRAP, CW and water irrigation treatments (Figure 6.3). In order to sustain vigorous and healthy growth plants require sufficient quantities of macro and micro nutrients (Marschner 1990, Epstein & Bloom 2005). The high rate algal pond and CW treatment processes utilise plants and algae which decrease the amount of nutrients in the effluent as it is utilised to support plant growth. This is probably the main reason why plants irrigated with HRAP and CW irrigation treatment water were significantly smaller than plants irrigated with NS, AD and PFP irrigation treatments. To further support this conclusion, plants subject to HRAP and CW irrigation treatments showed signs of nutrient deficiency. Their outer leaves were yellow-orange and/or dark red purple in colour, which is known as chlorosis and necrosis, and is a sign of nitrogen, phosphorus and potassium deficiency (Figure 6.11; Epstein & Bloom 2005). Effluent treatment processes that remove plant nutrients are counterproductive when using effluents as an irrigation source for plants because they remove valuable nutrients that are needed to support plant growth.

Brewery effluent is not an ideal plant nutrient solution and has certain characteristics that could inhibit plant growth. Anaerobically digested and post-PFP BE contained slightly higher concentrations of nitrogen and phosphorus than the NS (Table 6.2). However cabbages that were irrigated with BE subject to AD and PFP were smaller than cabbages irrigated with NS (Figure 6.3), but showed no signs of nutrient deficiency (Figure 6.10). Therefore certain characteristics of BE either inhibit the uptake of nutrients by cabbages or put stress on the plants resulting in less energy being spent on growth. It has previously been identified that the high conductivity, sodicity and pH in BE may decrease the growth and health of plants (Ajmal & Khan 1984, Juwarkar & Dutta 1990, Sweeney & Graetz 1991, Sukanya & Meli 2004, Senthilraja *et al.* 2013, Power 2014).

The pH of nutrient solution plays a major role in the availability of macro and micro nutrients to plants, with the optimal range for most plants being between five and seven (Lucas & Davies 1961, Epstein & Bloom 2005, Power & Jones 2015). The unadjusted BE irrigation treatments had pH values around 8.14 with HRAP irrigation treatment having a mean pH of 9.17 (Figure 6.2). Surprisingly no difference was observed in the growth, CCI and chemical composition of cabbages subject to BE irrigation treatments with or without pH adjustment. The pH range for good quality irrigation water is between 6.5 and 7.5 (Epstein & Bloom 2005). High pH values above 8.5 can cause the precipitation of Fe^{2+}, Mn^{2+}, PO_4, Ca^{2+} and Mn^{2+} to insoluble and unavailable salts (Tyson *et al.* 2007, Bauder & Brock 2001). However there was no difference in the growth, CCI or chemical composition of cabbages treated with pH unadjusted HRAP effluent (pH 9.17) and pH adjusted HRAP effluent (pH 6.5). Soils have the ability of resist pH change, which is known as their buffering capacity (Buckman & Brady 1967). The buffering capacity could have counteracted the pH adjustment of BE.

The salinity of irrigation water is one of the concerns when using effluents as irrigation waters since salinity causes reduced growth and yield of most crops (Shannon & Grieve 1999, Muyen *et al.* 2011). The mean EC of BE was 3301.85 $\mu s/cm^2$ (Figure 6.1), which should reduce cabbage crops yields by 10-20% (DWAF 1996). The mean mass of cabbages irrigated with AD and PFP effluents was 13% lower than cabbages irrigated with NS irrigation treatments. The high EC probably of AD and PFP irrigation treatments probably caused the reduced yield of cabbages when compared to NS irrigated cabbages because AD and PFP irrigation treatments contained higher concentrations of N and P than the NS irrigation treatment. Medium salinity levels in irrigation water (2000-3000 µs/cm) causes a decrease in yield in most crops (Shannon & Grieve 1999, DWAF 1996). This is primarily due to the osmotic effects by decreasing the osmotic potential between the root plasma and soil water (Munns & Termaat 1986, Jacoby 1994). This means that plants have to spend more energy to take up water from the soil, which increases respiration and has negative effects on growth (Munns & Termaat 1986, Jacoby 1994). The severity of the crop response salinity is species specific and is also mediated

by environmental factors such as humidity, temperature, wind, light and air pollution (Shannon *et al.* 1994). It is important to select salt tolerant crops when using effluents that contain moderate and higher salinities as they will be less affected.

6.4.2 Soil fertility

Over the course of the experiment the soil level in each pot dropped. The bulk density and compaction of soil in each pot increased from 1.01 g/cm^3 and 4.83 kg/cm2 to 1.08 g/cm^3 and 14.39 kg/cm^2 respectively. While the air filled porosity and infiltration rate of the soil in each pot decreased from 13.45% and 2.13 cm/min to 7.92% and 1.33 cm/min respectively. This was because the soil in the pots had not a settled soil and was not in a stable state and therefore it compacted over time especially, when irrigated because water weakens the bonds holding the soil aggregates together, causing them to compact (Van & Hill 1995). However, the increase in soil compaction and decrease in soil porosity was similar for all treatments.

Soil water potential quantifies the tendency of water to move from one area to another area and is mainly affected by the concentration of salts in the soil (Bauder & Brock 2001, Tuller *et al.* 2003). It gives provides a measure of how easily soil water will move into the root of a plant. Soils subject to irrigation with HRAP and CW more had significantly reduced water potentials at all soil moisture contents than soils subject to the other irrigation treatments. As the salinity of the irrigation water and/or soil increases, the water potential will decrease (Barbour *et al.* 1998; Bauder & Brock 2001). This means that plants have to spend more energy to get water from the soil which will in turn compromise the growth of the plant (Barbour *et al.* 1998, Bauder & Brock 2001). The high salinity of BE probably increased the energy that plants invested in obtaining water, and this is a possible cause for the decreased growth of plants in these treatments. Most plants (including cabbages) cannot access water in the soil when the water potential decreases below -1.5 MPa; this is the dashed black line in Figure 6.6 (Lambers *et al.* 2008, Chapin *et al.* 2011). This means that in soils irrigated with HRAP and CW treated BE, plants could not access water in the soil when the gravimetric soil moisture content dropped below 15% (Figure 6.6). With the other irrigation treatments, plants could not access soil water when the gravimetric soil moisture content dropped below 10%. The negative affect of saline irrigation waters on the availability of water to plants demonstrated that the treatment process that results in BE having the lowest salinity would be the most suitable for crop irrigation.

The salinity of the various irrigation treatments had an effect on the salinity and SAR of the soils which, in turn could affect the physical characteristics of the soil. At the beginning of the trial the soils had an SAR of 2.21 ± 0.05. After 12 weeks of irrigation, the soil SAR subject to BE irrigation treatments rose to 8.18 ± 0.17 while the SAR of soils subject NS and water irrigation remained the same throughout the trial. In most studies conducted on the use of wastewaters as an irrigation water source the SAR of the receiving soil has increased (Ajmal & Khan 1984, Kaushik *et al.* 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). Dakoure *et al.* (2013) irrigated eggplants grown on ferralsol soil with BE that had been treated using stabilisation ponds. After two seasons of irrigation (2006-2008) they found that the effluent caused an increase in the SAR and ESP of the soil accompanied by a strong degradation of hydro structural soil properties. Soil irrigated with effluent had a decreased soil structural porosity, an increased bulk density and pH when compared to soils irrigated with tap water (Dakoure *et al.* 2013). During this study the increase in SAR of the soil did not seem to negatively affect the physical structure of the soil with the exception of the stability of soil aggregates which was slightly lower than soils irrigated the NS irrigation treatment. However this trial was only run for 12 weeks and after prolonged irrigation of BE on increase in the SAR of the soil accompanied by a decrease in the soils physical structure would be expected. This emphasises

the point that the BE treatment which results in the lowest sodium, chloride and salinity concentration will be most suitable for irrigation.

There were signs of surface cracking in most of the soils in the experiment. The only treatment which did not show signs of surface cracking were soils irrigated with NS. Soils irrigated with BE or municipal water all had surface cracking. Irrigation water containing a high concentration of sodium (BE irrigation treatments) can cause extreme soil particle flocculation which in turn can cause surface cracking (Miller & Donahue 1995, Buckman & Brady 1967). Hanson *et al.* (1999) found that irrigation water with a conductivity less than 500 $\mu S/cm^2$ caused soil aggregate dispersion. The low EC of the water irrigation treatments dispersed soil aggregates causing the soil surface to clot and crack (Hanson *et al.* 1999). Whereas the moderate EC (1000-2000 $\mu S/cm^2$) of the NS irrigation water caused soil particle flocculation as the cations in the water help bind the micro aggregates together (Hanson *et al.* 1999).

A similar trend was observed with the infiltration rates of the soil, with soils subject to HRAP, CW and water treatments developing reduced infiltration rates. These irrigation treatments either had the highest sodium content (HRAP and CW) or the lowest conductivity (water). A high sodium content in irrigation causes extreme flocculation, resulting in the formation of a soil crust and decreased infiltration rates while the low conductivity (< 500 $\mu S/cm^2$) of the water irrigation treatments caused soil particle dispersion resulting in a decreased soil structure and infiltration rates (Miller & Donahue 1995, Hanson *et al.* 1999, Bauder & Brock 2001). A decrease in infiltration rates is normally associated with a decrease in porosity of the soil (Agassi *et al.* 1981, Abu Sharar *et al.* 1987, Hanson *et al.* 1999, Muyen *et al.* 2011). However this was not observed in this trial because the porosity of soils subject to all irrigation treatments were similar. This could be due to the method used to calculate porosity because the wetting cycles used to determine porosity could have caused the soil to compact and any differences in porosity would have been undetectable. In future studies, methods that do not require wetting the soil to determine air filled porosity of the soil are recommended.

To sustainably use effluents as an irrigation source the build-up of elements and molecules in the soil needs to a kept to a minimum. There was no increase or difference in the concentration of carbon, calcium, copper, magnesium, manganese, and zinc between soils receiving the experimental irrigation treatments or when comparing beginning and end concentrations. The application of distillery effluent and wastewater to soils did not increase the levels of elements in the soils to toxic levels and could be used in irrigated agriculture (Kaushik *et al.* 2005, Hati *et al.* 2007, Kiziloglu *et al.* 2007). This supports the idea that post-AD or post-PFP BE can successfully be used as an irrigation source.

Brewery effluent does have a relatively high conductivity, sodium and chloride content which could potentially build up in the soil. Soils subjected to BE irrigation treatments had in an increase in the sodium, chloride and conductivity. Most studies that investigated the use of industrial effluents as an irrigation source found that they increased the sodium, chloride and conductivity of the soil after six months of irrigation (Ajmal & Khan 1984, Kaushik *et al.* 2005, Kumar *et al.* 2010, Kumar & Chopra 2012, Dakoure *et al.* 2013). With continued irrigation the build-up of sodium and chloride will have negative effects on the physical structure of the soil (described above) and will result in osmotic stress on the plants thus compromising their growth and yield. Therefore it is important to use effluents with the lowest possible conductivity and to irrigate salt tolerant crops or crops that are able to remove sodium and chloride from the soil and water.

It is important to understand whether the application of BE onto soils will affect the community of microbes in the soil and thus the functions they provide (Black 1968, Abbot & Murphy 2007). No

significant difference was observed in the carbon source utilisation of soils subject to the various irrigation treatments (Figure 6.9). Soils were dominated by carbohydrate (36. 03%) utilising bacteria followed by amino acid (22.11%), carboxylic and acetic acid (19.31%), and polymer (18.45%) utilising bacteria. The literature shows both detrimental and enhancing effects of effluent irrigation on soil microbial population and communities, illustrating the complexity of relationships among soil microbial communities in agricultural soils (Sinsabaugh *et al.* 2004, Sinsabaugh 2010). From this study it can be concluded that the application of BE to agricultural soils does not affect the overall functioning and processes performed by the soil microbe community, in the short term. It may have changed the species composition of soil microbes but the overall metabolic community structure of microbes present was not affected. Future studies should investigate the changes in species composition of soil microbes using metagenomics and conduct the study over a longer timescale.

Soils subject to BE and NS irrigation treatments had significantly higher colony forming units per gram of soil than soils irrigated with tap water. To add to this the AWCD of the Biolog plates inoculated with soil subjected BE irrigation treatments was significantly higher those inoculated with soil irrigated with water. The same results were observed when looking at the diversity and richness of the Biolog plates. In previous studies the application of treated effluents onto soil had no effect or increased the microbial population in the soil (Kannan & Oblisami 1990, Saqqar *et al.* 1997, Hati *et al.* 2007, Senthilraja *et al.* 2013). These authors concluded that the increase in microbial populations could have been due to the increase in soil carbon. However, Saqqar *et al.* (1997) stated that the increase in microbial populations could be due to the addition of microbes present in the wastewater. Juwarkar & Dutta (1990) and Kaushik *et al.* (2005) observed a 50% reduction in soil microbial populations treated with raw distillery effluent. The reduction in soil microbes in tap water irrigated treatments could have been due to two factors: firstly, tap water is chlorinated and has been treated to kill microbes, therefore its application to soil should decrease soil microbe populations if residual hypochlorite ions were still at an effective level, and the pH range was between 7.0 and 7.6, which the hypochlorite ion requires in order to be active as an oxidising agent. Secondly, the tap water contained very little to no carbon, which means that no energy source was supplied to the microbes from the water, which would have resulted in a decrease in microbe numbers. The underlying cause however is not clear. Soils subject to NS irrigation treatments had lower soil microbe numbers that soils irrigated with BE treatments, however the difference was not significant. In this case, the NS solution provided a high level of nitrogen and phosphate. If organic carbon was present in the soil already, then carbon to nitrogen ratio and cognitive phosphate ratio could be met, which would result in a high level of microbial activity taking place in the soil.

In conclusion, the application of BE had no effect on the soil microbial populations in terms of numbers and metabolic diversity. However, the prolonged use of BE will result in a build-up of salt in the soil, which may have negative effects on soil microbial populations, as observed by (Condom *et al.* 1999, Dakoure *et al.* 2013), as well as shift in diversity to more salt tolerant species (Nelson *et al.* 1996, Pankhurst *et al.* 2001).

6.5 Conclusion

Brewery effluent can be used as an irrigation water source for cabbage production and contains sufficient nutrients to improve crop growth, since cabbages irrigated with BE grew significantly larger than those irrigated with water-only. Post-AD or post-PFP BE is the most suitable for cabbage crop irrigation because it contains the highest concentration of plant nutrients and the lowest conductivity. However BE is an inferior irrigation water source when compared to a commercial irrigation water source with added inorganic fertiliser, and the pH adjustment of BE did not improve

plant growth or the biological activity, chemical and physical fertility of the soil. Post-HRAP and CW BE were the least suitable for cabbage crop irrigation due to the lowest concentration of nutrients and the highest concentration of salts. The sodium and chloride concentrations, and overall salinity (conductivity) are the biggest concerns when using BE because the combination results in an increase in the SAR and conductivity of the soil, which puts osmotic stress on the plants, resulting in reduced growth. The application of post-AD and post-PFP BE did not significantly decrease the biological and physical factors of the soil. However after prolonged use it may negatively affect the soil's physical structure and reduce the soil's biological activity due to the sodium and chloride present in the effluent. Future studies should investigate the long term effects of irrigating soils with post-AD or post-PFP BE and the development of technology to combat it.

6.6 References

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7. Conclusions and recommendations

The drop in nutrient concentration in the high rate algal ponds used to treat brewery effluent was due to a combination of mechanisms that include algal assimilation, bacterial nitrification, ammonia volatilization and the abiotic nitrogen and phosphorus cycles. The microbial species in the ponds were characterised under various environmental conditions such as different flow rates (i.e. hydraulic retention times) and different times of year, and the corresponding mechanisms responsible for the changes in nutrient concentration were investigated under different environmental conditions too. It is the shift in population structure and corresponding mechanism of nutrient removal and dynamic nature of these communities and their ability to change rapidly that makes these systems so adaptable and able to treat dynamic effluent streams.

The benefits of fully understanding the mechanisms of nutrient removal from HRAP ponds used to clean brewery effluent and further developing this nutrient and water resource for downstream reuse could contribute to cost-reductions at breweries and other similar water users. It is also likely to result in more efficient water, nutrient and energy management, and the creation of downstream job opportunities with the potential of improving food security in local communities. This research continues to contribute towards social, economic and environmentally sustainable water management practices.

7.1 Recommendations for application of this technology in agriculture

The work presented here has, again, demonstrated the value of alternative effluent treatment technologies and that they can result in alternative downstream activities such as hydroponic vegetable production or conventional crop irrigation. Tomato plants grown in treated brewery effluent did not grow as well as those grown using inorganic hydroponic solution, but the manipulation of the pH of treated effluent resulted in a significant improvement in growth. Furthermore, the production of cabbages grown in the soil and irrigated with treated brewery effluent was significantly greater than those irrigated with a conventional water source. In both hydroponic tomatoes production and the irrigation of cabbage crops grown in the soil, the use of brewery effluent as a nutrient source was inferior to the use inorganic fertilizes; however, the compromised growth rates need to be considered in relation to the added value associated with the potential of producing an organic product.

Millions of litres of organic effluent are discharged to municipal sewers every day in South Africa and around the world. The technology that we are developing here and the results of this research demonstrate the potential of recovering the nutrients and water in this effluent for downstream use in agriculture.

7.2 Recommendations for the brewery to consider

There are challenges that need to be addressed regarding minimizing nutrient pollution and finding a solution for managing the alkalinity of the effluent that comes from the anaerobic digester. The alkalinity of the brewery wastewater remains one of the key concerns moving forward if it is to be used as nutrient supply for hydroponics, and this requires further investigation in the future. The alkalinity is less of a concern in conventional crop irrigation since the soil tends to act as a pH buffer. However, the potential build-up of salts in the soil, when using treated brewery effluent as a water and nutrient source in crop production, is another challenge that the breweries will face if the treated brewery effluent is to be used to irrigate crops.

Altering the up-stream management practices of the anaerobic digester and cleaning practices in the brewery could potentially address both the alkalinity and the problem associated with salts in the effluent. For example, alternative detergents could be used in the brewery, or effluent streams could be split, and alternative methods of pH control could be used in the anaerobic digester. These approaches should be considered in the design of future breweries and new management practices if brewery effluent is going to be made available for downstream use in agriculture in the future.

7.3 Recommendations for future research

While this work has contributed to our understanding of the shifts in community structure and associated mechanisms, it has also identified a gap in our knowledge: The majority of the organisms in high rate algal ponds (HRAP) under certain conditions remain "unknown" (in some instances about 50% of the bacteria and close to 75% of the eukaryotes were "unknown") and for us to fully understand the mechanisms responsible for nutrient dynamics in these ponds these organisms need to be characterised. This must be done in future research.

There is potential for using fish to remove algae from HRAP pond effluent. However, we have not reached the point where we can make recommendations to apply this research; further research is required. This future research needs to focus on developing methods/technologies aimed at mitigating the negative effects that HRAP water chemistry has on fish physiology; for example, adjusting pond effluent chemistry prior to exposing fish to the water or possibly using alternative fish species that are better adapted to withstand the extreme environmental conditions of HRAP effluent. There are species of fish that are morphologically more adapted to remove algae that have settled out of suspension; either way, future work should also focus on making sure that fish have greater access to algae by increasing the portion of the algal biomass that forms a biofloc that is either in suspension or settled. This work also needs to be designed to ensure that filtration rates of the fish can be adequately estimated, taking algal productivity and the effect that algal settlement and microalgal grazers have on algal biomass into account.

Similarly, more research into the use of duckweed in nutrient removal from brewery effluent is needed before practical recommendations can be made. The rate of nutrient removal by duckweed is highly dependent on temperature and it was interesting to observe that phosphate removal by duckweed is probably more consistent than phosphate removal by algae; however, the experiments here were not designed to make direct comparisons between algal and duckweed systems, but this could be looked at in future work. Duckweed was successfully used as a fish feed supplement and it was substantially easier to harvest the duckweed compared with unicellular algae.

When brewery effluent was used to produce cabbages in the soil, it did not negatively influence soil character; however, we are still concerned that the long-term build-up of salts in the soil might compromise the use of this effluent as a water and nutrient source. Although the built-up salt could be addressed by changing the cleaning chemicals used upstream in the brewery, it could also be addressed by investigating the use of halophytic plants as part of the treatment process. Future work could also focus on the use of crops that are known to reduce the build-up of salt in the soil.

Finally, brewery effluent can be used as a water and nutrient source in hydroponic vegetable production, with the added advantage of a hydroponic product that has the potential of organic certification. However, the growth of hydroponic crops in brewery effluent is inferior to that of hydroponic crops grown using inorganic fertilisers. Future research should be carried out to: (a) identify alterative crops that might be better suited to brewery effluent as a nutrient source; and (b) optimise the use of brewery effluent as a potential organic nutrient source in hydroponic crop production.