OPTIMIZING THE REMOVAL OF ALGAE FROM INDUSTRIAL EFFLUENT TREATMENT PONDS USING FISH

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Executive summary

High rate algal ponds (HRAP) are an alternative, environmentally sustainable method of effluent treatment. However, the removal of the microbial biomass that is suspended in the treated effluent remains a bottleneck in the use of this technology, particularly if the recovered water is intended for reuse or release into a natural water body. The "harvest" of microalgae from post-HRAP using filter-feeding fish, where the fish passively consume the algal biomass and essentially converting algae into fish biomass, has been previously demonstrated using Mozambique tilapia. However, HRAP systems used to treat brewery effluent are characterized by extreme water conditions such as oxygen and pH fluctuations and high alkalinity, all of which can compromise fish health. Furthermore, it has not been established if tilapia is the most suitable species to perform this function and whether it is possible to mitigate the extreme conditions in the HRAP effluent making it more suitable for fish culture and thus potentially improving the rates of algal removal by the fish.

Oxygen levels increase to supersaturation during the day in the HRAP, while pH increases from around 8 to 11, due to photosynthesis. Fish generally require pH conditions ranging between 6.5 and 9.0, so the upper pH levels of HRAP water are problematic for fish production. This is worsened if ammonia is present in the effluent as a greater portion of total ammonia exists as toxic unionized ammonia at elevated pH. In this regard, the control of HRAP pH effluent could increase the removal of microalgae by fish.

The overall aim of this project was to optimize the removal of microalgae from post-HRAP effluent treatment systems using fish that can filter-feed and to describe a process that best achieves algal removal. To achieve this end, the following was investigated:

1) which fishes are most suited to remove microalgae from HRAP;
2) what moderations need to be made to the effluent to make the environment more suitable for fish survival with a focus on flocculation, manipulation of pH using photosynthesis, CO_2 and acid;
3) the most suitable stocking density and duration needed to achieve a change in algal biomass; and
4) the rate at which these fishes are able to remove the algae.

Although African catfish (*Clarias gariepinus*) can filter-feed phytoplankton from the water column and can survive the extreme environmental conditions of HRAP, the data presented here did not support the hypothesis that they are suitable for removing algae from post-HRAP effluent. There were no differences in the algal biomass between treatments with and without catfish. Furthermore, the addition of the flocculant, chitosan, did not make the algae more available to the fish. As such, it was decided to focus on alternative species, including Mozambique tilapia (*Oreochromis mossambicus*) and freshwater mullet (*Pseudomyxus capensis*).

In preliminary trials, the use of CO_2 to manipulate the pH by constantly inoculating this gas into the algal tanks successfully moderated the pH of the algal tanks and maintained the pH within a range suitable for fish culture. However, the CO_2 also displaced O_2 in the water, which dropped progressively during the experiment to levels as low as 4.0 mg/L which are not suitable for fish culture. Although the tilapia did not show signs of stress during the trial, a number of fish died in the days after the trial and this was probably related to the physiological stress they were placed under due to the low oxygen concentrations.

High rate algal pond effluent was subsequently subjected to a single dose of either sulphuric acid or CO_2, and as a control, the pH of the effluent was left unadjusted in a third treatment. Each of these treatments was duplicated, with one set subjected to light conditions while the other set was covered to prevent photosynthesis from taking place. The oxygen levels in all treatments were allowed to increase prior to stocking them with fish. The use of CO_2 or acid as a single dose for pH adjustment was successful in lowering pH of HRAP effluent from about 10.0 to 6.5 at the start of the trial. In both cases pH continued to increase for the duration of the trial; however, after five days it had not increased above about pH 8.0 to 9.5. Acid resisted the high alkalinity of brewery effluent better than CO_2, as the values increased at a slower rate over the five-days of the experiment. The dark treatment exhibited lower pH values in all three pH adjustment treatments due to the decreased photosynthetic activity. However, oxygen concentration was consistently lower in the dark treatments compared with those in which the algae were able to photosynthesise. In all cases in the dark, oxygen concentration levelled off after the first day and remained constant at about 7.0 mg/l, which is suitable for fish culture. However, oxygen should be considered in studies with high densities of fish in future work as this might limit the carrying capacity of fish the tanks.

In the dark, algal removal increased significantly with an increase in tilapia stocking density from zero to 20 kg/m^3 ($y = -3.50x + 3.94$; $r^2 = 0.93$; $p = 0.0004$). Under light conditions, tilapia removed about 40% of the starting algal biomass, while in the dark about 70% of the starting algal biomass was removed from the water column.

Tilapia removed more algae from the effluent since it could be stocked at higher densities. Both tilapia (stocked at 20 kg/m^3) and mullet (stocked at 10 kg/m^3) removed algae at a significantly faster rate when the effluent pH was moderated using sulphuric acid compared with the treatment moderated with CO_2. This may be due to the lower pH that was achieved when using sulphuric acid compared to CO_2, or the initially lower DO levels when using CO_2. There was a significant increase in ammonia and nitrite concentration with time when fish were stocked into the tanks.

Based on the above experimental investigation, the algal removal process was tested under numerous conditions where fish species, fish stocking density, method of pH manipulation and the light and dark conditions were varied. A selection of these scenarios that resulted in the highest rates of algal removal are summarised in Figure 1.

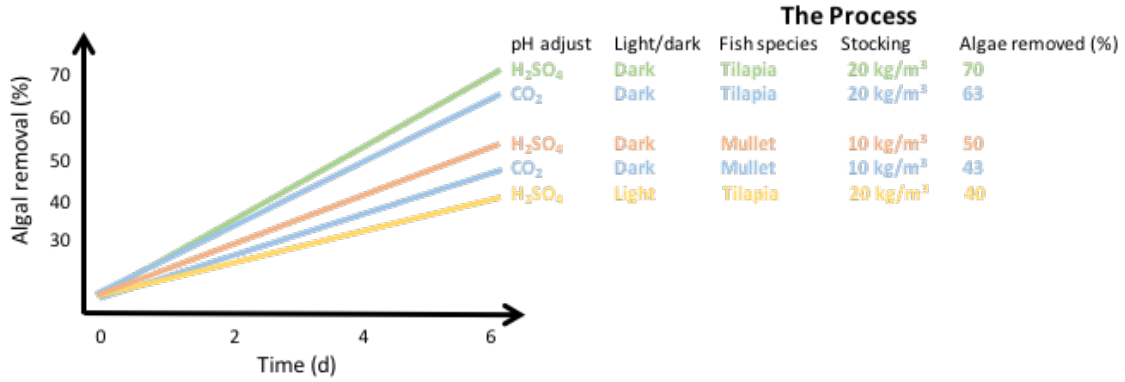

Figure 1 The rate of algal removal from high rate algal pond (HRAP) effluent subject to various processes of environmental manipulation over six days.

In conclusion, fish can be used to remove algae from post-HRAP effluent, making the treated water available for reuse. By manipulating the environment, such as covering the tanks to stop photosynthesis and algal productivity, using acid to buffer pond pH, and by selecting a species such as tilapia that can withstand the environmental fluctuations of an algal pond, and by

ensuring that oxygen in the water is not depleted, it was possible to remove a substantial portion of the algae from the pond. To achieve a 70% reduction of the starting algal biomass, over six-days, the following process provided the best result:

- post-HRAP effluent placed into stagnant pond;
- the pH of the effluent lowered to 6.5 using sulphuric acid;
- the tanks covered to eliminate light;
- fish tanks stocked with Mozambique tilapia; and
- using stocking density of 20 kg/m^3.

Based on current laboratory scale investigations, future work needs to test this process on a pilot-scale. In addition, the process described here is not limited to treatment of brewery effluent, but probably universally applicable for treatment of other similar effluent streams, and this also requires future investigation.

However, the use of fish to remove algae from HRAP effluent may be counterproductive if the fish themselves pollute the water with ammonia such that it has to be re-treated before it can be reused or released into the environment. Future research should therefore also focus on minimising the impact of deteriorating water quality when high stocking densities are used to remove algae. In this regard, the addition of zeolite filters or biological treatment or polishing in a wetland could be considered, after the algae is removed by the fish, to remove residual ammonia.

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Capacity building

The MSc student Mr Lwazi Nombembe (Figure 2A) is currently registered at Rhodes University, and is in the process of writing up his MSc using data collected as part of project. Honours students Mr Brett Johnston and Mr Mike Skeeles were officially registered to work on this project as part of their BSc Honours degrees; these students used this project for the content of their written project literature review, written project proposal and oral honours project proposals.

Mr Richard Taylor (Figure 2B, PhD student), Ms Nyiko Mabasa (Figure 2C, PhD student) and Mr Martyn de Jong (Figure 2D, MSc student), although working on different projects for their respective theses, were all employed on this project as research assistants on a part-time basis; and this project contributed to their training as professional scientists.

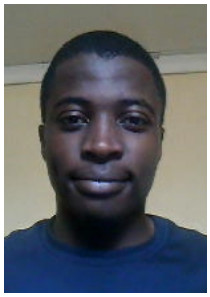
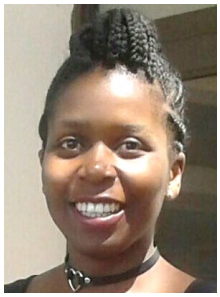
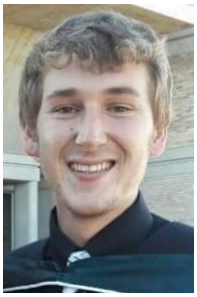
(A)  (B)  (C)  (D) 

Figure 2 The participation of students in this WRC funded project included MSc student Mr Lwazi Nombembe (A) and BSc Honours student Brett Johnstone (no photo) and Michael Skeeles (no photo); these students were officially registered as students working on this WRC project as part of their degree work. Mr Richard Taylor (B) is also a student involved in the programme (although a third-party funds his bursary and project) – he is, however, employed as a site manager for this project and was employed on this project on a part-time basis. A third party also funds PhD student Miss Nyiko Mabasa (C), but her work contributes to the overall programme. MSc student (D) Mr Martyn de Jong's thesis is not based on this WRC funded project, but he was employed to work on it as a part-time employee.

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Anaerobic digester (AD)

Analysis of variance (ANOVA)

Biological oxygen demand (BOD)

Chemical oxygen demand (COD)

Dissolved oxygen (DO)

High rate algal pond (HRAP)

Integrated algal ponding system (IAPS)

Primary facultative pond (PFP)

Treatment (T)

Total suspended solids (TSS)

Chapter 1: Introduction

Water is becoming an increasingly precious resource around the globe. As a result of various domestic, agricultural and industrial water uses, organic and inorganic waste can lead to water pollution and water quality degradation (Abdel-Raouf *et al.* 2012). The high organic loading in agro-food industry effluent represents a major threat to natural water systems (Raposo *et al.* 2010). Thus, appropriate wastewater treatment processes are becoming increasingly important around the world and have been extensively studied (Abdel-Raouf *et al.* 2012). Conventional methods of water treatment include centrifugation, in-pond chemical precipitation of suspended material, flotation and filtration (Raposo *et al.* 2010). These methods are largely limited to the removal of suspended solids from the effluent, whereas activated sludge and reverse osmosis are examples of treatment processes that are responsible for removal of dissolved materials. However, these methods are often expensive and require high technology that requires specific skills, time and space (Al-Rajhia *et al.* 2012). These hi-tech systems are increasingly being replaced by biological treatment systems because they are economically and environmentally sustainable alternatives that are, in many instances, more appropriate.

High rate algal ponding (HRAP) has gained considerable interest as an alternative water treatment process due to the *ecologically friendly* and cost-effective nature of the treatment process (Jones *et al.* 2014). It includes a dynamic, symbiotic relationship between microalgae, bacteria, fungi and other microorganisms (Jones *et al.* 2016; Mogane 2016). The mechanisms of nutrient removal in the HRAP include algal assimilation, bacterial nitrification and pH-mediated volatilization; however, the main mechanism relies on algal assimilation (Jones *et al.* 2016; Mogane 2016), where the system's photosynthetic capabilities convert solar energy and mainly inorganic carbon into useful biomass (Bosman & Hendricks 1980; Mogane 2016; Jones *et al.* 2016). The ability to turn this biomass into various products such as feed supplements or algal culture systems have been investigated (Jones *et al.* 2014 & 2016; Edmundson & Huesemann 2015), and the plausibility of converting this algal biomass into fish biomass has formed the basis of earlier studies (Shelef *et al.* 1984; Edwards 1980; Jones *et al.* 2016). Brewery effluent is rich in organic and inorganic compounds containing carbon, nitrogen and phosphorous which can lead to eutrophication and nutrient pollution if left untreated (Al-Rajhia *et al.* 2012). Therefore, it is essential that the effluent be appropriately treated to reduce its impact on the environment.

The poor water quality and high concentrations of organic matter in the form of algae in the post-HRAP water represent a major pollution risk to the environment (Jones *et al.* 2014). High concentrations of algae in water can alter the physical and chemical properties of the water body (Edmundson & Huesemann 2015). The growth of these organisms within the effluent is affected by a number of factors such as temperature, solar irradiance intensity and duration, CO_2 concentration and availability of nutrients (Simate *et al.* 2011). Post-HRAP effluent water is characterized by high algal density, high water temperatures in summer, high pH and a low biological oxygen demand (BOD). Algae in the HRAP are responsible for lowering the ammonia and phosphorous concentrations in the effluent (Jones *et al.* 2016). During photosynthesis, algal cells consume CO_2 faster than it can be replaced by bacterial respiration, which results in an increase in pH and other factors such as BOD (Abdel-Raouf *et al.* 2012). At night, the algal cells metabolize a portion of their organic carbon stores (that were synthesised during the day) and consume O_2 (that was produced during the day) and CO_2 is respired, which results in the drop of the pH and BOD in the culture medium (Abdel-Raouf *et al.* 2012). While the algae are integral in the effluent treatment process (Jones *et al.* 2016), its removal is essential as the presence of algae can influence water quality parameters such as pH and chemical oxygen demand (COD) once released into the environment, leading to the treated effluent not meeting the necessary standards for discharge into a water body.

The harvesting of algae from the post-HRAP effluent, once the nutrients have been sequestered, represents a major bottleneck in the treatment process (Jones *et al.* 2016), and forms the basis the current study. The dynamic nature of the algal community structure is responsible for the resilience of the HRAP in treating effluent streams that vary in composition, since the algal/bacterial community structure alters in response to changes in the environment and effluent (Jones *et al.* 2016; Mogane 2016). Unlike in the monoculture of larger, commercially produced unicellular algae, which can be more easily harvested from the effluent (e.g. *Spirulina* sp.), many of the naturally occurring algal cells, which proliferate in the effluent in the HRAP system, are very small (i.e. often less than 20 μm) (Jones *et al.* 2016; Mogane 2016). This makes their removal from the treated effluent stream very difficult.

The removal of algae with the use of phytoplanktivorous-fish, as an *in-situ* filter-feeder, has been studied as a possible alternative to conventional methods (Jones *et al.* 2016). However,

filter-feeding in fish is a passive process (Dempster *et al.* 1995), and the water quality parameters of the effluent, following HRAP treatment, have been found to stress the fish, reducing the fish's filter-feeding capabilities (Dempster *et al.* 1995; Jones *et al.* 2016). Physiological stress can have detrimental effects on the growth, reproduction, ability to feed and various physiological processes in fish (Moriarty 1973). For example, gastric secretion, which is necessary for the digestion of algal cell walls, can be compromised in stressed fish (Moriarty 1973), and it has been hypothesized that this might be responsible, at least in part, to reduced digestion efficiency and a reduction in the rate of algal removal in post-HRAP effluent (Jones *et al*. 2016). The fluctuating and extreme environmental conditions associated with high algae concentrations during the day are moderated when photosynthesis stops at night. During algal respiration there is an increase in CO_2 concentration in the effluent, which is inversely proportional to the change in O_2 concentration and water pH, both of which drop during the night (Kayombo *et al.* 2002). In order to increase the success of harvesting the algae from the post-HRAP effluent, the harvesting environment within the tanks need to be made more suitable for *in situ* filter-feeding fish.

Chapter 2: Literature review

2.1 Using microalgae as a biological treatment for brewery effluent

Microalgae are microscopic or single-cell aquatic organisms that live in both fresh and marine water environments (Al-Rajhia *et al.* 2012). The use of microalgae in an aquaculture system offers an important biological alternative to the predominantly chemistry-based water treatment systems or biological systems, which require high technology and advanced equipment and specialised skills to execute (Al-Rajhia *et al.* 2012). The intensive growth and harvesting of microalgae, as a method for the tertiary treatment of sewage, was first suggested and studied by Bogan *et al.* (1960). Subsequently, the removal of industrial nitrogenous waste with HRAP was developed with a multistage system that was followed by algal harvesting (Bosman & Hendricks 1980). This has the added advantage of removing nutrients such as nitrogen and phosphorous from the water and thus preventing environmental eutrophication (Al-Rajhia *et al.* 2012). Therefore, biological treatment of effluent water using microalgae has emerged as an important and successful alternative to some of the more conventional treatment methods.

Brewery effluents are rich in both organic and inorganic compounds such as phosphates, proteins, ammonia, nitrite and nitrate that need to be removed from the water before it can be discharged into the environment (Raposo *et al.* 2010; Jones *et al.* 2014). Furthermore, brewery effluent is usually high in sugars, soluble starch, ethanol and fatty acids (Raposo *et al.* 2010). Following treatment within the HRAP, the bacteria present in the microbial consortium produce CO_2 during metabolism, which is required by the microalgae for photosynthesis (Al-Rajhia *et al.* 2012). The HRAP systems lower the COD of a system due to the consumption of CO_2, but algal biomass production results in elevated BOD and pH levels (Al-Rajhia *et al.* 2012). So, although the system lowers the COD, algal assimilation also contributes to the COD and thus the algae need to be removed or harvested post-treatment.

Tertiary wastewater treatment using HRAP is achieved through the aerobic degradation of the organic matter present in the effluent (Abdel-Raouf *et al.* 2012). The concentrations of the inorganic substances that are produced (i.e. CO_2; PO_4^{3-}; NH_3) can vary according to various chemical and biochemical reactions, including algal assimilation, bacterial nitrification, NH_3 volatilization and PO_4^{3-} precipitation (Simate *et al.* 2011).

The algal biomass produced from the culture can be used as animal feed, as fertilizer to enrich soils with nutrients or they can be used to extract valuable compounds such as antioxidants or important enzymes (Abdel-Raouf *et al.* 2012). To avoid the recycling of nutrients within the water system and to recover the biomass generated from the HRAP, the harvesting and physical recovery of the algal cells represents one of the most technical and important difficulties to overcome. This has led to the investigation of applying *in situ* filter-feeding fish to remove the microalgae left in the effluent water and to convert that algal biomass into fish biomass (Jones *et al.* 2016).

2.2 The effect of HRAP on water quality parameters that might influence fish health

Once the effluent has been treated in the HRAP system, there are a number of physical and chemical parameters that could negatively affect the health of fish, and thus their ability to remove microalgae from the effluent. For example, the high pH and high ammonia (Table 1) could be detrimental to many fishes, and this concern needs to be addressed if fish are to be used to remove algae from this effluent (Jones *et al.* 2016).

High algal concentrations in post-HRAP effluent influence the BOD and pH within the system (Abdel-Raouf *et al.* 2012). The BOD is a measure of the respiratory demand of the bacteria and algae when metabolizing the organic matter within the effluent (Abdel-Raouf *et al.* 2012). This is an important parameter to consider as the BOD of the microorganisms can cause periodic hypoxic conditions within the system; this can lead to anaerobiosis and fish kills (Smith & Piedrahita 1988). These parameters need to be closely controlled in order to minimize their effect on the post-HRAP effluent, that is, after the effluent has been treated in the HRAP and when the algae are being removed from the treated effluent by the fish. This will contribute to moderating the environmental conditions of the post-HRAP effluent that are unfavourable for fish (e.g. high pH and oxygen fluctuation) and that potentially limit the suitability of post-HRAP effluent for fish culture.

Table 2.1 The temperature, pH, chemical oxygen demand (COD), ammonia, nitrate, phosphate and chloride of brewery effluent that was subject to anaerobic digestion and treatment in a post- HRAP (Jones *et al*. 2014).

Parameter	Post-HRAP effluent		
	Minimum	Maximum	Mean
Temperature (°C)	18.10	29.70	22.31
pH	6.64	10.50	9.82
COD (mg/L)	97.50	250.00	171.21
Ammonia (mg/L)	0.00	5.00	1.08
Nitrate (mg/L)	-	-	0.01
Phosphate (mg/L)	1.63	28.10	17.30
Chloride (mg/L)	-	-	417.65

Elevated pH values can negatively affect fish physiology, possibly leading to fish kills (Smith 1998). The reason for the diurnal increase in pH in the HRAP was established earlier (Abdel-Raouf *et al.* 2012); this increase is often in excess of pH 9.0 (Table 2.1). The pH of a water system relies on the dissolution of CO_2 in water to form carbonates and H^+ (Moran *et al.* 2010). This alone negatively affects fish health.

In addition, the toxicity of ammonia to fish is influenced by water pH. Unionised ammonia is highly toxic to fish and affects survival and growth (Smith 1998; El-Shafai 2004). The pH fluctuations in the HRAP are due to the photosynthetic and respiratory activity of the highly concentrated microalgae in these ponds (Tadesse et al. 2004). During the day, photosynthesis causes the uptake of carbon dioxide (CO_2) and may result in dissolved oxygen supersaturation (Tadesse et al. 2004). The CO_2 consumption by algae is met with the concomitant dissociation of bicarbonate, resulting in a hydroxyl ion production that is responsible for raising the pH of the water (Tadesse et al. 2004). As pH increases, the proportion of total ammonia that exists as unionised ammonia increases; and, as such, the increase in pH that occurs when photosynthesis takes place in HRAP ponds also increases the unionised ammonia concentration, making the effluent less suitable for fish.

The increase in dissolved oxygen during the day as a result of the photosynthetic production of oxygen is directly proportional to the increase in pH of the water, due to the increase in hydroxyl ions in the water (Wurts 2003). The reverse takes place at night, when cellular respiration results in the consumption of free oxygen and the production of CO_2, resulting in a drop in pH (Edmundson & Huesemann 2015). The pH of a water system was used by Kayombo *et al.* (2002) as a performance indicator, since a pH above 8.0 is produced by a photosynthetic rate that demands more CO_2 compared with that replaced by respiration and decomposition. At a pH above 8.0, ammonia concentrations are high, which affects the rate of photosynthesis and productivity of algae (Kayombo *et al.* 2002). The pH can be controlled through the addition of strong acids, alkalis and by flooding the system with dissolved CO_2 (Lee & Tay 1991). As the hydroxyl ions accumulate within the water medium at high pH values, the relative proportion of free ammonia and other toxic chemicals, such as nitrate accumulate in the water creating an environment that is unsuitable for filter-feeding fish (Edmundson & Huesemann 2015).

This positive correlation between the concentration of CO_2 and pH within isolated water systems has been used to improve algal productivity. It is common practice during mass cultivation of algae to supply CO_2 enriched air to prevent carbon limitation (Richmond 2013). However, the presence of high concentrations of CO_2 can result in the depression of photosynthetic activity and the growth rate of microalgae (Lee & Tay 1991). High CO_2 partial pressure in the algal pond of *Chlorella pyrenoidosa* significantly lowered the specific growth rates of the algae as well as inhibiting photosynthetic output (Lee & Tay 1991). However, controlling pond water pH with the addition of CO_2 also enhances algal production by preventing ammonia inhibition of algal growth (Park & Craggs 2010). Furthermore, Yang and Gao (2003) found that increased CO_2 concentration significantly enhanced the growth rates of three freshwater algal species: *Chlamydomonas reinhardtii, Chlorella pyrenoidosa* and *Scenedesmus obliquus*. The specific growth rates and photosynthetic ability of all these microalgae were influenced by the higher CO_2 concentrations that were associated with the lower pH (Yang & Gao 2003). The enhanced growth rate with increased CO_2 is likely to be related to lower energy consumption and a constant carbon supply (Yang & Gao 2003). This resulted in an increase in the net photosynthetic ability and light-saturation point of all three species of microalgae, but also resulted in a drop in the post HRAP effluent pH (Yang & Gao 2003; Park & Craggs 2010).

The diurnal drop in oxygen at night and the increase in pH during the day are the two most extreme environmental parameters that require further investigation or manipulation before fish can be used to remove algae from post-HRAP effluent.

2.3 Microalgae at night: characterizing night biomass loss in algal concentrations

In the presence of light, algae are known to photosynthesise using solar energy to convert CO_2 into oxygen and glucose. The growth and abundance of microalgae is thus dependent on the presence of solar irradiation (i.e. light) in order for photosynthesis to take place. This process results in algae consuming CO_2 faster than it can be produced by bacterial respiration; bacteria live in symbiosis with the algae in the HRAP system. The CO_2 deficiency that occurs during photosynthesis results in the release of bicarbonate ions from the algae (Tadesse *et al.* 2004). This bicarbonate dissociation coupled with the consumption of CO_2 by algae through photosynthesis increases the concentration of hydroxyl ions in the water column, elevating the pH (Tadesse *et al.* 2004).

At night, however, a significant fraction of daily photosynthetic productivity can be lost due to respiration (Edmundson & Huesemann 2015). During periods of no light, the algal cells respire, which results in the consumption of O_2 and the production of CO_2 (Edmundson & Huesemann 2015). The night-time pH will be expected to drop, as respiration replaces photosynthesis (Edmundson & Huesemann 2015).

The potential exists to manipulate the pH of an algal tank by manipulating the light to which the system is exposed. Thus, it might be possible to mitigate the negative effect of elevated pH in post-HRAP effluent on fish, by manipulating the light in the algal/fish tanks.

2.4 Three candidates: Mozambique tilapia, African catfish and mullet

Mozambique tilapia, *Oreochromis mossambicus,* is a hardy species found throughout southern Africa and can tolerate a wide range of environmental conditions (Dempster *et al.* 1995). Members of the genus *Oreochromis* are known to be omnivorous and their diet ranges from microalgae, aquatic vegetation and detritus to invertebrates and even small vertebrates (Likongwe *et al.* 1996, Dempster *et al.* 1995). Dempster and company found that under most

conditions, when subjected to a diet consisting solely of algal biomass, tilapia become unable to fulfil basic maintenance and thus begin to lose weight (Dempster *et al.* 1995). Furthermore, filter-feeding alone does not result in sufficient intake of algae in this fish, and filter-feeding needs to be supplemented by feeding on algal aggregations in the water column or flocculent surface scums of cyanobacteria (Dempster *et al.* 1995).

Mozambique tilapia was previously investigated for its ability to consume microalgae in post HRAP effluent as well as testing the growth rate of the fish exposed to the varying effluent concentrations either with or without supplemented feed (Jones *et al.* 2016). Tilapia removed microalgae from the tanks; however, the unfavourable environmental conditions (high pH and changes in oxygen concentration) of the post-HRAP effluent negatively affected fish growth and nutrient assimilation (Jones *et al.* 2016). Mozambique tilapia is also readily available and has economic value, which provides further support for their use as *in situ* filter-feeding fish for the current project.

Clarias gariepinus is a freshwater fish commonly referred to as the sharp-tooth or African catfish (Skelton 1993). Its distribution ranges throughout Africa and other regions including Turkey and the Middle East (Yalcin *et al.* 2001), and its natural habitat includes lakes, rivers and seasonal floodplain swamps and small ponds (Skelton 1993; Kaunda-Arara *et al.* 2010). Since *C. gariepinus* has the widest longitudinal distribution range of all freshwater fish, it is among the most used sources of protein throughout Africa, especially in the rural areas (Hecht, 1981).

The African catfish is an opportunistic predator (Potts *et al.* 2008), with a thin-walled and rather short intestine consistent with a need for a high protein diet. However, it is a generalist feeder since it employs several feeding modes, depending on food availability – individual foraging, individual shovelling, formation feeding and surface feeding – and it feeds on a variety of organisms (Bruton 1979), ranging from vertebrates to plants and plankton (Skelton 1993). It is juveniles that tend to feed at the lower trophic levels (Kadye & Booth 2012). African catfish are known to filter-feed in groups at the water surface using gillrakers (Hecht, Uys & Britz 1988). In addition to a wide mouth, which assists in suction, the African catfish is equipped

with long gillrakers, relative to non-filter-feeding fishes, which trap particles from the water that passes over the gills.

The African catfish has a modified gill arch that makes it possible to breathe air (Skelton 1993) and thus survive when there is insufficient dissolved oxygen in the water. This, together with its ability to survive in the harsh environmental conditions of evaporating pools on African mudflats (Skelton 1993), and its ability to filter-feed (Skelton 1993), makes it a suitable candidate species for investigation in the current study.

Many mullets are more suitable to uptake microalgae compared to African catfish and Mozambique tilapia due to the morphology of their gillrakers; however, they are less well adapted to surviving in extreme environments. The freshwater mullet (*Myxus capensis*) is endemic to South Africa and lives in coastal estuaries and rivers, with a distribution from the Breë River to Kosi Bay (Skelton 2001). Despite very few studies analysing the food preference of freshwater mullet, Odum (1986) investigated the particle selection of the striped mullet (*Mugil cephalus*), a close relative. Odum (1986) identified microalgae (including epiphytic and benthic diatoms, dinoflagellates as well as green and blue-green algae) as the fish's primary source of nutrition. Furthermore, Odum (1986) illustrated the fish's ability to select particles smaller than 15 μm and discovered that very fine particles (< 10 μm) exceeded over 80% of the stomach contents. This fine particle selection is largely due to the fish's pharyngeal filtering capabilities in which pharyngeal taste buds identify particles rich in microalgae (Odum 1986). However, not all mullets have the same ability to filter-feed.

2.5 Use of a flocculants to settle algae: Chitosan

Algae are microscopic organisms and are thus difficult to consume. The consumption of algae by filter-feeding fish such as the Mozambique tilapia is made possible by the gill structure together with the tendency of the algae to form clumps or flocs (Jones *et al.* 2016). The mean space between Mozambique tilapia gillrakers (i.e. the organ used to filter algae from the water) is about 600 μm (Jones *et al.* 2016), which is substantially larger than the unicellular algae in the system (Jones *et al.* 2016; Mogane 2016). However, algal/bacterial/fungal flocs occur naturally in the HRAP and these flocs are usually about 1000 μm in diameter or sometimes larger, making the microalgae available to the filter-feeding fish (Jones *et al.* 2016).

Algal cells can be manipulated to form aggregations, and this increases the particle size and the ease of recovery of the microalgal biomass (Richmond 2013). Clumping of microbial cells resulting from increasing the pH of the medium or through the addition of an electrolyte, is known as coagulation; clumping of cells resulting from the addition of a polymer is known as flocculation (Narasimhan 2010). Typically, microalgae cells carry a negative charge in solution, which prevents natural aggregation of cells in suspension (Richmond 2013). Multivalent metal salts like ferric chloride ($FeCl_3$), aluminium sulfate ($Al_2(SO_4)_3$) and ferric sulfate ($Fe_2(SO_4)_3$) can all be used to flocculate algae within a cultured system.

The introduction of polymetric cations, such as the flocculant chitosan, results in the reduction of negative charge and thus aggregation of cells leading to increase in cell size and algal availability (Richmond 2013). The negative charge of the algae is attracted to the positive charge of the cationic flocculants, which results in algae particles being bound together to form clumps within the suspended medium (Narasimhan 2010). Flocculation performance is a function of a variety of different factors, including the dose applied, the concentration of the biomass, the molecular mass of the polymer, the charge density on the molecule, the pH of the culture, as well as the extent of mixing within the tank (Richmond 2013). The use of bioflocculants, which are biopolymers obtained from a natural source, can enhance the flocculation of cells whilst at the same time being environmentally safe and relatively inexpensive compared to other equivalent chemicals.

Auto-flocculation occurs as a result of changing conditions within the algae cultivation such as sudden loss of CO_2 or an increase in pH (Richmond 2013). The natural flocculation at high pH occurs when the pH value is close to the theoretical value of magnesium hydroxide precipitation (Narasimhan 2010). Auto-flocculation can be simulated by adding non-toxic NaOH to achieve desired pH values, or by the addition of caustic soda or lime (Richmond 2013). The auto-flocculation of microalgae cells under highly alkaline conditions is species-specific, and depends on other factors such as water temperature, cell size and algal concentration (Richmond 2013).

Chitosan, a cationic polymer (deacetylated polymer of β-N-acetyl-D-glucosamine), is a commonly used bioflocculant prepared from the crushed exoskeletons of marine crustaceans (Narasimhan 2010). It is preferred over other conventional flocculants as it does not produce any toxic side effects and low concentrations of chitosan can be used to create high algal clumping within wastewater (Narasimhan 2010).

The potential exists to increase the efficiency with which filter-feeding fish or deposit-feeding fish can remove algae from the system, if the algae form flocs in the water column or flocs that settle out of suspension.

2.6 Conclusion

The small size of the algal cells that characterise the algal-bacterial community complex in HRAP effluent systems remains a bottleneck in the use of HRAP as an effluent treatment solution, and the harvest and physical recovery of these algal cells represents one of the most technical difficulties that still needs to be addressed. It has been demonstrated in this literature review that filter-feeding fish can remove algae from these systems; however, the rate of this removal has not been fully investigated and the environment created by the HRAP is not always suitable for fish culture. This environment includes a diurnal drop in oxygen concentration and a diurnal increase in pH beyond the limits of most fishes. The work that follows in this study needs to focus on: (1) alternative fish species that might be better able to survive in these fluctuating environments and that (2) might be more suited to algal removal; (3) making the algae more available through flocculation; or (4) by algal clumping by manipulating post-HRAP effluent pH using CO_2 or light, for example; which will, in turn, (5) moderate the direct negative effects that high pH has on fish physiology.

Chapter 3 – Preliminary method development

3.1 Use of a flocculant and African catfish to remove algae from treated effluent

3.1.1 Introduction

African catfish have evolved to survive extreme environmental conditions. It is not uncommon to find them surviving at high stocking densities in small pools, in dried out river beds or floodplains in its natural environment. These pools are frequently low in oxygen, very turbid and are sometimes eutrophic; that is, conditions that are not dissimilar to those in high rate algal ponds (HRAP) used to treat effluent. In addition, these fish have gill structures that make it possible for them to filter plankton in the water column and to obtain nutritional benefit from this process. This fish is not an obligatory filter-feeder, since it obtains most of its nutrition from other sources; so, unlike fish that have to rely on filter-feeding and have thus developed gill structures that are highly efficient at filter-feeding, the gillrakers of catfish are less developed for this purpose. The flocculation of suspended solids in the water column is likely to make this material more available to fish, since the flocs of algae will be larger. The aim of the first part of this study was to determine if African catfish can remove algae from post-HRAP effluent and to determine if this removal is influenced by the presence of a natural flocculant.

3.1.2 Materials and methods

To determine the effect that flocculation had on algal biomass removal by fish, hatchery-reared African catfish (*Clarias gariepinus*) were stocked into six 500 L fish-tanks. The tanks formed part of a recirculating aquaculture system housed in the Ibhayi Brewery's Project Eden greenhouse tunnel in Port Elizabeth (Jones *et al.* 2014). Prior to stocking, the tanks were filled with brewery effluent that had undergone treatment in the integrated algal ponding systems (IAPS) at Ibhayi Brewery (Jones *et al.* 2014). The effluent was drawn from the system after the high rate algal ponds (post-HRAP), which contained the full complement of algal cells; that is, algae were not allowed to settle out of suspension prior to filling the tanks. An additional six 500 L tanks were left unstocked (i.e. without fish) and were filled with the same post-HRAP effluent containing algal cells. All tanks were closed off from the recirculating system for the duration of the experiment. Chitosan flocculant was added to three of the tanks with fish and

three of the tanks without fish; the remaining six tanks (three with and three without fish) did not receive the chitosan flocculant.

The pH, the concentration of algal cells measured as total suspended solids, chlorophyll-a concentration, chemical oxygen demand (COD), temperature, dissolved oxygen, ammonia, nitrite and phosphate were recorded at the start and end of the data collection period and, in some instances, at two-hour intervals over the period of the experiment, which lasted for six hours. Differences in water quality and algal concentration were compared between treatments using repeated measures, two-way-factorial analysis of variance (ANOVA) at $p < 0.1$.

3.1.3 Results and discussion

The presence of fish in the algal tanks appeared to moderate changes in pH, although pH was not influenced by a significant interaction between the presence/absence of fish and flocculant (multifactor, repeated measures ANOVA: $F_{(1,8)}=0.714$; $p=0.422$; Table 3.1).

Table 3.1 Mean pH (± standard error) of post-HRAP effluent with and without flocculant both in the presence and absence of catfish over four hours (multifactor, repeated measures ANOVA: $F_{(1,8)}=0.714$; $p=0.422$).

Fish	Flocculant	Time (h)	pH
Present	Present	0	9.45 ± 0.14
Present	Present	4	9.34 ± 0.07
Present	Absent	0	9.37 ± 0.20
Present	Absent	4	9.56 ± 0.28
Absent	Present	0	9.42 ± 0.23
Absent	Present	4	9.87 ± 0.25
Absent	Absent	0	9.44 ± 0.15
Absent	Absent	4	9.88 ± 0.26

The presence of fish in the tank did, however, appear to mitigate the increase in pH compared with treatments without fish, but again this was not significant (repeated measures ANOVA: $F_{(1,8)}=4.881$; $p=0.058$). If this trend exists, it was probably due to a reduction in photosynthesis when fish were in the tanks removing algae.

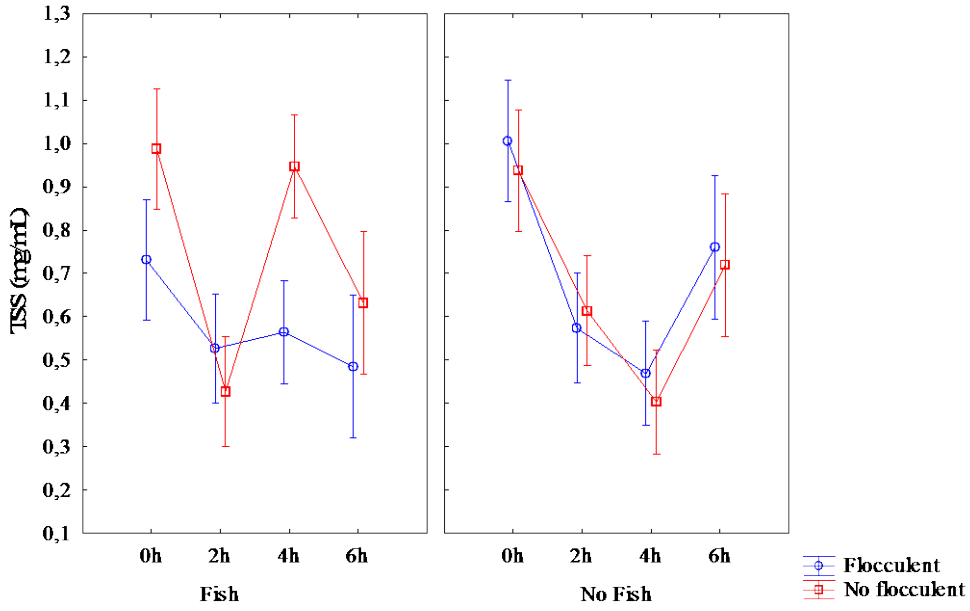

Figure 3.1 The change in mean total suspended solids (TSS; ± standard error) of post-HRAP effluent with and without flocculant, both in the presence and absence of catfish over six hours (multifactor, repeated measures ANOVA: $F_{(1,8)}$=0.734; p=0.542).

The algal concentration was determined by measuring the total suspended solids (TSS) in the effluent and its chlorophyll-a concentration. The TSS concentration was not influenced by an interaction between factors over time (multifactor, repeated measures ANOVA: $F_{(1,8)}$=0.734; p=0.542; Figure 3.1). There is, however, some evidence of an interaction between the presence/absence of fish and flocculant: whereas the presence/absence of a flocculant did not influence TSS when fish were absent, the presence of fish resulted in lower TSS when the effluent was dosed with a flocculant, compared with treatments where flocculant was absent (multifactor ANOVA: $F_{(1,8)}$=3.667; p=0.092; Figure 3.2). This suggests that the fish were better able to remove algae in the presence of the flocculant; although the trend is apparent, it was not significant at p=0.92 (Figure 3.2). The chlorophyll-a results did not corroborate a trend at all, since the presence of fish in the tanks did not appear to influence these data (Figure 3.3).

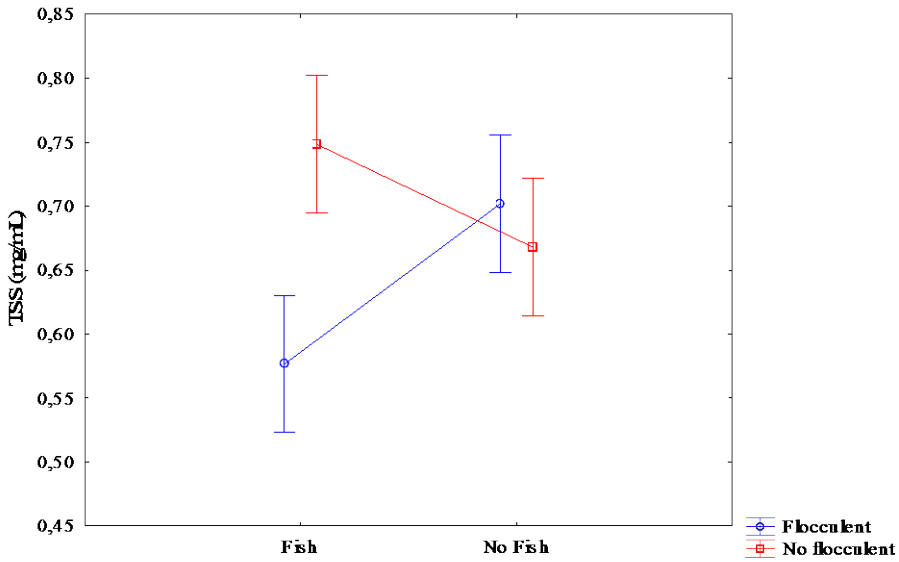

Figure 3.2 Mean total suspended solids (TSS; ± standard error) in post-HRAP effluent subject to a flocculant or not, both in the presence and absence of catfish (multifactor ANOVA: $F_{(1,8)}$=3.667; p=0.092).

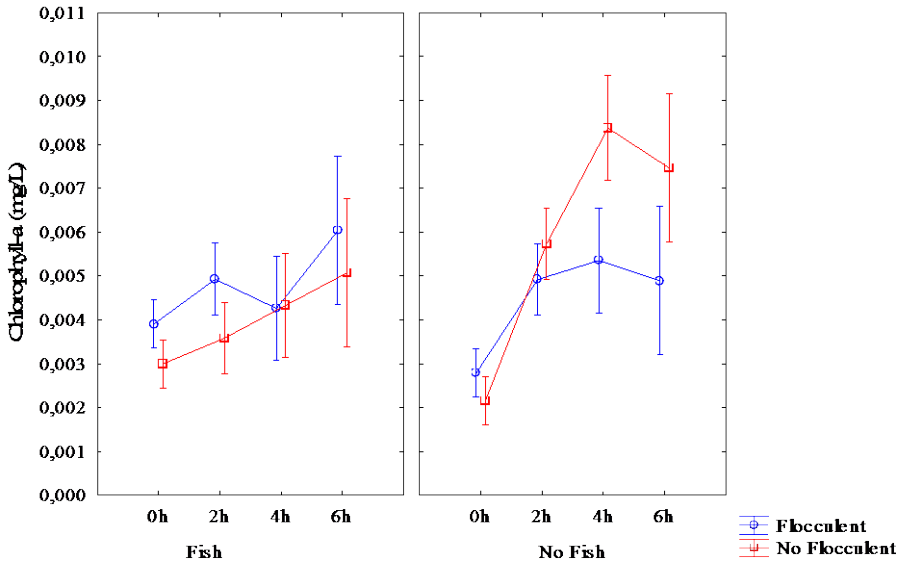

Figure 3.3 The change in mean chlorophyll-a concentration (± standard error) in post-HRAP effluent with and without flocculant both in the presence and absence of catfish over six hours (multifactor, repeated measures ANOVA: $F_{(1,8)}$=0.629; p=0.603).

Table 3.2 The change in mean chemical oxygen demand (COD; ± standard error) of post-HRAP effluent with and without flocculant both in the presence and absence of catfish over six hours (multifactor, repeated measures ANOVA: $F_{(1,8)}$=5.300; p=0.050).

Fish	Flocculant	Time (h)	COD
Present	Present	0	300.00 ± 00.00
Present	Present	6	153.00 ± 14.73
Present	Absent	0	205.33 ± 11.70
Present	Absent	6	139.33 ± 03.33
Absent	Present	0	291.33 ± 08.67
Absent	Present	6	147.67 ± 02.40
Absent	Absent	0	283.33 ± 12.33
Absent	Absent	6	152.33 ± 05.70

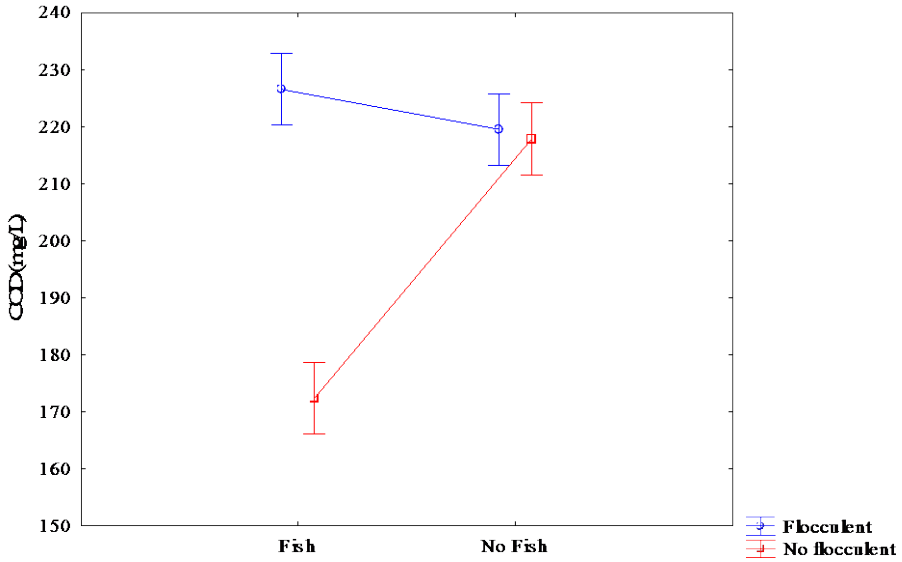

Figure 3.4 Mean chemical oxygen demand (COD; ± standard error) of post-HRAP effluent subject to a flocculant or not, both in the presence and absence of catfish (multifactor ANOVA: $F_{(1,8)}$=17.475; p=0.003).

The chemical oxygen demand (COD) was influenced by an interaction between the presence and absence of flocculant and fish (multifactor, repeated measures ANOVA: $F_{(1,8)}$=5.300; p=0.050; Table 3.2). The COD was significantly lower when fish were present in the tank and without flocculant, whereas the addition of flocculant did not contribute to a lower COD in the

presence of fish (multifactor ANOVA: $F_{(1,8)}$=17.475; p=0.003; Figure 3.4), and this difference was most apparent at the start of the trial (Table 3.2).

The mean temperature, dissolved oxygen, ammonia, nitrite and phosphate concentrations were not affected by the presence or absence of flocculant and fish (Table 3.3; multifactor, repeated measures ANOVA: p>0.10).

Table 3.3 The mean temperature, dissolved oxygen, ammonia, nitrite and phosphate concentrations (± standard error) in post-HRAP measured over six hours. These data are the combined means of all treatments combined as there were no significant interactions between or within factors (multifactor, repeated measures ANOVA: p>0.10).

	Time (h)			
	0	2	4	6
Temperature (°C)	27.81 ± 0.08	-	27.52 ± 0.37	-
Dissolved oxygen (mg/L)	4.52 ± 0.22	-	4.43 ± 0.41	-
NH_4 (mg/L)	0.25 ± 0.06	0.12 ± 0.02	0.52 ± 0.17	0.17 ± 0.05
NO_2 (mg/L)	0.08 ± 0.01	0.08 ± 0.01	0.22 ± 0.52	0.22 ± 0.10
PO_4 (mg/L)	11.40 ± 3.48	4.43 ± 1.33	11.15 ± 3.72	15.79 ± 1.35

3.1.4 Conclusion

Although African catfish can filter-feed phytoplankton from the water column and are very hardy fish that can survive the extreme conditions of high rate algal ponds (HRAP), the data presented here did not support the hypothesis that they are suitable for removing algae from post-HRAP effluent. Furthermore, the addition of the flocculant did not make the algae more available to the fish.

The work that followed in this study thus focused on alternative species that are better able to filter-feed, and on moderating the extreme conditions so that more sensitive fishes can be used to filter the algae.

3.2 pH moderation using carbon dioxide and algal removal using tilapia

3.2.1 Introduction

This experiment was designed as a first attempt to alter the pH in the high rate algal pond (HRAP) effluent using carbon dioxide. This was a preliminary 'method-development' exercise that aimed to mitigate the pH spike that develops in the HRAP during the course of the day as a result of photosynthesis, and it aimed to see if this influenced the rate that Mozambique tilapia were able to remove algae from the effluent.

3.2.2 Materials and methods

To determine the effect that pH moderation of effluent treated in high rate algal ponds (HRAP) had on algal removal by fish, 60 hatchery-reared Mozambique tilapia (*Oreochromis mozambicus*) were stocked into six 40 L fish-tanks (i.e. five fish per tank). The tanks formed part of a recirculating aquaculture system housed in the Ibhayi Brewery's Project Eden greenhouse tunnel in Port Elizabeth (Jones *et al.* 2014). Prior to stocking, the tanks were filled with brewery effluent that had undergone treatment in the integrated algal ponding system (IAPS) at the brewery (Jones *et al.* 2014); the effluent was drawn from the treatment system after the HRAP, which contained the full complement of algal cells (i.e. algae were not allowed to settle out of suspension prior to filling the tanks). An additional six 40 L tanks were left unstocked (i.e. without fish) and were filled with the same post-HRAP effluent containing algal cells. All tanks were closed off from the recirculating system for the duration of the experiment. Carbon dioxide was continually bubbled at 2.25 litres CO_2 per litre of water per hour into three of the tanks containing fish and three of the unstocked fish tanks, to maintain pH between about 7.5 and 8.5. The pH in the remaining six tanks (i.e. three with fish and three without fish) was left unadjusted.

The CO_2 concentration, pH, oxygen and the concentration of algal cells using total suspended solids (TSS), turbidity and chlorophyll-a concentration as indicators were recorded at the start, at hourly intervals and at the end of the trial, which lasted for five hours. Differences in water quality and algal concentration were compared between treatments using repeated measures, two-way-factorial analysis of variance (ANOVA) at $p < 0.05$.

3.2.3 Results and discussion

As expected, there was a significant difference in pH between pH-moderated and un-manipulated tanks over five hours (Figures 3.5 and 3.6). The pH was maintained between about 7.5 and 8.5 in the tanks subject to pH moderation using CO_2, whereas it remained high at about 10.5 to 11.5 in tanks without pH moderation (repeated measures ANOVA: $F_{(5,40)}$=392.95; p<0.001; Figure 3.6). There was no interaction in pH amongst the factors (i.e. fish/no fish and pH manipulation/no pH manipulation treatments) over the period of the experiments (repeated measures ANOVA: $F_{(5,40)}$=0.392; p=0.851); meaning that the presence or absence of fish had no effect on the pH of the tanks. The pH in the moderated treatments was maintained within a range suitable for fish culture.

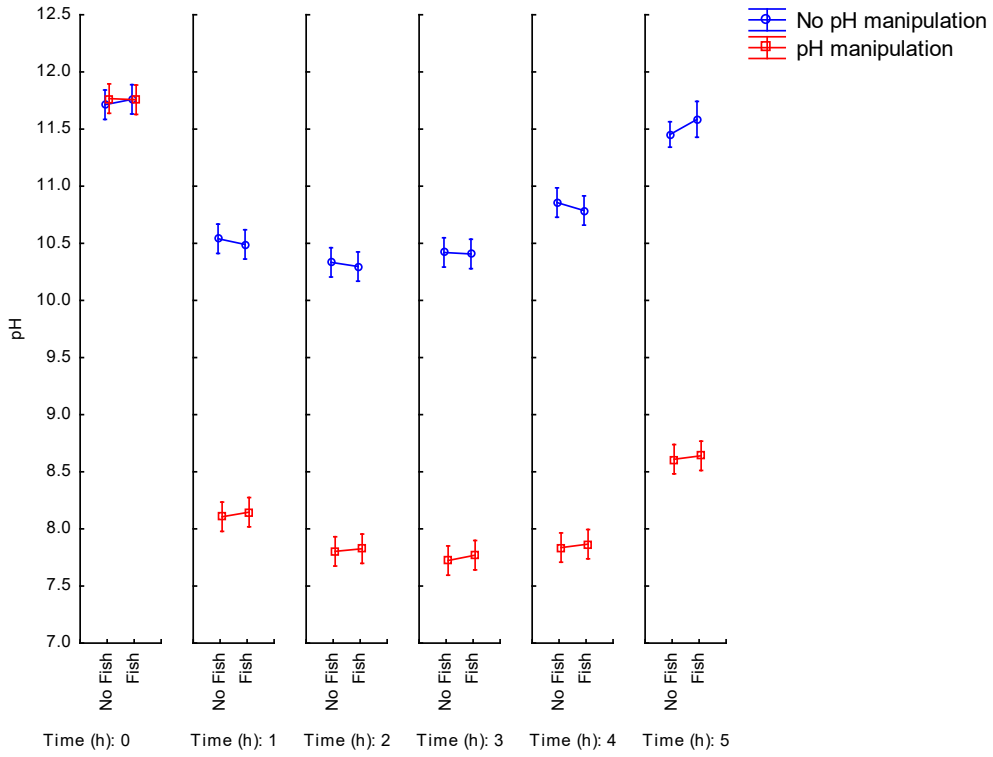

Figure 3.5 The mean (± standard error) pH in tanks of post-HRAP effluent, that either include pH manipulation (with CO_2) or no pH manipulation, each with fish or without fish over five hours (repeated measures ANOVA: $F_{(5,40)}$=0.392; p=0.851).

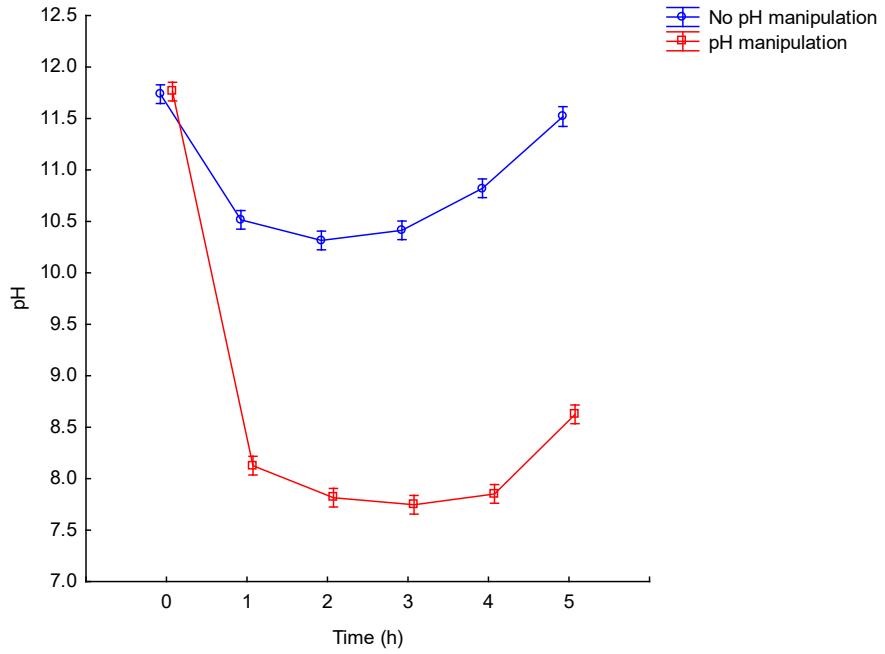

Figure 3.6 The mean (± 95% confidence interval) pH in tanks of post-HRAP effluent, that either include pH manipulation or no pH manipulation over five hours (repeated measures ANOVA: $F_{(5,40)}$=392.95; p<0.001). Here fish are excluded as a factor since the presence/absence of fish did not affect pH – these data are the combined data of tanks with and without fish.

The successful manipulation of the pH was due to the CO_2 that was added to the tanks which, as was expected, mirrored the pH data. Carbon dioxide was similar between treatments at the start of the trial, increasing sharply in the treatments that received CO_2 gas (repeated measures ANOVA: $F_{(5,40)}$=7391.1; p<0.001; Figure 3.7). Again, the presence or absence of fish had no influence on the CO_2 concentration and the factors did not interact over the duration of the trial (repeated measures ANOVA: $F_{(5,40)}$=0.695; p=0.630; Figure 3.8).

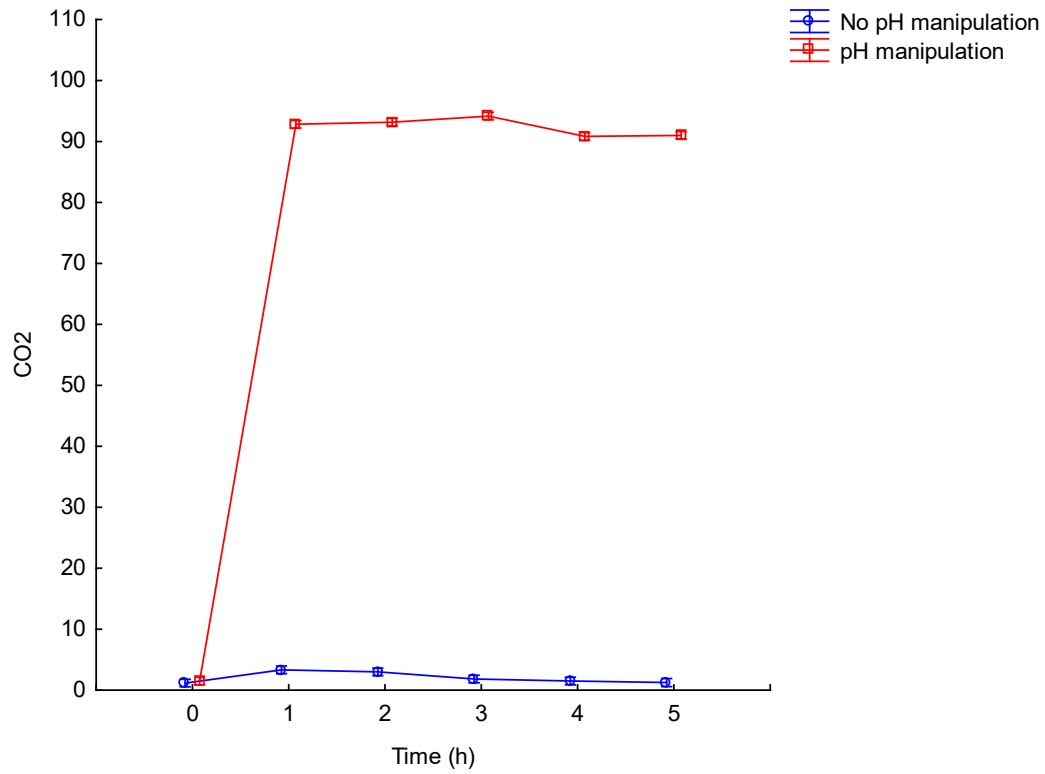

Figure 3.7 The mean (± 95% confidence interval) carbon dioxide concentration in tanks of post-HRAP effluent, that either include pH manipulation or no pH manipulation over five hours (repeated measures ANOVA: $F_{(5,40)}=7391.1$; $p<0.001$).

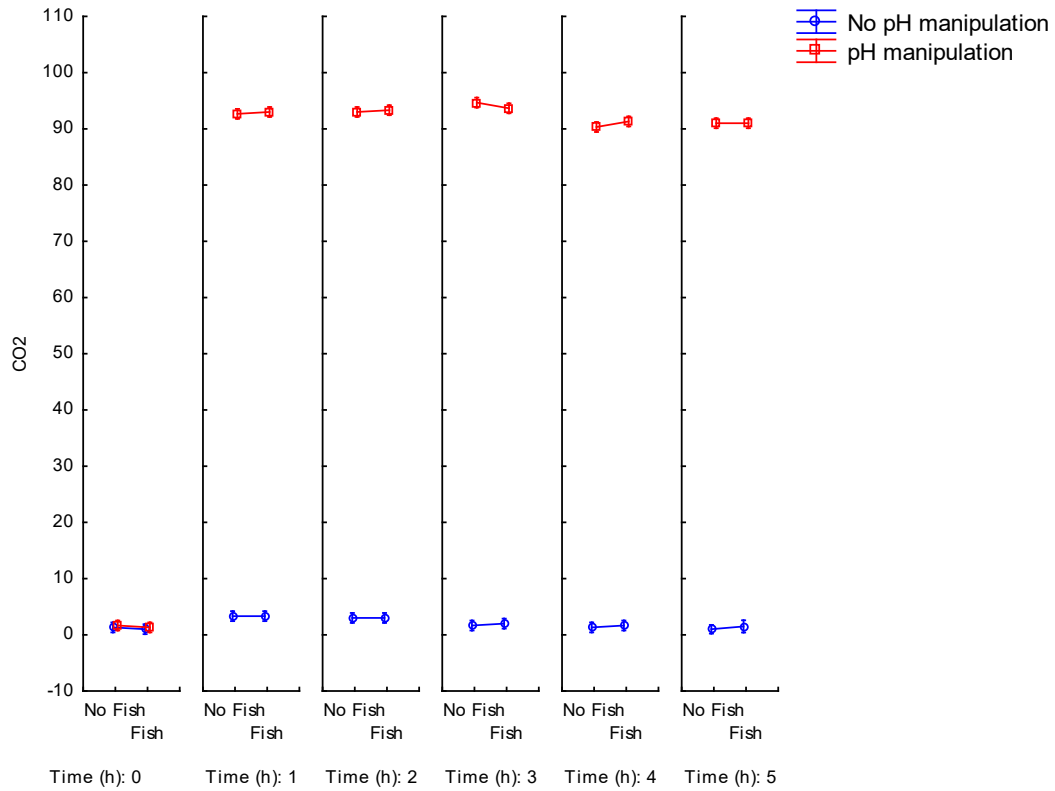

Figure 3.8 The mean (± standard error) carbon dioxide concentration in tanks of post-HRAP effluent, that either include pH manipulation or no pH manipulation and fish or no fish over five hours (Repeated Measures ANOVA: $F_{(5,40)}$=0.695; p=0.630).

Dissolved oxygen differed significantly between pH moderated and un-manipulated treatments. Dissolved oxygen was higher in the un-moderated tanks and increased in those treatments, whereas it decreased with time in the treatments that received CO_2-addition (repeated measures ANOVA: $F_{(5,40)}$=74.965; p<0.001; Figure 3.9). This trend was the same for treatments with and without fish (repeated measures ANOVA: $F_{(5,40)}$=0.599; p=0.701; Figure 3.10).

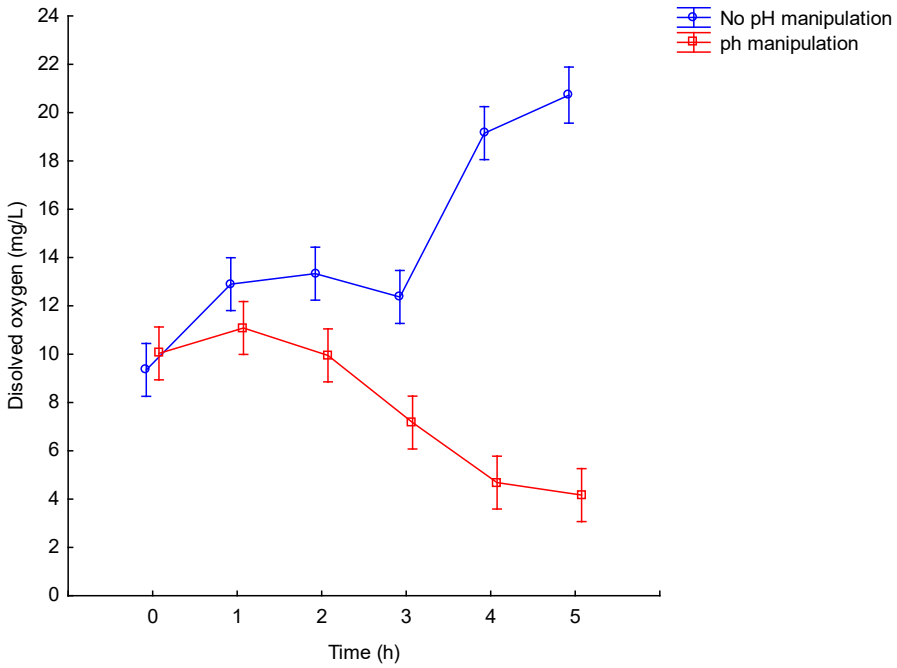

Figure 3.9 The mean (± 95% confidence interval) dissolved oxygen in tanks of post-HRAP effluent, that either include pH manipulation or no pH manipulation over five hours (repeated measures ANOVA: $F_{(5, 40)}$=74.965; p<0.001).

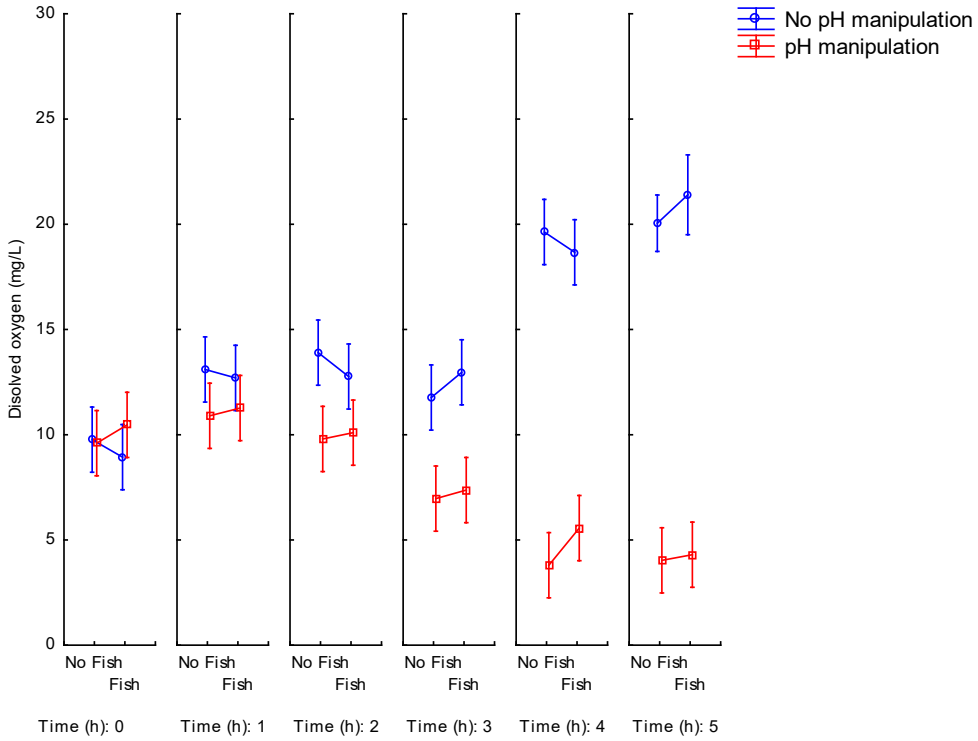

Figure 3.10 The mean (± standard error) dissolved oxygen in tanks of post-HRAP effluent, that either include pH manipulation or no pH manipulation and fish or no fish over five hours, highlighting the lack of a significant difference amongst the factors (repeated measures ANOVA: $F_{(5,40)}$=0.599; p=0.701).

Total suspended solids, chlorophyll-a concentration, the chemical oxygen demand (COD) and turbidity (i.e. all indirect measures of algal concentration) remained uninfluenced by interactions between factors and the factors (i.e. presence/absence of fish and pH moderation) (Table 3.4; multifactor, repeated measures ANOVA: $p<0.05$). However, in the presence of fish there was a downward trend in suspended solid concentration from about 3h to 5h (Table 3.4) and this trend was also apparent in a drop-in chlorophyll-a concentration over the same period (Table 3.4), indicating that the trial might have ended prematurely.

Turbidity is also an indicator of algal concentration, and it differed between treatments over time. However, this difference was not a result of an interaction between the factors that were being investigated here (repeated measures ANOVA: $F_{(3,24)}=0.048$; $p=0.986$; Figure 3.11), whereas it was affected by each factor on their own: turbidity was on average higher in all pH manipulated tanks, whereas it was lower if tanks where pH was allowed to increase (i.e. the un-manipulated treatments) (repeated measures ANOVA: $F_{(1,8)}=6.850$; $p=0.031$; Figure 3.12). A significant difference in turbidity was also observed between tanks with fish and tanks without fish over five hours, where it decreased more when fish were present compared with when they were absent (repeated measures ANOVA: $F_{(3,24)}=3.401$; $p=0.034$; Figure 3.13).

Table 3.4 The mean (± standard error) total suspended solids, chlorophyll-a, chemical oxygen demand (COD) and turbidity of post-HRAP effluent subject to pH fluctuation (no pH manipulation) or pH moderation using carbon dioxide dosing (pH manipulation), both in the presence or absence of tilapia over five hours (multifactor, repeated measures ANOVA: $p<0.05$).

		Time (h)						3-way interaction		Fish/no fish		ph manip./non	
		0	1	2	3	4	5		p	F	p	F	
Total suspended solids (mg/mL)													
No pH manipulation	No Fish	1.37 ± 0.14	2.11 ± 0.14	1.66 ± 0.06	1.89 ± 0.09	1.45 ± 0.05	1.76 ± 0.10	1.385	0.251	2.346	0.059	0.251	0.93
No pH manipulation	Fish	1.86 ± 0.44	0.99 ± 0.10	2.09 ± 0.30	2.37 ± 0.26	1.81 ± 0.06	1.40 ± 0.07						
pH manipulation	No Fish	1.46 ± 0.03	1.29 ± 0.10	1.63 ± 0.25	2.06 ± 0.28	1.30 ± 0.14	1.42 ± 0.01						
pH manipulation	Fish	1.42 ± 0.11	0.89 ± 0.10	1.71 ± 0.15	1.29 ± 0.09	1.70 ± 0.12	1.15 ± 0.07						
Chloropyll-A (ug/L)													
No pH manipulation	No Fish	3.43 ± 0.36	2.45 ± 0.06	3.90 ± 0.53	2.82 ± 0.26	2.37 ± 0.33	4.26 ± 0.79	0.761	0.583	0.949	0.460	2.218	0.07
No pH manipulation	Fish	3.74 ± 0.22	3.10 ± 0.26	4.02 ± 0.29	3.75 ± 0.47	2.88 ± 0.08	2.48 ± 0.29						
pH manipulation	No Fish	3.33 ± 0.41	1.95 ± 0.27	2.66 ± 0.20	2.62 ± 0.32	2.97 ± 0.42	1.36 ± 0.10						
pH manipulation	Fish	3.69 ± 0.08	3.11 ± 0.16	2.95 ± 0.10	3.15 ± 0.40	3.43 ± 0.36	1.89 ± 0.06						
COD (mg/L)													
No pH manipulation	No Fish	83.67 ± 1.02					84.33 ± 1.68	0.002	0.969	0.717	0.422	0.860	0.38
No pH manipulation	Fish	83.67 ± 2.36					88.00 ± 2.31						
pH manipulation	No Fish	84.33 ± 1.68					81.33 ± 1.71						
pH manipulation	Fish	82.33 ± 1.58					82.67 ± 1.58						
Turbidity (cm)													
No pH manipulation	No Fish	4.67 ± 0.19	3.83 ± 0.10		3.03 ± 0.18		2.03 ± 0.02	0.048	0.986	3.401	0.034	2.400	0.09
No pH manipulation	Fish	4.50 ± 0.17	4.00 ± 0.17		3.80 ± 0.09		2.83 ± 0.32						
pH manipulation	No Fish	3.67 ± 0.10	3.17 ± 0.10		2.50 ± 0.17		2.00 ± 0.17						
pH manipulation	Fish	3.67 ± 0.19	3.23 ± 0.13		3.30 ± 0.17		2.83 ± 0.27						

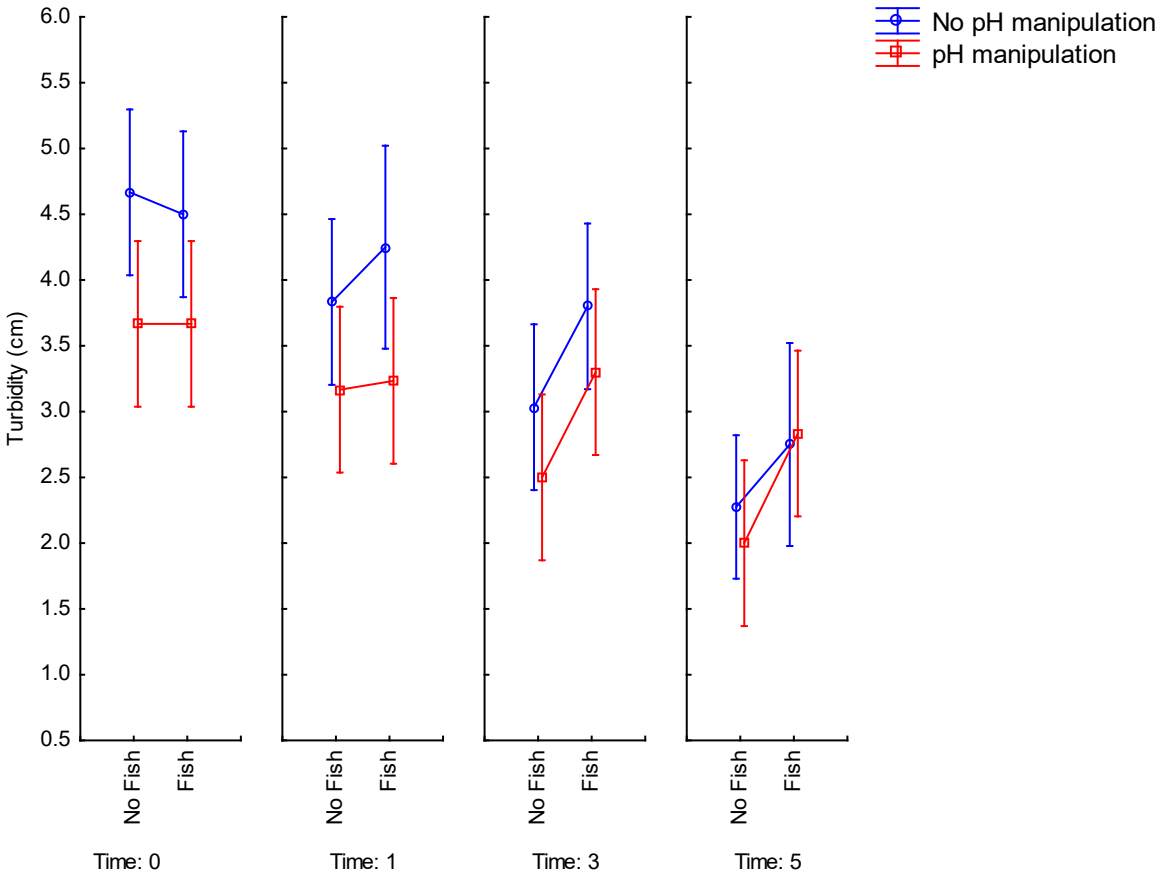

Figure 3.11 The mean (± standard error) turbidity in tanks of post-HRAP effluent, that either include pH manipulation or no pH manipulation and fish or no fish over five hours indicating a lack of a significant difference amongst the factors (repeated measures ANOVA: $F_{(3,24)}$=0.048; p=0.986).

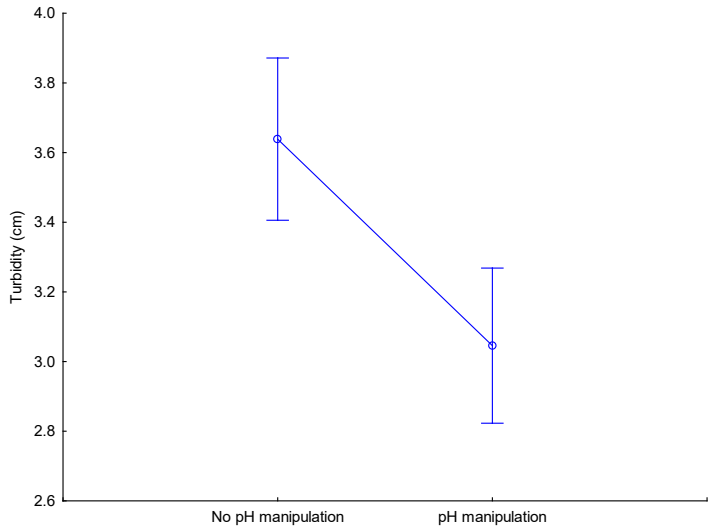

Figure 3.12 The mean turbidity (± 95% confidence interval) in tanks of post-HRAP effluent that either include pH manipulation or no pH manipulation (repeated measures ANOVA: $F_{(1,8)}$=6.850; p=0.031).

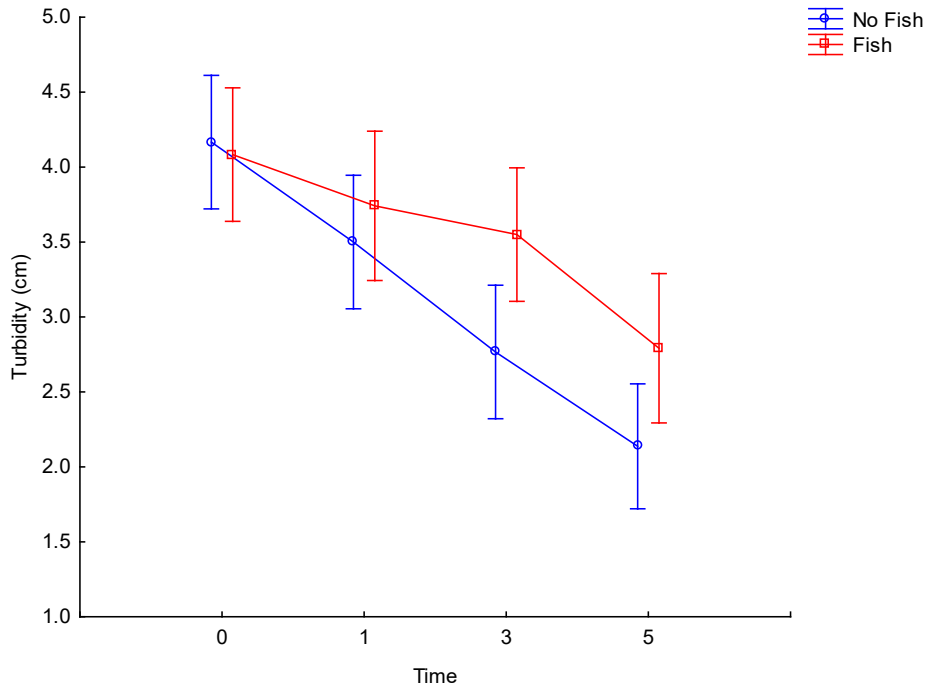

Figure 3.13 The mean turbidity (± 95% confidence interval) in tanks of post-HRAP effluent, that either include fish or no fish over five hours (repeated measures ANOVA: $F_{(3,24)}$=3.401; p=0.034).

3.2.4 Conclusion

The use of CO_2 to manipulate the pH by constantly inoculating CO_2 gas into the algal tanks successfully moderated the pH of the algal tanks and maintained this pH within a range suitable for fish culture. However, the CO_2 also displaced O_2 in the water. Although the fish did not show signs of stress during the trial, a number of fish died in the days after the trial and this was probably related to the physiological stress they were placed under due to the low oxygen concentrations that were observed here.

In line with earlier discussion, fish that are under physiological stress are probably less likely to feed and this is likely to have a negative impact on the rate that algae were removed. However, using turbidity as an indication of algal concentration, these results confirm early work indicating that tilapia can be used to remove algae from HRAP effluent. It was hypothesised that if a greater number of fish had been used or if the experiment had been extended for a longer period, the significant change in turbidity observed here might have been seen in other parameters such as total suspended solids and chlorophyll-a also. To test this, the following experiments in this study were designed to optimize the period taken to reduce algal concentration and the corresponding stocking density of the fish.

This work also suggests that the moderation of pH might improve this rate of removal by the fish. Therefore, the following experiments in this study were designed to investigated if pH moderation, without compromising oxygen levels in the water, makes the environment more suitable for fish culture.

3.3 Alternative methods to moderate pH in algal ponds

3.3.1 Introduction

The continual addition of CO_2 into high rate algal ponds (HRAP) effluent brought the pH into a range that was suitable for fish culture, but the CO_2 displaced oxygen in the water, and this lack of oxygen compromised the fish. Alternative methods of moderating the pH in HRAP were needed, which do not result in reduced oxygen levels. Three alternative methods to decrease pH levels were identified: addition of CO_2 as once-off dose, addition of an acid as a once-off dose, and/or the decrease in light availability. It was hypothesised that the addition of CO_2 or an acid would increase the number of H^+ ions in the water, thereby decreasing the pH,

and this would not compromise oxygen levels for an extended period if administered as a once-off dose. Similarly, the decrease of light availability would decrease the photosynthetic rate and would increase the production of OH^- ions, which will decrease pH levels.

The overall aim of this experiment was to investigate alternative methods to moderate pH in post algal pond effluent. The objectives were to:

1) determine whether addition of CO_2, reducing light availability or the combination of the two reduces pH within HRAP effluent;
2) determine whether addition of acid, reducing light availability or the combination of the two reduces pH within HRAP effluent; and to
3) determine if CO_2 or acid, with the right light availability combination, is best suited to reduce pH within HRAP effluent.

These experiments did not involve fish and they did not investigate algal removal rates. They were limited to testing different methods of pH moderation in HRAP effluent only.

3.3.2 Methods and materials

Experimental system

This experiment took place in a greenhouse facility at the Ibhayi brewery situated in Port Elizabeth, South Africa. A portion of the brewery's effluent was initially treated in an anaerobic digester (AD) and was subsequently passed into the Project Eden integrated algal ponding system for further treatment. This algal ponding system included a primary facultative pond (PFP) and a series of high rate algal ponds. The HRAP were operated with a hydraulic retention time of 2.4 days. Post-HRAP effluent was pumped to eighteen 40 L tanks, each with their own air supply.

Experimental design and procedure

The experiment included a two-by-three multifactorial design. The pH in the tanks was either moderated using CO_2 or sulphuric acid or the pH was left un-moderated, and each of these treatments was subject to either light or dark conditions (Table 3.5). Each treatment was replicated three times.

Table 3.5 Two-by-three multifactorial experiment with six treatments (T1-T6), with three pH adjustments each represented under either light or dark conditions.

pH adjustment	Light	Dark
Carbon dioxide	T1	T2
Sulphuric acid	T3	T4
None	T5	T6

All tanks were filled with 40 L of HRAP effluent prior to the start of the experiment. At the start of the trial CO_2 was bubbled into the tanks until the pH decreased to 6.5 for the CO_2 for pH adjusted treatments. Eighteen millilitres of 98% sulphuric acid was used to adjust the pH to 6.5 in sulphuric acid treatments. Tanks in the dark treatments where covered with a sheet of grey polyvinyl-chloride to block light entering the tanks.

Water quality parameters

Dissolved oxygen (DO) and pH where recorded in the morning (08:00), in the early afternoon (12:00) and in the evening (16:00) for five days. The pH and DO of the water was recorded using an electronic probe (Hanna, HI 991300, United Kingdom).

Statistical analysis

The of treatments were compared using a one-way and multifactor repeated measures analysis of variance (ANOVA). When significant differences were detected treatment, means were compared using a Tukey multiple range test at $p<0.05$. 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 was conducted on hydrogen ion concentration when comparing pH data, and graphs were displayed as pH values

3.3.3 Results and discussion

Light conditions and pH adjustment method effect on pH

The pH of HRAP water was influenced by an interaction between presence of light and pH adjustment regime (multifactor repeated measures ANOVA, $F_{(34,204)}=10.31$, $p<0.0001$; Figure 3.14). In the light, when there was no pH adjustment, diurnal pH fluctuations ranged between 10.0 in the morning and 10.25 (Figure 3.14). In the dark, with no pH adjustment, the pH decreased steadily from 10 to 9 over the following five days (Figure 3.14).

The increase in pH in the light was due to the photosynthetic activity of the microalgae (Tadesse *et al.* 2004). Photosynthesis causes the uptake of CO_2 and release of O_2. The uptake of CO_2 caused bicarbonate (H_2CO_3) to dissociate, resulting in hydroxyl (OH^-) production which increased the pH of the water (Tadesse *et al.* 2004). The pH decrease in dark conditions is due to respiration of microalgae (Tadesse *et al.* 2004). Here, CO_2 is produced which increases the hydrogen ions (H^+) in the water, which decreases the pH of the water (Tadesse *et al.* 2004).

The addition of CO_2 and acid resulted in an increase in pH over the five days, in the light and under dark conditions (Figure 3.14). This increase was due to the high alkalinity of the HRAP water, which was generated from the addition of sodium hydroxide to the brewery effluent prior to anaerobic digestion and the generation of carbonate, bicarbonate and ammonium alkalinity during anaerobic digestion (Van Rensburg *et al.* 2003; Power 2014). This high alkalinity of brewery effluent counteracts the pH adjustment through CO_2 and acid addition.
In the dark, the pH in acid adjusted treatments increased at a slower rate than the CO_2 adjustment (Figure 3.14). The pH adjustment with CO_2 would have increased the dissolved CO_2 concentration when compared to acid treatments. Therefore, one would expect more CO_2 to have gassed out of the liquor when compared to the acid treatments. When CO_2 is exposed to the atmosphere, the volatile carbon dioxide expressed as carbonic acid is gassed off whereas the carbonate alkalinity is more stable and remains in the water (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). This results in a decrease in acidity and an increase in pH (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). Directly after pH adjustment the pH in CO_2 adjustment treatments increased faster than acid adjustment treatments due to the outgassing of super saturated carbon dioxide.

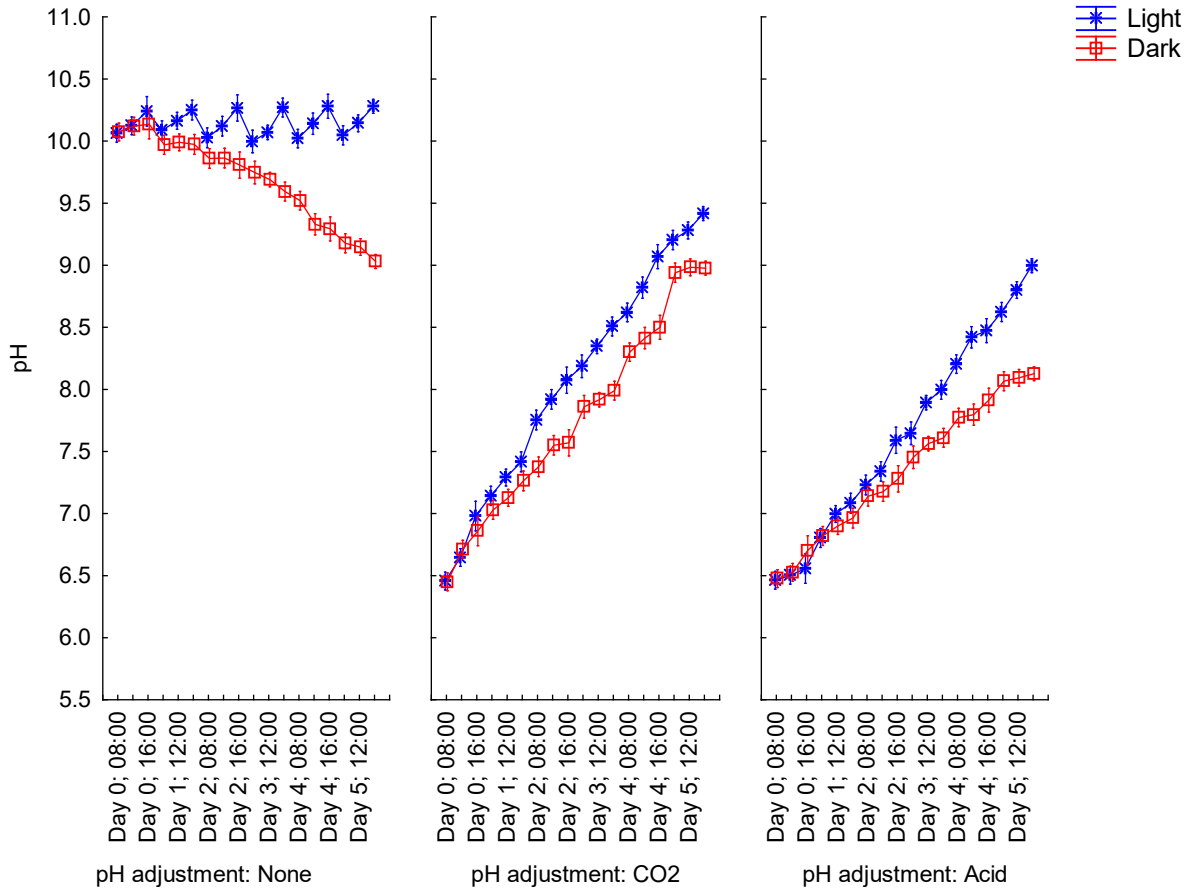

Figure 3.14 The mean (± 95% confidence interval) pH of algal tanks subject to different pH adjustment regimes and light or dark conditions over a five day period (multifactor repeated measures ANOVA, $F_{(34,204)}$=10.31, p<0.0001).

Light conditions and pH adjustment method effect on dissolved oxygen

Dissolved oxygen concentration of HRAP effluent was influenced by an interaction between the pH adjustment regime and the presence of light (multifactor repeated measures ANOVA, $F_{(34,204)}$=6.26, p<0.0001; Figure 3.15).

In all the tanks subject to light, the DO concentration increased to around 13 mg/L during the day and decreased to about 9.0 mg/L at night (Figure 3.15). Dissolved oxygen in dark treatments for no pH adjustment and pH adjustment using acid values initially decreased on the first day from 10 to 9 mg/l and then stabilised at 9 mg/l for the remainder of the experiment (Figure 3.15). Photosynthetic activity was responsible for the production of oxygen in the light

(Tadesse *et al.* 2004). The absence and presence of light influenced the dissolved oxygen of the water; at night, respiration utilised oxygen and this resulted in the decrease in oxygen concentration, which was replaced due to photosynthesis in the light (Tadesse *et al.* 2004), whereas in the dark the oxygen was not replaced, due to the absence of photosynthetic activity.

The pH adjustment by adding CO_2, in both light and dark treatments, resulted in low initial dissolved oxygen levels (4.26 ± 0.18 mg/l; Figure 3.15). This is due to oxygen changing from its aqueous form to a gaseous form and being expelled out of solution into the atmosphere. The DO concentrations then rose and stabilised at 7.8 ± 0.15 mg/l for dark treatments and fluctuated between 9 and 13 mg/l for light treatments. The use of CO_2, to decrease pH decreases the DO concentration in water, which can limit the density of fish the water can support.

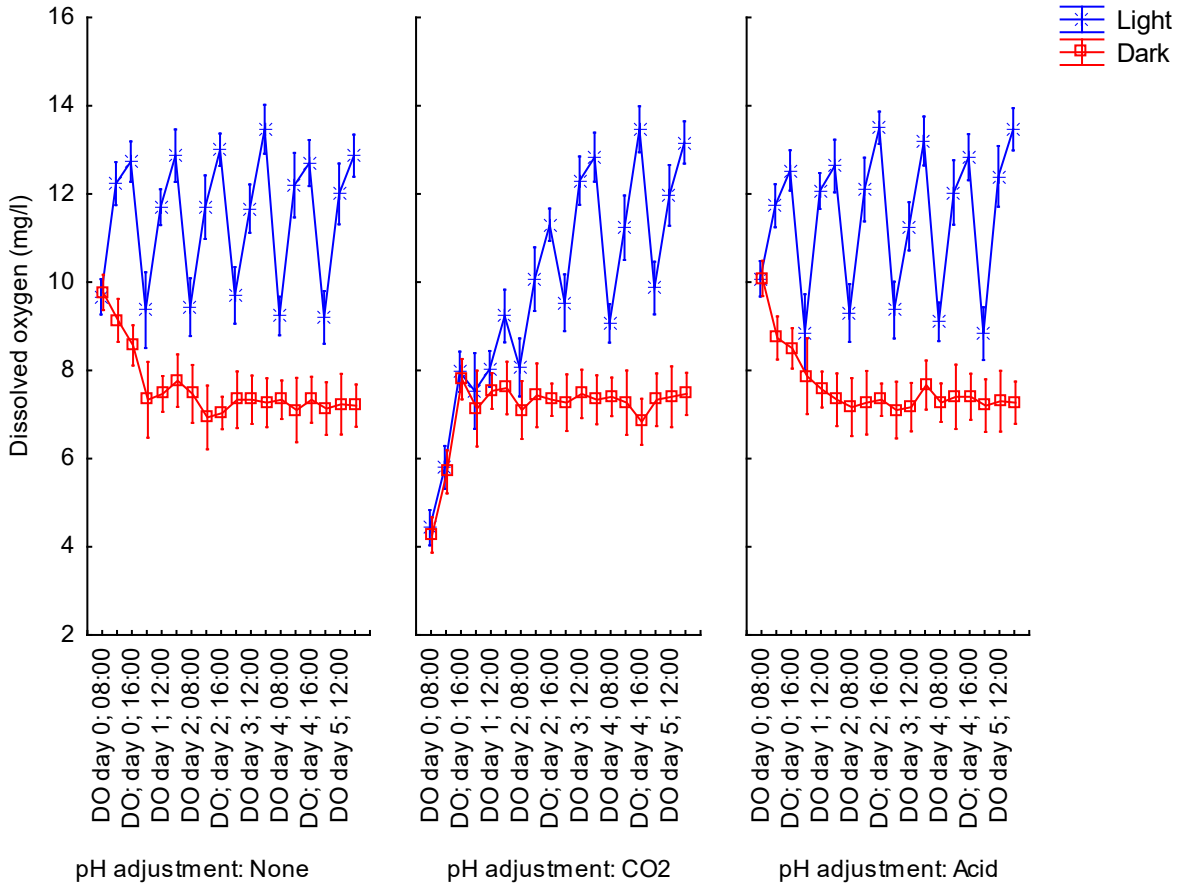

Figure 3.15 The mean (± 95% confidence interval) dissolved oxygen concentration of HRAP effluent subject to different pH adjustment regimes and light or dark conditions over a five day period (multifactor repeated measures ANOVA, $F_{(34,204)}=6.26$, $p<0.0001$).

3.3.4 Conclusion

The use of carbon dioxide and acid for pH adjustment were successful in lowering pH of HRAP effluent. However, acid was able to resist the high alkalinity of brewery effluent better than carbon dioxide, as the values increased at a slower rate over the five days of the experiment. The dark conditions are more effective in moderating pH in algal ponds, compared with treatments exposed to sunlight. This is due to the decreased photosynthetic activity, which is responsible for increasing pH. However, lower oxygen concentration in the dark treatments should be considered in experiments with high densities of fish in future work as this might limit the carrying capacity of fish in the tanks.

3.4 Optimizing fish stocking density and time required to remove algae from HRAP

3.4.1 Introduction

It became apparent in the earlier trials carried out in this project that the rate of algal removal was related to a combination of the concentration of the algae, the biomass of the fish in the tank and the time of exposure that the fish had to the algae. Furthermore, this is likely to differ among different species of fish. However, the optimal fish stocking density and the time of exposure had not been established. Therefore, the aim of this experiment was to determine (1) optimal fish biomass and (2) to optimize the fish/algae exposure time, and to do this for two fishes: Mozambique tilapia and freshwater mullet.

3.4.2 Methods and materials

Wild caught freshwater mullet (*Myxus capensis*) were taken from the Sundays River and transported to Ibhayi Brewery in an oxygenated 1000 L water tank. The fish were acclimated to captive conditions for three months in six 500 L recirculated water tanks within the

greenhouse facility at the brewery. Captive-bred Mozambique tilapia (*Oreochromis mossambicus*) were purchased from a commercial hatchery and were transported to Ibhayi brewery in oxygenated 1000 L water tanks. The fish acclimated to the brewery greenhouse facility water conditions for two months before the trial.

The experiment was conducted in the same facility, using the same HRAP water as described in Section 3.3.2. A preliminary test found that the mullet died if they were stocked above 10 kg/m^3 so the mullet was excluded from this trial. Fourteen of the 40 L tanks were filled with HRAP effluent, and 18 mL of sulphuric acid was added to each tank to reduce pH to 6.5.

Tilapia were purged for 48 h prior to the experiment and subsequently stocked into twelve of the tanks at stocking densities ranging from 0.63 to 20.00 kg/L (Table 3.6). The remaining two tanks were left unstocked. Each stocking density was represented in light and under dark conditions.

Table 3.6 Tilapia stocking densities used in preliminary trial. Each density was presented in the light and under dark conditions, which resulted in 14 treatments (T1-T14).

Stocking density (kg/m^3)	Light	Dark
0.00	T1	T8
0.63	T2	T9
1.25	T3	T10
2.50	T4	T11
5.00	T5	T12
10.00	T6	T13
20.00	T7	T14

The pH and DO of the water was recorded once a day using an electronic probe (Hanna, HI 991300, United Kingdom). Algal concentration was recorded once a day. Before a sample was taken the water in the tanks was thoroughly mixed to ensure even distribution of algae in the water column. Algal concentration was recorded as dry weight biomass per volume of water, where 50 mL of sample was filtered through 0.2 µm filter paper. The filter paper was previously placed in an oven set at 105 ^{o}C, dried for an hour and weighed before being used to filter a sample. The filter paper was then placed in the oven and allowed to dry for at least 24h, until

a constant weight was achieved, before being reweighed. Algal concentration was then calculated using Equation 3.1:

$$\text{Algal concentration (g/L)} = (\text{final weight (g)} - \text{initial weight (g)}) / \text{volume (L)} \quad [3.1]$$

Ammonia, nitrite and nitrate concentrations were recorded daily using a spectrophotometer and commercially available test kits (Merck Spectroquant Pharo 100 spectrophotometer, product number 100706, Darmstadt, Germany). Each sample was filtered through an eight-micron filter paper prior to analysis and the following test kits were used:

- Low-range ammonium test (Merck Pty Ltd, product: 1.14752.0001);
- Nitrite test (Merck Pty Ltd, product: 1.14776.0001);
- Nitrate test (Merck Pty Ltd, product: 1.09713.0001).

3.4.3 Discussion and results

Effect of fish biomass and light conditions on algal concentration

There was no change in algal biomass when fish were absent, under both light ($p=0.23$) and dark ($p=0.43$) conditions (Table 3.7). In the dark and in the light, the two highest stocking densities (10 and 20 kg/m^3) resulted in a significant drop in algal biomass ($p<0.01$; Figure 3.16; Table 3.7). At these stocking densities, algal removal was greater in the dark when compared to the light treatments. A stocking density of 20 kg/m^3 showed the largest decrease in algal concentration from 0.65 mg/L to 0.38 mg/L in the light treatment ($y=-0.055x+0.7183$; $r^2=0.97$; $p<0.01$) and a decrease from 0.60 mg/L to 0.20 mg/L in the dark treatment ($y=-0.008x+0.7044$ $r^2=0.94$; $p<0.01$; Figure 3.16; Table 3.7). This difference was due to algal productivity in the light (i.e. reproduction and growth of algae due to photosynthesis) which did not occur in the dark. In the light, there was a significant increase in algal biomass even when fish were present at 5.0 kg/m^3 ($y=0.040x+0.5169$; $r^2=0.88$; $p=0.01$), whereas in the dark this did not occur, and there was a significant decrease in algal concentration at 1.25 kg of fish per cubic meter ($y=-0.010x+0.6327$; $r^2=0.81$; $p=0.02$; Table 3.7).

In the light, algal concentration increased after five days at the lower stocking densities (Figure 3.16C), whereas in the dark after five days there was a significant reduction in algal biomass

with increasing stocking ($y = -3.50x + 3.94$; $r^2 = 0.93$; $p = 0.0004$; Figure 3.16D). In the dark, removal rate was highest at -69% at 20 kg/m^3; in the light at the same stocking density the algal biomass had decreased by -40% (Figures 3.16C&D). The algal removal rates of the fish in the dark are a more accurate indication of the rate of algal removal because there was no algal productivity in these treatments.

Table 3.7 Change in algal concentration at different fish stocking densities in the light and under dark conditions, over five days (regression analysis, $p<0.05$).

Light/Dark	Density (kg/m^3)	Model	r^2	p
Light	0		0.33	0.23
Light	0.63	y = 0.010x + 0.5597	0.67	**0.04**
Light	1.25		0.00	0.98
Light	2.5	y = 0.041x + 0.5034	0.75	**0.02**
Light	5	y = 0.040x + 0.5169	0.88	**0.01**
Light	10	y = -0.033x + 0.6771	0.95	**>0.01**
Light	20	y = -0.055x + 0.7183	0.97	**>0.01**
Dark	0		0.16	0.43
Dark	0.63		0.60	0.07
Dark	1.25	y = -0.010x + 0.6327	0.81	**0.02**
Dark	2.5		0.44	0.15
Dark	5		0.57	0.08
Dark	10	y = -0.030x + 0.6067	0.99	**>0.01**
Dark	20	y = -0.008x + 0.7044	0.94	**>0.01**

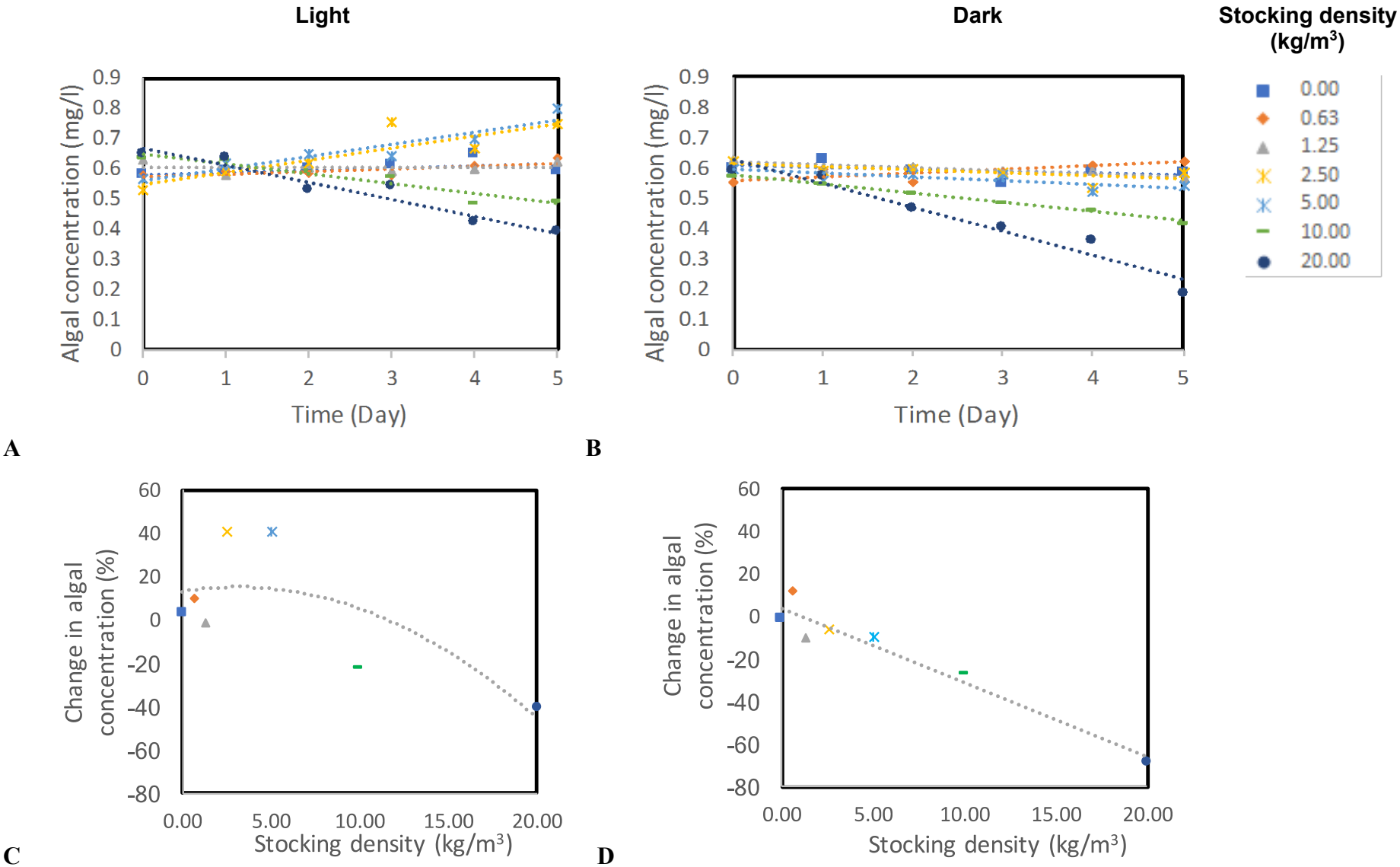

Figure 3.16 The algal concentration of HRAP effluent subject to different stocking densities of tilapia, under (A) light and (B) dark conditions, over five-days (the models for all significant slopes are presented in Table 3.7); and (C) percent change in algal biomass after five days in (C) the light ($p = 0.09$) and (D) the dark ($y = -3.50x + 3.94$; $r^2 = 0.93$; $p = 0.0004$).

Effect of stocking density on water quality

The different stocking densities show similar trends for pH and dissolved oxygen and both parameters remained similar across the stocking densities (Table 3.8). Ammonia and nitrite increased with an increase in stocking density ($y = 0.0556x + 0.023$, $r^2 = 0.84$ and $y = 0.0504x + 0.069$, $r^2 = 0.74$ for ammonia and nitrite, respectively; Table 3.8).

Table 3.8 The pH, dissolved oxygen (DO) ammonia and nitrite at the start and end of the stocking density trial.

Parameter	Day	Stocking density (kg/m^3)						
		0	0.63	1.25	2.5	5	10	20
pH	0	6.53	6.59	6.57	6.51	6.56	6.47	6.58
	5	9.17	8.92	8.99	9.03	9.16	8.87	8.98
DO (mg/L)	0	10.15	10.41	12.53	11.28	11.71	10.64	10.56
	5	11.42	11.01	11.67	11.82	11.74	11.59	11.72
NH_4-N (mg/L)	0	0.07	0.10	0.06	0.07	0.06	0.06	0.06
*	5	0.06	0.06	0.10	0.08	0.10	0.93	1.02
NO_2-N (mg/L)	0	0.02	0.02	0.02	0.02	0.03	0.02	0.02
**	5	0.02	0.03	0.02	0.03	0.58	0.92	0.87

* $y = 0.0556x + 0.023$; $r^2 = 0.84$

** $y = 0.0504x + 0.069$; $r^2 = 0.74$

3.4.4 Conclusion

Mullet could not be stocked at more than 10 kg/m^3 in HRAP effluent without compromising health and survival, whereas the stocking density of tilapia was successfully increased to 20 kg/m^3 without negative effects on the fish. The rate of algal removal over five days was approximately 40% of the starting algal biomass in the light; however, this was increased to nearly 70% over the same period if photosynthesis was stopped and the experiment was carried out in the dark.

Chapter 4 – Optimizing the process to remove algae from HRAP using fish

4.1 Introduction

High rate algal ponds are efficient effluent treatment systems; however, the removal of microalgae cells after treatment remains a constraint in the use of this technology. Conventional methods of removing particulate matter include: in pond chemical precipitation, coagulation, flocculation, centrifugation and filtration (Parks *et al.* 2011). These methods can be costly, energy intensive and can contaminate the settled matter (Parks *et al.* 2011).

The application of in situ filter-feeders has been proposed to remove microalgae and to convert algal biomass into fish biomass, which can be removed from the system more easily (Jones *et al.* 2014). The use of fish culture to remove microalgae has been studied previously; however, extreme environmental conditions in the experiment, which included pH and oxygen fluctuations, compromised the study since the fish were subject to physiological stress. If the harsh environmental conditions could be mitigated, it would effectively reduce physiological stress on fish and removal efficiency of microalgae by fish may be improved.

Two fishes capable of filter-feeding were identified and included Mozambique tilapia (*Oreochromis mossambicus*) and freshwater mullet (*Myxus capensis*). Mozambique tilapia can tolerate a range of environmental conditions, stocking densities and diet (Dempster *et al.* 1996). However, previous studies show that these fish were negatively affected by the extreme environments, which could have compromised their general health and feeding behaviour (Jones *et al.* 2014). Freshwater mullet has been suggested as a more suitable fish species to remove microalgae due to the morphology of their gill structure. This fish is endemic to South Africa and lives in coastal estuaries and rivers (Skelton 2001). There have been few studies on freshwater mullet food preference but related striped mullet (*Mugil cephalus*), which share a similar environment, consume microalgae as a primary dietary source (Odum 1986). The striped mullet has the ability to select particles smaller than 15 µm and particles smaller than 10 µm make up 80% of the stomach contents, due to pharyngeal filtering in the gills (Odum 1986). However, the mullet is probably less able to cope with the extreme environment in the HRAP system, so moderation of this environment will be necessary for survival. Under the right environmental conditions, these fish may provide the solution to algal biomass conversion to fish biomass.

The overall aim for the experiment was to determine if using CO_2 or sulphuric acid to reduce pH will increase algal removal by tilapia and mullet. The objectives were to:

1) compare algal removal rates when HRAP pH was moderated using CO_2 or acid; and to
2) determine which species of fish was better suited for algal removal in HRAP effluent.

4.2 Methods and materials

Mullet and tilapia described in Section 3.4.2 were used in this experiment. The experiment was conducted in the same facility, using the same HRAP water, which was described in Section 3.3.2. It was carried out in the dark by placing a grey polyvinylchloride sheet over each tank.

A 500 mL zeolite filter was placed in each tank to remove the ammonia excreted by the fish. The factors in this experiment included pH manipulation (i.e. CO_2 addition or sulphuric acid addition) and fish species (i.e. mullet or tilapia; Table 4.1). Each treatment was replicated three times. Mullet and tilapia were stocked at 10 kg/m^3 and 20 kg/m^3 in the experimental rectangular tanks. The pH of the water was adjusted to 6.5 using CO_2 or 98% sulphuric acid as described in Section 3.3.2. This adjustment was made at the start, and no further pH manipulation took place for the six-day duration of the experiment.

Table 4.1 Experimental treatments (T1-T6) used to determine the effect of pH adjustment and fish type on algal removal rate.

Fish	Method of pH adjustment	
	CO_2	Sulphuric acid
Tilapia	T1	T2
Mullet	T3	T4
No fish	T5	T6

The pH and dissolved oxygen of the water were recorded using an electronic probe (Hanna, HI 991300, United Kingdom). Algal, ammonia and nitrite concentrations were recorded once a day using the methods described in Section 3.4.2.

Data were analysed using the same statistical analysis as described in Section 3.3.2.

4.3 Results and discussion

Effect of fish type and pH adjustment method on algal concentration

The concentration of algae in HRAP effluent was not influenced by an interaction between fish type and pH adjustment method (multifactor repeated measures ANOVA, $F_{(12,71)}$=1.51, p<0.1421; Figure 4.1). Over six days, tilapia removed significantly more algae than mullet from HRAP effluent (multifactor repeated measures ANOVA, $F_{(12,72)}$=44.32, p<0.0001; Figure 4.2), which was to be expected since tilapia were stocked at double the density. Fish removed algae at a significantly faster rate from tanks where the pH was adjusted with sulphuric acid when compared to tanks with CO_2 pH adjustment (multifactor repeated measures ANOVA, $F_{(1,12)}$=22.73, p<0.0005). The change in algal biomass levelled off after three days for both fishes subject to CO_2 adjustment, with no significant changes between day 3 and day 5 (Figure 4.1). Similarly, algal removal levelled off for mullet after day 3 in the acid-adjustment treatment; however, the algal concentration was still on a downward trend at day 6 for the tilapia in the acid treatment, which suggests that they might have removed even more algae had the experiment been extended beyond six days (Figure 4.1). The algal concentration remained constant between both CO_2 and acid pH adjusted treatments when no fish were present (Figure 4.1).

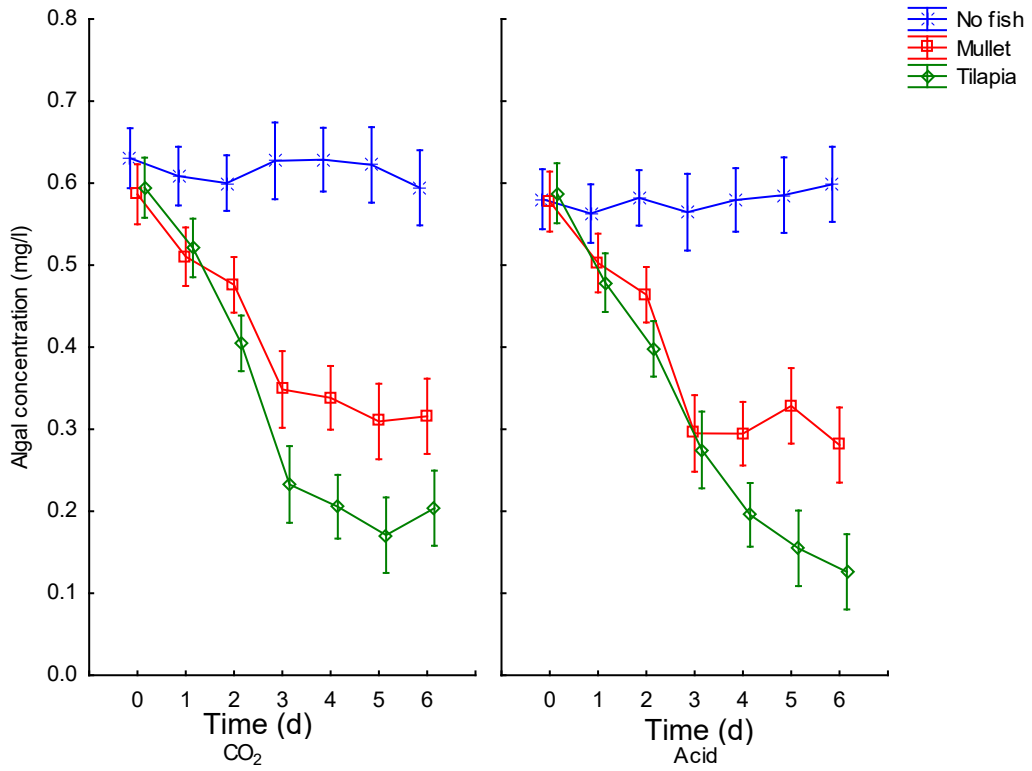

Figure 4.1 The mean (± 95% confidence interval) dry weight algal concentration of HRAP water containing mullet or tilapia where the pH was either adjusted with carbon dioxide or sulphuric acid (multifactor repeated measures ANOVA, $F_{(12,72)}=1.51$, $p=0.1421$).

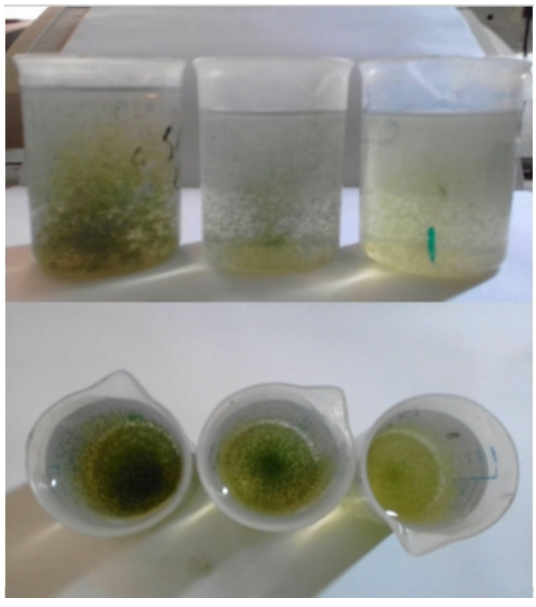

Figure 4.2 Water samples showing algae concentration of water subject to no fish, mullet and tilapia; after six days (from left to right).

Overall, tilapia removed more algae than mullet; however, tilapia had a higher biomass per unit volume of water since they were stocked at double the density. When comparing the species corrected for the difference in fish biomass, mullet was more effective in removing algae (per unit weight of fish). This is probably due to the prevalence of algae, diatoms and detritus in the mullet's natural diet (Bok 1983), whereas tilapia are more generalist feeders (i.e. have a wider range of food sources) and do not rely on a microalgae diet. Dempster *et al.* (1996) found that when algal biomass is the only available food source, tilapia struggle to fulfil basic maintenance, which may decrease foraging activity. This may explain why mullet are more effective at algal removal when viewed as algae removal per biomass of fish. Tilapia were still more efficient since they can be stocked at a higher rate and this stock removed more algae.

The mullet stocking density was limited to 10 kg/m^3 due to high mortality rate observed above 10 kg/m^3. A possible solution to this problem is using a similar species such as the flathead mullet (*Mugil cephalus*). In a study to determine the potential of freshwater mullet and flathead mullet for aquaculture, it was found that *M. cephalus* had higher growth rates in freshwater than in saltwater, while *M. capensis* had decreased growth rates in freshwater compared to saltwater (Bok 1983). Growth rates were occasionally up to six times greater for *M. cephalus* and it displayed greater growth rates even in turbid waters (Bok 1983). Maximum size of *M. cephalus* are recorded as approximately 60 cm while *M. capensis* is approximately 45 cm (Bok 1983; Skelton 2001). Survival rates in freshwater were also higher for *M. cephalus* when compared to *M. capensis* (Bok 1983). Therefore, future research could focus on using *M. cephalus* as a candidate species as it may provide increased conversion of algal biomass into fish biomass. The Bok (1983) study was done at relatively low stocking densities. Future studies should investigate *M. cephalus* at higher stocking densities.

Effect fish type and pH adjustment method on water quality

The pH of HRAP water was not influenced by an interaction between fish type and pH adjustment method (multifactor repeated measures ANOVA, $F_{(12,72)}$=0.87, p=0.58). Type of fish also had no influence on the pH of HRAP effluent (multifactor repeated measures ANOVA, F(2,12)=1.81, p=0.2582). On the other hand, the pH of HRAP water adjusted with sulphuric acid was significantly lower than the pH of HRAP water adjusted with carbon dioxide (multifactor repeated measures ANOVA, $F_{(2,12)}$=742.57, p<0.0001; Figure 4.3), and by the end

of the study the mean sulphuric acid pH was still below 9.0 making it suitable for holding fish and maintaining fish health.

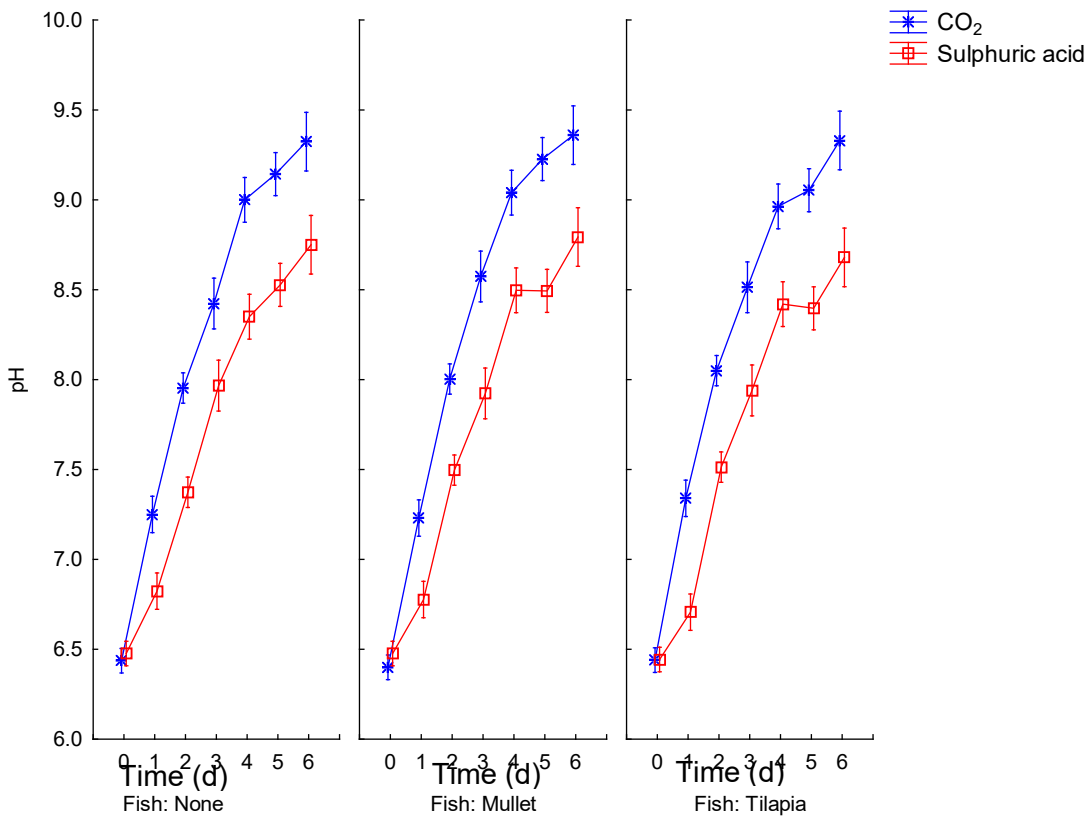

Figure 4.3 The mean (± 95% confidence interval) pH of HRAP water containing no fish, mullet or tilapia, subject to pH adjustment using carbon dioxide or sulphuric acid (multifactor repeated measures ANOVA, $F_{(12,72)}$=0.87, p=0.58).

The use of CO_2 to decrease the pH of HRAP effluent to 6.5 resulted in significantly lower starting DO concentrations (4.5 ± 0.12 mg/L) when compared to sulphuric acid (11.4 ± 0.13 mg/L; Table 4.2). After five days, the interaction between pH adjustment and fish type had no influence on the DO concentrations of HRAP effluent (Table 4.2). Algal water containing fish had a lower DO concentration, when compared to the no fish treatments, with tilapia treatments having the lowest DO levels, which was due to their high stocking density (Table 4.2). The ammonia and nitrite concentrations were similar between all treatments at the start of the experiment (Table 4.2). After six days, tanks that contained fish had significantly higher ammonia and nitrate concentration than tanks with no fish (Table 4.2), but these were maintained at a level suitable for fish due to the zeolite filters.

Algal removal and an increase in ammonia and nitrite represent a trade-off. This means that in order to increase algal removal, fish biomass must increase, which in turn increases ammonia and nitrite levels. High levels of ammonia and nitrite are toxic to fish as they may lead to kidney damage, reduced growth rates, possible brain malfunction as well as a reduction in the oxygen-carrying capacity of the fish (Lewis and Morris 1986; Hargreaves and Kucuk 2001; Randall and Tsui 2002). This suggests that the water needs to be further treated before it can be reused; this could include passing the effluent through a biological filtration system or the inclusion of larger zeolite filters to mitigate the build-up of ammonia.

4.4 Conclusion

The pH of the effluent can be moderated using either CO_2 or sulphuric acid since both resulted in similar rates of removal. Sulphuric acid maintained a lower pH compared with CO_2 over the six-day duration of this trial.

Tilapia and mullet both reduced the concentration of algae that was used to treat brewery effluent. Although mullet was better equipped to remove the algae, tilapia removed more algae as it was possible to stock this fish at twice the stocking density of the mullet without compromising their health and survival. Tilapia removed approximately 70% of the suspended solids that were greater than 8.0 µm in size from the HRAP effluent, when stocked at a density of 20 $kg.m^{-3}$ and when pH was maintained below 9.0.

Table 4.2 The mean (± 95% confidence interval) start and end water quality parameters of HRAP effluent containing no fish, mullet or tilapia subject to pH adjustment with either sulphuric acid or carbon dioxide. Values in the same row represented by a different superscript symbol represent significantly different treatment means (ANOVA, $P<0.05$).

Parameter	Day	Tilapia		Mullet		No fish		F/H value	P Value
		Acid	CO_2	Acid	CO_2	Acid	CO_2		
DO (mg/L)	0	11.57 ± 0.09 [a]	4.57 ± 0.09 [b]	11.37 ± 0.09[a]	4.60 ± 0.15 [b]	11.47 ± 0.18 [a]	4.33 ± 0.15 [b]	H = 13.75	0.017
	6	6.77 ± 0.03 [a]	5.73 ± 0.29 [b]	6.70 ± 0.06 [a]	6.33 ± 0.21 [a]	7.77 ± 0.32 [c]	7.17 ± 0.25 [c]	F = 30.88	0.001
NH_4-N (mg/L)	0	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	F = 0.69	0.520
	6	1.69 ± 0.09 [a]	1.81 ± 0.09 [a]	1.34 ± 0.04 [a]	1.06 ± 0.01 [a]	0.03 ± 0.01 [b]	0.03 ± 0.01 [b]	H = 15.79	0.007
NO_2-N (mg/L)	0	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	F = 0.53	0.949
	6	0.85 ± 0.06 [a]	0.85 ± 0.05 [a]	0.40 ± 0.02 [b]	0.46 ± 0.04 [b]	0.02 ± 0.01 [c]	0.01 ± 0.00 [c]	H = 15.96	0.007

Dissolved oxygen (DO)

Chapter 5 – Conclusion and recommendations

The maximum biomass for mullet was 10 kg/m^3 due to mortality occurring at stocking densities higher than this. The ideal stocking density for tilapia was 20 kg/m^3 fish because algal removal was the greatest. In addition, algal removal was greater in dark treatments compared to light treatments due to the lack of photosynthesis and algal productivity in the dark.

The biomass of fish needed to remove algae resulted in an increase in dissolved ammonia and nitrite concentrations, and the algae in the tanks did not mitigate this build-up. These compounds are toxic to fish and under high stocking densities may reach lethal concentrations. An ammonia removal filter needs to be incorporated into the system to decrease the build-up of ammonia if high stocking densities are used in future work. As such, it was suggested that a zeolite filter be used to remove the ammonia excreted by the fish if the higher densities are used.

Tilapia are able to decrease the algal concentration in water more effectively than mullet. This may be due to tilapia having a higher tolerance of the extreme environmental conditions, which allowed for higher stocking densities used in the experiments. When viewing algal removal per mass of fish, mullet may well be more effective in removing algae from HRAP. Other species of mullet, for example *M. cephalus*, may allow for higher biomass as they display greater survival and growth rates when compared to *M. capensis*. This should be considered in future research.

Fish removed algae at a significantly faster rate when sulphuric acid was used for pH adjustment, compared to when carbon dioxide was used for pH adjustment. This may be due to the lower pH when using sulphuric acid compared to carbon dioxide, or the initially low DO levels when using carbon dioxide.

In conclusion, fish can be used to remove algae from post-HRAP effluent, making the treated water available for reuse. By manipulating the environment, such as covering the tanks to stop photosynthesis and algal productivity, using acid to buffer pond pH, and by selecting a species such as tilapia that can withstand the environmental fluctuations of an algal pond, and by ensuring that oxygen in the water is not depleted, it was possible to remove a substantial portion

of the algae from the pond. To achieve a 70% reduction of the starting algal biomass, over six-days, the following process provided the best result:

- post-HRAP effluent placed into stagnant pond;
- the pH of the effluent lowered to 6.5 using sulphuric acid;
- the tanks covered to eliminate light;
- fish tanks stocked with Mozambique tilapia; and
- using stocking density of 20 kg/m^3.

Based on current laboratory scale investigations, future work needs to test this process on a pilot-scale. In addition, the process described here is not limited to treatment of brewery effluent, but probably universally applicable for treatment of other similar effluent streams, and this also requires future investigation.

However, the use of fish to remove algae from HRAP effluent may be counterproductive if the fish themselves pollute the water with ammonia such that it has to be re-treated before it can be reused or released into the environment. Future research should therefore also focus on minimising the impact of deteriorating water quality when high stocking densities are used to remove algae. In this regard, the addition of zeolite filters or biological treatment or polishing in a wetland could be considered, after the algae is removed by the fish, to remove residual ammonia.

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