

The integration of effluent treatment using constructed wetlands, with crop production and aquaculture

A thesis submitted in fulfilment of the requirements for the degree:

Master of Science

at



Rhodes University

by

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March 2019

Abstract

Breweries are major consumers of water and produce nutrient rich wastewater. Therefore, it is important to research technologies that optimise the reuse and recycling of this waste. This study compared different constructed wetlands (CWs) in terms of their potential for cleaning brewery effluent and simultaneously producing crops *Beta vulgaris* and fish *Oreochromis mossambicus*. Filling and draining times (FDT; 15 min, 30 min and 60 min) in tidal CWs were compared in Experiment 1. The 15 min FDT (6.52 ± 0.09 mg/l) and 30 min FDT (5.74 ± 0.09 mg/l) had higher dissolved oxygen (DO) than the 60 min FDT (5.40 ± 0.09 mg/l; $p < 0.05$). This resulted in the 15 and 30 min FDT treatments reaching ammonia effluent discharge standards sooner than the 60 min FDT. Total plant harvest increased with increasing FDT; therefore, 15 min FDT was used as the FDT in tidal treatments in the following experiments. The aerated CW (5.81 ± 0.07 mg/l) and tidal CW (5.67 ± 0.07 mg/l) treatments had higher DO concentrations than the unaerated CW treatment (3.76 ± 0.07 mg/l; $p < 0.05$) in Experiment 2. This resulted in lower ammonia concentrations on day 5 in the aerated and tidal CWs compared with the unaerated treatment ($p < 0.05$). The tidal CW (23.97 ± 2.57 kg) had a total harvest that was approximately four times higher than the unaerated CW ($p < 0.05$), which had the highest frequency of chlorosis and plant mortality; and was unable to treat ammonia to discharge standards. In Experiment 3, the aerated and tidal CW were compared with municipal-water as water sources for aquaculture. There were no differences in fish growth ($p > 0.05$). However, there were differences in water quality; with the municipal treatment having the lowest pH, EC and nitrate concentration ($p < 0.05$); but all water quality parameters remained in a range suitably for fish production. Due to the tidal CW having the highest plant harvest and lowest frequency of chlorosis and mortality; it was the most suitable CW technology to clean the brewery effluent, and to produce *B. vulgaris* and water that could be used downstream in aquaculture.

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List of abbreviations

AD	Anaerobic digester
AHSSF	Aerated horizontal sub-surface flow
AS	Activated sludge
BOD	Biological oxygen demand
CCI	Chlorophyll concentration index
CF	Condition factor
COD	Chemical oxygen demand
CW	Constructed wetland
CWs	Constructed wetlands
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
EPA	Environmental protection agency
FCR	Feed conversion ratio
FWS	Free water surface
HF	Horizontal flow
HSSF	Horizontal sub-surface flow
LCA	Life cycle analysis
Pty Ltd	Proprietary limited
PVC	Polyvinyl chloride
SAB	South African Breweries
SGR	Specific growth rate
SSF	Sub-surface flow
UHSSF	Un-aerated horizontal sub-surface flow
VF	Vertical flow
VSSF	Vertical sub-surface flow

Acknowledgements

I would first like to thank my family, and Viv Dames and Prof. Jo Dames for their support and guidance through this journey.

I would like to thank my supervisor Prof. Cliff Jones. Firstly, for sourcing funds from Rhodes University Research Committee account and secondly, for his patience, advice and willingness to guide me whenever it was needed. Thank you to William Selapa for providing funding through DAFF for my water quality test kits, the staff of the Department of Ichthyology and Fisheries Science for their academic and administrative support. Special thanks must go to Bulelwa Mangali and Yvain Erasmus for taking care of all financial admin during the last two years. Thank you to Prof. Brad Ripley from the Botany Department for letting me use the equipment in his laboratory.

Special thanks must be given to employees at Ibhayi Brewery, especially Ntsapo, Deon, Pravendhran, Trevor and Corné for providing information and support throughout my time spent at the brewery. Thank you to the employees of Proxa: Olivia, Fortune, Khaya and Renier who were always welcoming and supportive when I needed their advice. A very special thank you to my amalume Ronald for your ability to cheer me up on the bad days, all your help with my system and the tunnel, all the jokes and for your friendship over the last two years.

Finally, a very special thank you must go to Richard Taylor for all your help and advice with my project, all the days and nights spent fishing and the daily braais. It was a pleasure living with you for the past two years.

Chapter 1: Introduction.

Water, energy and food shortages are an increasing concern in South Africa and the rest of the world (Rodda *et al.* 2011, Rasul 2014). Industrialisation led to wealth and power to numerous cultures, however, it did so at the expense of the environment, biodiversity, and the stability of Earth's self-regulating systems (Todd *et al.* 2003). This has also led to more wastewater production and has reduced the amount of suitable water for human consumption (Hanjra and Qureshi 2010); and increase in the pollution and degradation of most freshwater systems (Hanjra and Qureshi 2010, Rodda *et al.* 2011). Due to water shortages, it is vital that research is done to develop technologies that will optimise the reusing and recycling of water and nutrients (Hanjra and Qureshi 2010, Simate *et al.* 2011).

Breweries use large quantities of water and produce nutrient rich wastewater (Braeken *et al.* 2004). Strict regulations are costly and problematic for breweries as the treatment process is expensive and energy demanding (Simate *et al.* 2011). Brewery effluent contains nitrogen and phosphorus which are essential plant nutrients (Freeman 2005, Power and Jones 2016) but are considered pollutants and prevent its use as an aquaculture water source. These nutrients provide brewery effluent with the potential for reuse in crop growth (Kumar *et al.* 2010) where the crops contribute towards nutrient removal, allowing the resultant effluent to be reused downstream, in activities such as aquaculture.

Constructed wetlands (CWs) have been mentioned as suitable effluent treatment systems (Vymazal 2010, Vymazal 2011a). They are engineered systems that are designed to utilise natural processes by mimicking natural wetland systems (Kivaisi 2001). Using these systems for wastewater treatment gives the potential to utilise wastewater for biomass production and seasonal agriculture (Kivaisi 2001). Advantages of CWs include: low energy input, high

level of treatment, low maintenance, reliable water quality, oxygen input and carbon dioxide consumption via vegetation (Sundaravadiel and Vigneswaran 2001).

Rhodes University has been working with South African Breweries (SAB Ltd) using integrated systems to recover water and nutrients in brewery effluent for downstream reuse (Power and Jones 2016, Taylor *et al.* 2018). Horizontal sub-surface flow wetlands have been used to treat brewery effluent to standards suitable for aquaculture. However, due to the low oxygen concentration of effluent in the wetlands, the type of plants that can be grown in the wetland and the rate of nutrient removal is limited. In addition, the problem with conventional wetland plants is that they do not have high economic value. A potential solution that may ensure the treatment process generates income, would be a constructed wetland system that provides suitable conditions to produce cash crops and fish downstream.

1.1 Problem identification

In order to produce cash crops, most of which are terrestrial, a CW requires sufficient oxygen and nutrients for the plants to grow, while ensuring that the plants are safe for human consumption. Effluent treated in CW can be used in fish culture; however, for downstream aquaculture, the water needs to be treated sufficiently to maintain fish health and growth.

Effluents vary in terms of water quality parameters and microorganisms. The effects of these are important to understand when testing a technology to treat the effluent for reuse and recycling as different technologies offer different treatment options and therefore, produce different environmental conditions. When this effluent is untreated and discharged into the environment it may lead to eutrophication, primary production decrease and fish kills due to depleted oxygen levels (Oluwande *et al.* 1983, Emongor *et al.* 2005). It is therefore important to treat the effluent before discharging into the environment.

Horizontal sub-surface flow wetlands have been used to treat effluent in the past (Vymazal 2009). The problem with these systems is that only aquatic macrophytes can be grown as the filtration beds often suffer from a lack of oxygen (Stottmeister *et al.* 2003, Vymazal 2009). Aquatic macrophytes can survive as they are adapted to low oxygen and extreme rhizosphere conditions (Stottmeister *et al.* 2003). These plants tend to decrease oxygen loss to the rhizosphere and have air spaces, known as aerenchyma tissue, in various parts of the leaves, stems and roots which allows them to grow in anaerobic or anoxic environments (Vymazal 2011b). The roots of most commercial crops require oxygen for respiration which it obtains directly from the atmosphere and cannot survive waterlogged conditions (Stottmeister *et al.* 2003). Therefore, it is important to monitor dissolved oxygen as post AD effluent has low oxygen levels due to denitrification, which requires anaerobic conditions, being the dominant process (Botheju and Bakke 2011).

Some raw effluents have high concentrations of salt (Muyen *et al.* 2011) and this concentration can increase during treatment due to the evaporation of water (Muyen *et al.* 2011). The total dissolved salts in effluent can be detrimental to plants if > 1500 mg/l and may result in salt build up which affect plant yields (Muyen *et al.* 2011, Schröder and Lieth 2002). High salinity adds osmotic stress to plants and can inhibit water and nutrient uptake (Schröder and Lieth 2002). Post brewery effluent has high concentrations of salt (Taylor *et al.* 2018) and therefore, it is important to monitor and determine its effect on plant and fish health in the system.

The pH of water is known to affect the availability and form of nutrients to plants (Power and Jones 2016). Breweries effluent pH varies from 3-12 (Simate *et al.* 2011); and post-anaerobically digested effluent is on average 7.82 (Power and Jones 2016, Taylor *et al.* 2018).

Previous studies show that adjusting pH to 6.0 - 6.5 doubled the height and significantly increased the weight of tomato plants (*Solanum lycopersicum*; Power and Jones 2016). It is important to monitor pH to see if the constructed wetland can self-regulate this water quality parameter.

The levels of these nutrients in brewery effluent depend on the amount of raw material and yeast in the effluent (Braeken *et al.* 2004). Nutrients found in brewery effluent (e.g. nitrogen and phosphorous) are considered essential elements for plant growth (Epstein and Bloom 2005, Kamthunzi 2015). The form of nitrogen-based metabolites are also important as high levels of unionised ammonia is toxic to plants and fish (Randall and Tsui 2002, Kamthunzi 2015). Therefore, it is important to assess the nutrients and the form of nitrogen-based metabolites to ensure there is sufficient amounts for plants to uptake while maintaining concentrations below toxic levels for fish.

The different types of CWs and the flow-regime of effluent through them will result in differences in water quality parameters. These water quality parameters may affect plant growth as well as the suitability of the water for use in aquaculture. Thus, a constructed wetland needs to treat wastewater sufficiently while maintaining integrity of the water for plant production and other downstream water uses.

1.2 Literature review

Less than a century ago it was assumed that there was sufficient fresh water in the world to sustain the human population (Simate *et al.* 2011). However, since the 1960s the number of water scarce countries has increased, especially in developing countries (Kivaisi 2001). In addition to this, water pollution as well as degradation of water resources are also growing issues across the world (Wallace 2000, Wu *et al.* 2015). Major water pollution sources include

untreated sewage and industrial wastes (Kivaisi 2001); and the decrease in quality of fresh water complicates the water scarcity concern (Kivaisi 2001). The increase in industries over the last century has increased wastewater production and has thus limited clean water for human consumption (Hanjra and Qureshi 2010). With an increase in population, industrial activities and stricter environmental requirements, the cost of water is increasing (Simate *et al.* 2011, Mo and Zhang 2013).

At the same time, the demand for food is also increasing. The global population will increase by approximately 3.7 billion people by 2050, and this will not only contribute substantially to concerns related to fresh water availability (Mo and Zhang 2013, Kumar and Cho 2014), but to concerns related to food security and suitable soil for agriculture (Kumar and Cho 2014).

Urbanisation is one of the most important trends in the twenty first century which means the land available for agriculture will decrease further (Parkinson and Tayler 2003). Therefore, opening new agriculture landscapes are unfeasible (Kumar and Cho 2014).

Within the framework of integrated natural resource management, wastewater is not only an effluent, but a renewable resource (Qadir *et al.* 2010). Emphasis has been placed on a more holistic approach to industrial waste disposal by reducing the quantity of waste disposed and by recycling and reusing the wastewater close to where it is produced (Parkinson and Tayler 2003). Water reuse is defined by Environmental Protection Agency (EPA; 2004) as “...the method of recycling treated wastewater for beneficial purposes, such as agricultural and landscape irrigation, industrial processes, toilet flushing, and groundwater replenishing.” When wastewater is sufficiently treated and recycled it can become an alternative source of water that can beneficially and cost-effectively reduce fresh water demand (Simate *et al.*

2011). Therefore, wastewater reuse has become an important consideration for industries (Simate *et al.* 2011).

The beer brewing wastewater must be disposed of or treated in the most economically viable and safest process to meet discharge regulations set to protect human life as well as the environment (DWAF 1998, Simate *et al.* 2011; Table 1.1). The treatment of water is becoming one of the largest resource consumers in the USA as these treatment facilities require close to a quarter of all public energy use (Mo and Zhang 2013). Wastewater treatment plants also consume numerous materials over time which can be defined as indirect energy. This accounts for almost two thirds of energy that is directly consumed in the water treatment process (Mo and Zhang 2013). The wastewater treatment process and penalties for failing to meet regulations have proven to be problematic and costly for most breweries (Simate *et al.* 2011, Mo and Zhang 2013).

Breweries are seeking ways to minimise water use as well as finding economically viable ways to treat and reuse wastewater (Simate *et al.* 2011). There is a need to identify potential alternative water, nutrient and energy resources and to develop the techniques needed to exploit their value (Simate *et al.* 2011, Power *et al.* 2016). Therefore, treatment technologies must be developed that improve the efficiency of cost, maintenance and that provide indirect benefits.

Table 1.1 General wastewater limit values applicable to discharge of wastewater into a water resource (DWAF 1998).

Parameter	General limit
Chemical oxygen demand (mg/l)	75
pH	5.5 - 9.5
Ammonia as nitrogen (mg/l)	3
Nitrate/nitrite as nitrogen (mg/l)	15
Phosphorous	10
Electrical conductivity (mS/m)	70 - 150 above intake

1.2.1 Brewery effluent treatment process

The anaerobic digester has low energy inputs (relative to activated sludge), and has low sludge production, with the added benefit of biogas production (Power and Jones 2016). Anaerobic digestion includes a series of biological conversion processes (Angelidaki and Sanders 2004). They convert organic carbon into a gaseous mixture comprising of mainly carbon and hydrogen, where methane (CH₄) is the most reduced carbon state and carbon dioxide (CO₂) is the most oxidised carbon state (Lyberatos and Skiadas 1999). Other by-products produced in the process include; nitrogen, nitrogen oxides, hydrogen, ammonia, hydrogen sulphide and other volatile compounds (Angelidaki and Sanders 2004). An integrated community of bacteria is responsible for this process (Lyberatos and Skiadas 1999). The effluent is then treated in an up-flow anaerobic sludge blanket reactor before being polished for reuse in an activated sludge system or discharged to the municipality for further processing (Taylor *et al.* 2018). The activated sludge (AS) treatment system, is a process where aeration is used to provide oxygen to the aerobic microorganisms that break down organic material (Appels *et al.* 2008). The water is then sent to a clarifier where solids are settled and removed. The effluent then passes through a multimedia, disc, ultra and carbon filters before being sent to a storage tank. It is then either sent to the reverse osmosis plants or used in cleaning. Reverse osmosis is a process where contaminants are removed by moving water through a semi-permeable membrane (Fathizadeh *et al.* 2011). The brewery has two reverse osmosis plants, one at the boiler house to feed the boilers and the other at the packaging line for use in the bottle washer for pre-rinsing as well as the pasteuriser.

1.2.2 Project Eden

The study will be performed at Project Eden's experimental facility at Ibhayi Brewery (SAB Ltd, Port Elizabeth, South Africa). Project Eden was designed to treat brewery effluent using alternative technologies that add value to the products that are produced in the treatment process (Jones *et al.* 2014, 2016). The facility consists of an integrated algal ponding system, a CW, aquaculture, hydroponic and crop production facilities (Jones *et al.* 2016).

Effluent from the anaerobic digester is sent to a primary facultative pond (PFP). Here, large molecules are broken down (Botheju and Bakke 2011). The removal of these molecules results in lowered chemical oxygen demand (COD) and biological oxygen demand (BOD) (Jones *et al.* 2014). Solids entering the PFP along with excess biomass, produced from an anaerobic sludge layer at the bottom of the pond, are broken down during anaerobic digestion. The result of this is the release of soluble organics into the water column. (Marais 1970, Tchobanoglous and Schroeder 1987). When in the water column, the soluble organics are metabolised by heterotrophic bacteria using oxygen, which is then replenished by oxygen from the photosynthesis of algae (Marais 1970, Tchobanoglous and Schroeder 1987). The bacteria produce carbon dioxide which the algae use for photosynthesis; this relationship results in the decrease of BOD and COD in the effluent (Marais 1970).

The water is then sent through high-rate algal ponds (HRAP). Here, organic matter is degraded by bacteria and fungi. This process results in the release of carbon dioxide (CO₂), phosphate (PO₄) and ammonia (NH₃; Jones *et al.* 2016). The concentrations of these substances are then decreased through various processes such as bacterial nitrification, algal assimilation, NH₃ volatilisation, continuation of the abiotic nitrogen and phosphorous cycles and PO₄ precipitation (Jones *et al.* 2016). These systems are effective at removing nitrogen and

phosphorous from the effluent (Jones *et al.* 2016). The water treated by HRAP have been used in aquaculture and hydroponics (Power and Jones 2016, Jones *et al.* 2018); but a problem with this system was the removal of solids (i.e. microalgal biomass; Jones *et al.* 2016), and the resulting treated effluent had high pH and EC averages of 9.28 and 2937.29 $\mu\text{S}/\text{cm}^2$ respectively (Jones *et al.* 2016). Therefore, research has been focused on using CWs for treatment of brewery effluent, where nutrients can directly be converted to crop biomass; and at the same time, treating the effluent to levels suitable for fish aquaculture.

1.2.3 Brewery effluent potential in agriculture

Brewery effluent contains high concentrations of organics, which includes nitrogen and phosphorous (Braeken *et al.* 2004, Vymazal 2014). Nitrogen and phosphorous are part of the 17 essential nutrients necessary for plant growth and health (Freeman 2005, Kamthunzi 2015). Brewery effluent has the potential to be a water source for growing crops (Kumar *et al.* 2010, Jones *et al.* 2014). Lettuce and tomatoes have been grown using brewery effluent in hydroponic systems (Jones *et al.* 2014, Power and Jones 2016) and in irrigated agriculture (Taylor *et al.* 2018), but all on an experimental-scale.

Post anaerobically digested brewery effluent has high conductivity (3019.95 $\mu\text{S}/\text{cm}^2$) and high pH (± 8.00 ; Jones *et al.* 2016). High conductivity can cause stress to plants and fish that are adapted to low salinities (Hasegawa *et al.* 2000, Nielsen *et al.* 2003). The potential build-up of salt when brewery effluent is used in agricultural crop irrigation or to grow crops in CWs, and its effect on crop production, requires further investigation.

Adjusting pH has shown to significantly improve plant health and growth (Power and Jones 2016). The pH of the growth media can influence the availability of nutrients (Peterson 1982). Constructed wetlands have been used to regulate pH of mine water (Kadlec and Wallace

2008), and generally have a neutral pH, provided there is limited algal growth, and can potentially lower brewery pH to improve crop production (Vymazal 2005).

1.2.4 Constructed wetlands for wastewater treatment

In the past, natural wetlands were used to treat wastewater, and are still used for this purpose under controlled conditions (Vymazal 2010). The growing knowledge of wetland function and value to the natural environment has resulted in a decrease in their use for wastewater treatment (Vymazal 2010). Constructed wetlands are the preferred option to treat various forms of wastewater and are regarded as “green” technologies (Vymazal 2011a, Wu *et al.* 2015). They are engineered systems that mimic the natural processes that wetlands offer but in a controlled environment (Kivaisi 2001, Sundaravadivel and Vigneswaran 2001, Vymazal 2010).

Constructed wetlands utilise aquatic plants, soil and microorganisms to treat contaminated wastewater through physical, chemical and biological mechanisms (Kivaisi 2001, Wu *et al.* 2015). One of the main biological components of these wetlands are aquatic macrophytes, which aids purification reactions by enhancing a range of removal processes which includes direct uptake of nitrogen, phosphorous and other nutrients (Wu *et al.* 2015). This contributes to the removal of nutrients by microorganisms in the CW (Wu *et al.* 2015). Nutrient removal depends on the type of CW as the type of microbes present and chemical transformations rely on specific environmental conditions such as redox potential and carbon availability for microbial growth (Vymazal 2007). Examples of nitrogen removal include: ammonia volatilization, nitrification, denitrification, nitrogen fixation, plant and microbial uptake, ammonification, nitrate-ammonification, anaerobic ammonia oxidation, fragmentation, sorption, desorption, burial and leaching (Vymazal 2007).

Constructed wetlands were developed for several reasons, namely: 1) to offset rate of conversion of natural wetlands from agriculture to urban development; 2) for flood control; 3) for the production of food and fibre; and 4) wastewater treatment to improve water quality (Sundaravadivel and Vigneswaran 2001). These systems have also been created with multi-use objectives such as using treated water for reuse in agriculture (Kivaisi 2001, Vymazal 2014). They are land intensive, cost effective, easily operated, easily maintained and have potential for other applications compared to conventional treatment systems (Kivaisi 2001, Wu *et al.* 2015). More conventional wastewater treatment technologies for example; activated sludge process, membrane bioreactors and membrane separation are expensive and do not facilitate widespread application due to their high-tech nature (Wu *et al.* 2015).

1.2.5 Types of constructed wetlands

Constructed wetlands are categorised by the wetland's hydrology and flow path (Vymazal 2014). They can be classified into free water surface (FWS) and sub-surface flow (SSF) CWs based on hydrology (Kivaisi 2001, Wu *et al.* 2015; Figure 1.1).

The FWS wetlands simulate natural wetlands where wastewater flows over a saturated substrate (Wu *et al.* 2015), and often consist of shallow basins or channels, with a substrate suited to support rooted vegetation (Vymazal 2014). These have aerobic, anaerobic and anoxic zones (Vymazal 2014; Figure 1.2), and wastewater flows through the substrate where plants grow (often limited to aerobic zones). The SSF wetlands can be distinguished between vertical and horizontal wetlands according to their flow direction (Wu *et al.* 2015; Figure 1.1).

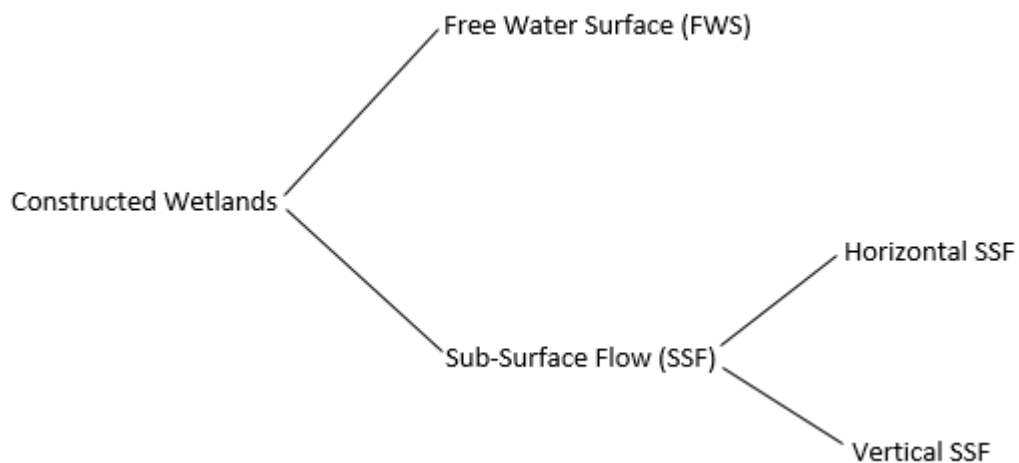


Figure 1.1 Simplified classification of constructed wetlands based on hydrology.

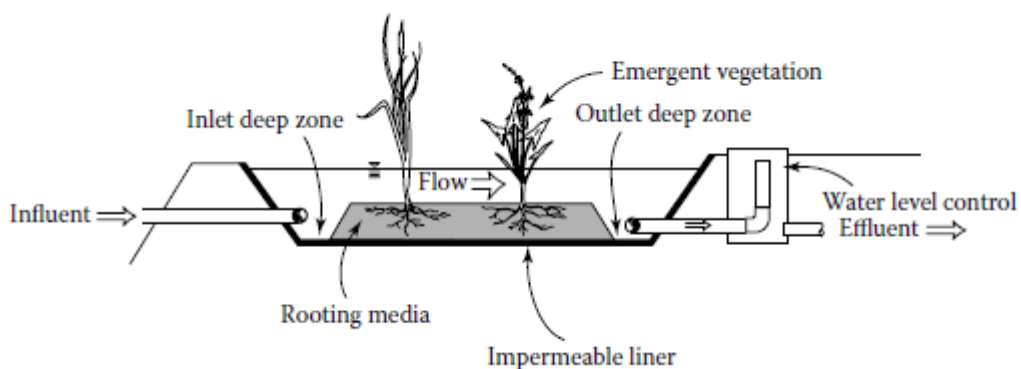


Figure 1.2 Basic elements of a free water surface wetland (Kadlec and Wallace 2008).

In horizontal sub-surface flow (HSSF) wetlands wastewater flows at a constant depth below the surface of the substrate where emergent vegetation is grown (Garcia *et al.* 2010, Vymazal 2010, Vymazal 2014; Figure 1.3). During the flow of the water through the HSSF system, wastewater runs through aerobic, anoxic and anaerobic zones (Vymazal 2014). Vertical sub-surface flow (VSSF) wetlands have a flat bed of gravel where plants are grown; which is fed with wastewater until it is flooded (Vymazal 2014; Figure 1.4). Thereafter the wastewater

drains completely, and this allows the bed to fill with air which oxygenates the system (Vymazal 2010, Vymazal 2014). These wetlands have better oxygen transfer from the atmosphere into the system when compared to HSSF wetlands, which is known to improve nitrification (Zurita *et al.* 2009).

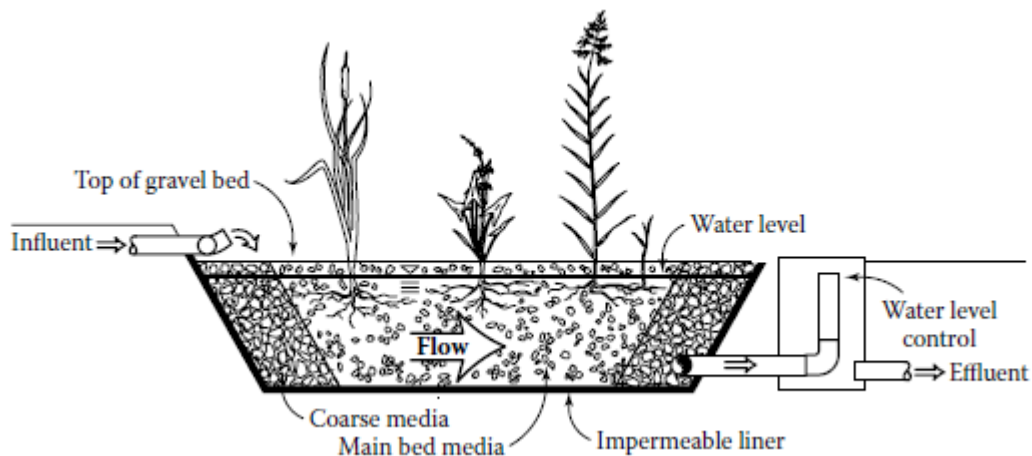


Figure 1.3 Horizontal sub-surface flow wetland schematic (Kadlec and Wallace 2008).

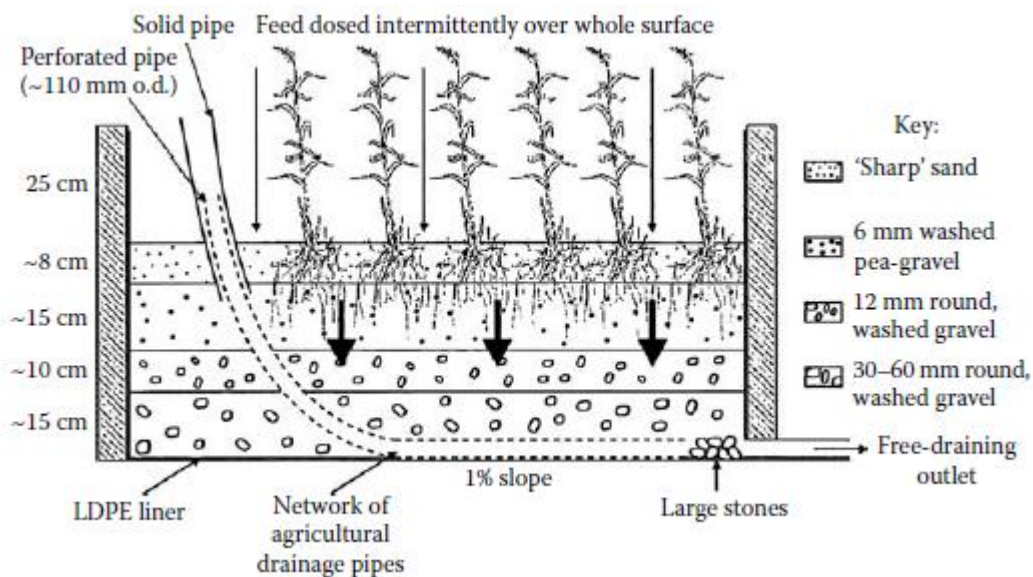


Figure 1.4 Typical arrangement of a vertical flow constructed wetland (Kadlec and Wallace 2008).

1.2.6 Plants in wastewater treatment

Planted wetlands outperform unplanted wetlands due to the microbial community within the rhizosphere (Vymazal 2011b). Plants are not only important in the treatment of effluent but can be harvested for economic gain (Vymazal 2011b). They are generally grown in soil as it acts as a medium for water uptake, nutrient uptake and support (Kamthunzi 2015) but they can also be grown in soilless environments, where substrates (i.e. sand, clay, gravel, perlite peat moss etc.) replace soil, on condition that the plants receive sufficient water, nutrients and support (Kamthunzi 2015). Plants require 17 essential elements (Epstein and Bloom 2005, Kamthunzi 2015; Table 1.2). These elements can be divided into two groups; macronutrients and micronutrients (Epstein and Bloom 2005, Kamthunzi 2015; Table 1.2). Macronutrients are required in high quantities and include; carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium and magnesium (Epstein and Bloom 2005; Table 1.2). These nutrients are used to produce nucleic acid, proteins, carbohydrates, phospholipids and other molecules (Epstein and Bloom 2005). Micronutrients are required in low quantities as they have roles in enzymatic activity (Epstein and Bloom 2005) which include; iron, manganese, zinc, copper, boron, molybdenum, chlorine and nickel (Epstein and Bloom 2005; Table 1.2). The function of these nutrients is shown in Table 1.3.

Table 1.2 Essential elements for plant growth and their concentration in dry tissue (Epstein and Bloom 2005, Freeman 2005, Kamthunzi 2015).

Essential elements			
Macronutrients		Micronutrients	
Nutrient	Concentration in dry tissue	Nutrient	Concentration in dry tissue
Carbon	45%	Iron	0.01%
Oxygen	45%	Zinc	0.002%
Hydrogen	6%	Manganese	0.005%
Nitrogen	1.5%	Copper	0.0006%
Potassium	1%	Chlorine	0.01%
Calcium	0.5%	Nickel	0.000005%
Phosphorous	0.2%	Boron	0.02%
Magnesium	0.2%	Molybdenum	0.0001%
Sulphur	0.1%		

Table 1.3 Essential elements for plant growth and their functions.

Essential elements			
Macronutrients		Micronutrients	
Nutrient	Function	Nutrient	Function
Carbon	Photosynthesis and organic molecules (Freeman 2005).	Iron	Chlorophyll synthesis, heme proteins, ferredoxin and iron-sulphur proteins. and enzyme cofactor (Epstein and Bloom 2005, Freeman 2005).
Oxygen	Cellular respiration and organic molecules (Freeman 2005).	Zinc	Enzyme activation, function, structure or regulation. (Epstein and Bloom 2005, Freeman 2005).
Hydrogen	Organic compounds; electrical balance and electrochemical gradients (Freeman 2005). Water is required to maintain turgor pressure in plants (Roberto 2005).	Manganese	An enzyme activator, water-splitting enzyme in photosystem II and enzyme superoxide (Epstein and Bloom 2005).
Nitrogen	Amino acids, nucleic acids, nucleotides, proteins, hormones, coenzymes and other metabolic entities (Epstein and Bloom 2005).	Copper	Enzymes involved in photosynthesis and respiration, component of lignin (Epstein and Bloom 2005, Freeman 2005).

Table 1.3 Continued...

Macronutrients		Micronutrients	
Nutrient	Function	Nutrient	Function
Potassium	Enzymes, cell osmotic adjustment and organic molecules (Epstein and Bloom 2005, Freeman 2005).	Chlorine	Enzyme system of photosystem II in which water is split and oxygen released (Epstein and Bloom 2005).
Calcium	Enzymes and cells, role in cell wall structure; membranes, movements of ions through membranes; transduction of signals (Epstein and Bloom 2005).	Nickel	Urease, an enzyme needed for nitrogen metabolism (Epstein and Bloom 2005).
Phosphorous	Metabolites dealing with energy acquisition (ATP). Nucleic acids, phospholipids, and several coenzymes (Epstein and Bloom 2005).	Boron	Binds to polysaccharides of the cell wall required for xylem differentiation. Ribonucleic acid (Epstein and Bloom 2005).
Magnesium	Chlorophyll, plant proteins and enzymes (Freeman 2005).	Molybdenum	Nitrogen acquisition and utilisation. Enzymes nitrate reductase and nitrogenase (Epstein and Bloom 2005).
Sulphur	Enzymes, proteins and coenzymes (Epstein and Bloom 2005).		

Nutrient deficiencies can cause a range of problems and it can be difficult to identify which nutrient is deficient. These deficiencies can be diagnosed through the presence of necrosis and chlorosis on plant leaves (Marchner 2011; Figure 1.5).

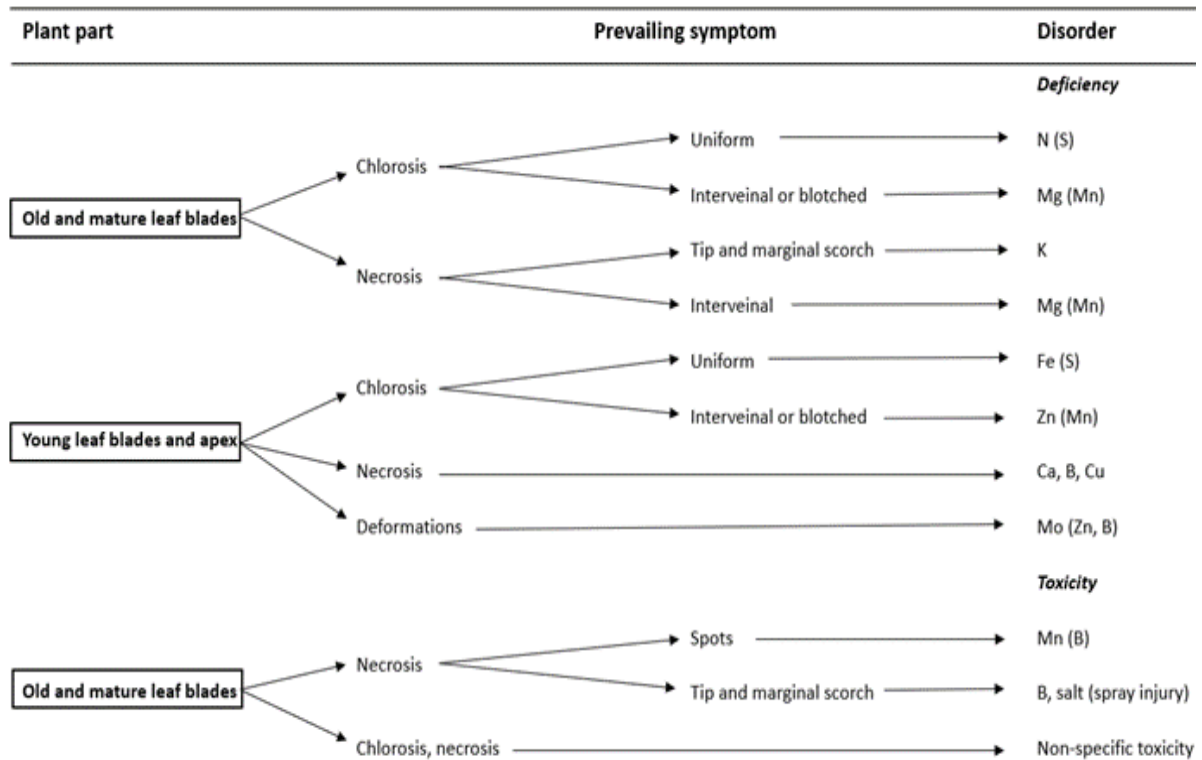


Figure 1.5 Some principles of visual diagnosis of nutritional disorders (Marschner 2011).

Plants used in CWs need to have the following properties: 1) tolerance to high organic levels and nutrient loading; 2) sufficient root and rhizome surface area for bacterial attachment and oxygenation; and 3) large aboveground biomass for harvesting to remove nutrients from the system (Vymazal 2011b). The role of plants in horizontal sub-surface flow (HSSF) wetlands are to: 1) release oxygen from roots to rhizosphere; 2) provide a substrate (roots and rhizomes) for bacteria; 3) provide carbon for microbial metabolism; 4) take up nutrients; and to 5) insulate the system at cooler temperatures (Vymazal 2010, Stefanakis and Tsihrintzis 2012, Wu *et al.* 2014). The oxygen input from roots in HSSF wetlands is low when compared to

aerated wetlands (Wu *et al.* 2014). Wetland and marsh plants work best in HSSF CWs as certain characteristics allow them to grow in extreme rhizosphere conditions (Stottmeister *et al.* 2003). The ability of the root system to supply oxygen from the atmosphere, through their aerenchyma tissue, is the reason why these plants survive anoxic conditions (Stottmeister *et al.* 2003). Marsh plant biomass is limited in terms economic utilisation as their production is seasonal and often without economic value (Vymazal 2011b).

Land plants (which include most vegetable cash crops) have adapted to dry conditions and cannot survive waterlogged conditions in horizontal sub-surface wetlands (Stottmeister *et al.* 2003). In tidal wetlands, the draining of the wetland increases the oxygen concentration which benefits root growth (Jia *et al.* 2010, Vymazal 2011a). The nitrification process in aerobic wetlands (converting ammonia into nitrate) improves nutrient quality and reduces ammonia toxicity to crops (Kamthunzi 2015). There is potential to grow economically viable crops (e.g. spinach and tomatoes) as studies have shown that aerobic conditions are preferred for crop production when compared to an anaerobic environment (Dan *et al.* 2011, Kamthunzi 2015). Spinach, *Beta vulgaris*, has a short maturity period and can be harvested all year round to remove nutrients out the system through aboveground biomass (Kamthunzi 2015). This plant has some salt tolerance, as yields have shown to increase at a low to moderate salinity (Osawa 1963). These factors make spinach a good candidate for the study.

1.2.7 Post anaerobic digester - Water quality parameters and their treatment in constructed wetlands

The water quality averages for post anaerobically digested brewery effluent are provided in Table 1.4. These parameters will all be measured in this study; therefore, their descriptions are provided in the sections that follow.

Table 1.4 Average water qualities of post anaerobically digested (AD) effluent (Cilliers 2012).

Parameter	Post AD effluent
Ammonia (mg/l)	42.53
Nitrite (mg/l)	0.07
Nitrate (mg/l)	1.97
Phosphate (mg/l)	12.49
pH	7.82
Electrical conductivity ($\mu\text{s}/\text{cm}^2$)	2761
Chemical oxygen demand (mg/l)	153.21
Chloride (mg/l)	482.25
Temperature ($^{\circ}\text{C}$)	22.69
Dissolved oxygen (mg/l)	-

Nitrogen

Nitrogen is one of the essential plant macronutrients (Epstein and Bloom 2005, Marschner 2011). It functions as components of amino acids, nucleic acids, nucleotides, proteins, hormones, coenzymes and other metabolic functions (Epstein and Bloom 2005, Marschner 2011). Nitrogen is available to plants in the form of ammonium or nitrate and growth may be affected by the form of nitrogen available for uptake (Borgognone *et al.* 2012). Plants adapted to low pH take up ammonium while others that are adapted to high pH prefer nitrate (Masclaux-Daubresse *et al.* 2010). High levels of nitrogen can have negative effects by delaying fruit ripening and increasing susceptibility to pests (Epstein and Bloom 2005, Roberto 2005).

Post AD brewery effluent is known to be high in nitrogen in the form of ammonia (Table 1.4). However, high levels of unionised ammonia can be toxic to plants (Kamthunzi 2015) and vertebrates, including fish, and can cause convulsions in animals and death of both plants and animals (Randall and Tsui 2002). This is due to the ammonia ion displacing K^+ , depolarising neurons and causing an influx of Ca^{2+} which results in cell death within the central nervous system (Randall and Tsui 2002).

Removal of ammonia is greater in vertical flow CWs due to the increase in oxygen and therefore, the rate of nitrification (Garcia *et al.* 2010, Stefanakis and Tsihrintzis 2012). Nitrification is the oxidation of ammonia into nitrite, and then into nitrate; thereby, improving plant nutrient quality and reducing toxicity (Kamthunzi 2015). The amount of nitrogen in post AD effluent should be sufficient for plant uptake and the CWs should be able to treat the effluent to levels low enough for survival of vertebrates and thus for fish culture.

Phosphate

The process of anaerobic digestion results in high level of phosphates in the effluent (Table 1.4). Phosphates are an essential macronutrient for plants as its functions include: a role in all metabolites dealing with energy acquisition (ATP) and is a component of nucleic acids, phospholipids, and several coenzymes (Epstein and Bloom 2005, Marschner 2011). If plants are deficient in phosphate they will be negatively affected: growth can be stunted, and leaves become discoloured, fruit production and root growth can also be reduced (Epstein and Bloom 2005). High phosphate concentrations can cause copper and zinc deficiencies by reducing the availability of these nutrients (Roberto 2005, Marschner 2011).

The removal of phosphate in CWs is generally poor. The removal mechanisms include absorption, adsorption, plant uptake and microbial uptake (Stottmeister *et al.* 2003, Vymazal

2007). Phosphorous removal remains low in CWs unless specific substrates with high sorption capacity are used (Vymazal 2010). Materials such as gravel or crushed rock, which are frequently used in CWs, have low sorption capacity (Vymazal 2007); whereas, those such as light weight clay aggregates and furnace steel slags have shown improved phosphorous removal (Vohla *et al.* 2005). However, these materials sorption capacity decreases over time (Vymazal 2010).

Oxygen

Post AD effluent has low dissolved oxygen concentrations due to the process being free of oxygen. Average concentrations range between 0.0 and 0.8 mg/l (Sooknah and Wilkie 2004, Botheju and Bakke 2011). Oxygen is important for water treatment, plant growth and health and fish growth and health (Jackson 1979, Stenstrom and Poduska 1980, Kadlec and Wallace 2008, Jia *et al.* 2010).

Nitrification reactions are affected by oxygen availability (Stenstrom and Poduska 1980, Wu *et al.* 2011b, Vymazal 2014). In some instances, dissolved oxygen concentration of 4 mg/l is sufficient for maximum nitrification while others have mentioned 1 mg/l as being sufficient (Stenstrom and Poduska 1980).

Plants require oxygen for growth and health, as well as root respiration which also affects growth (Jackson 1979, Freeman 2005, Marschner 2011). Those that are deficient will suffocate, leading to cell death and later, the death of the whole plant (Freeman 2005).

Fish also require oxygen for survival, growth and health. Oxygen concentration in the water influences feed consumption, metabolic rate and energy expenditure, and therefore, the

growth of fish (Buentello *et al.* 2000). Water that is oxygen deficient will result in suffocation and death due to the energy demands of essential functions not being met (Kramer 1987).

Another important parameter that involves oxygen is chemical oxygen demand (COD). The COD is the amount of a chemical oxidant, usually potassium dichromate, required to oxidize organic matter (Kadlec and Wallace 2008). In other words, COD is the amount of oxygen that can be consumed by the oxidation of pollutants in the water. Constructed wetlands are said to be able to lower this demand (Kadlec and Wallace 2008). Vertical, horizontal and free-water surface flow wetlands can lower COD by > 90.00 %. However, this removal depends more on hydraulic retention time, vegetation, depth, seasonality and temperature than type of constructed wetland (Kadlec and Wallace 2008).

Electrical conductivity and salts

Brewery effluent can be high in salts, depending on the chemicals used for cleaning processes at the brewery. This can be problematic for plant and fish culture. The EC is not known to be treated by wetlands, as the outlet EC can be 98% of the inlet EC (Kadlec and Wallace 2008). However, EC can be lowered via dilution if low hydraulic loading is coupled with high rainfall (Kadlec and Wallace 2008), and it can also be mitigated with the use of halophytes in the wetland which can assimilate salts in their leaves (Shelef *et al.* 2012).

High levels of EC can limit plant growth and health due to the energy the plant has to use to take up water and nutrients as well as limiting the availability of plant nutrients (Qadir and Schubert 2002, Muyen *et al.* 2011). The high EC of brewery effluent is a potential constraint in using brewery effluent in crop production. The high salinity of brewery effluent needs to be addressed to maximise yield of plants as it may decrease growth and health (Jones *et al.* 2016).

Chloride is widely regarded as having biological interactions with the ecosystem but does not usually result in concentration changes in water (Kadlec and Wallace 2008). It is one of the essential plant nutrients as it plays a role in enzyme activation of photosystem II (Freeman 2005, Epstein and Bloom 2005). Therefore, it can decrease in water via plant uptake (Xu *et al.* 2004). Water that is deficient in this compound will cause plants to wilt and water with high levels of chlorine can cause calcium deficiencies (Freeman 2005).

As fish differ in their ability to maintain internal osmotic pressure, the optimum EC for production is species specific (Bhatnagar and Pooja 2013). Unfavourable salinities can impede growth and cause death (Bhatnagar and Pooja 2013). Freshwater fish body fluids are hyperosmotic, which means they have higher salt concentrations in their bodies when compared to the surrounding water body (Greenwell *et al.* 2003). Therefore, water intake across the gills must be balanced by the excretion of water by the kidney and renal salt loss must be minimised to decrease the loss of salt at the gills due to diffusion (Evans 2001). The renal loss of ions is compensated by active transport of sodium and chloride across the gills; these are transported into gill cells via an electrochemical gradient formed by the excretion of H^+ ions and HCO_3^- respectively (Evans 2001, Greenwell *et al.* 2003).

Species of fish have different tolerances to salinity (Boeuf and Payan 2001). Rainbow trout, *Onchorhynchus mykiss*, are limited to low salinities of 0 to 34 psu (Boeuf and Payan 2001), while Mozambique tilapia, *Oreochromis mossambicus*, are capable of surviving salinities up to 120 psu (Boeuf and Payan 2001), which is due to the fish's ability to form specialised chloride cells in the gills and opercular membrane that are used to secrete excess salt (Uchida *et al.* 2000). High levels of chloride can burn the gills of fish resulting in long term effects and is therefore important to monitor in aquaculture (Bhatnagar and Pooja 2013).

pH

pH is an important parameter as it affects plants and fish growth and health. Ammonia becomes more toxic to plants and fish as the pH and temperature increases (Randall and Tsui 2002). High pH can also limit nutrient availability for plants (Tyson *et al.* 2007). The effect of pH on plants grown in soil (Figure 1.6) differs from plants grown in soilless cultures (Figure 1.7). Power and Jones (2016) found that tomatoes grown in treated brewery effluent double in yield when the pH was adjusted to 6.5, since the optimal pH range for most plants is 5-7 (Epstein and Bloom 2005). Lucas and Davis (1961) and Bugbee (2003) state, on the other hand, that a pH range of 5.5-5.8 is recommended for plant growth due to the availability of most nutrients being the highest in this range.

Fish can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0, and a pH of less than 4.0 or greater than 11 results in death of most fishes (Bhatnagar and Pooja 2013). The suitable pH range for fish culture is between 6.7 and 9.5, and Bhatnagar and Pooja (2013) state the ideal pH range for aquaculture is 7.5-8.5.

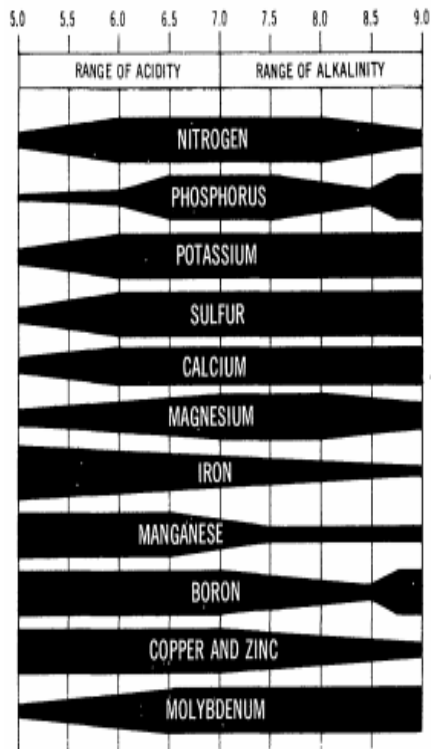


Figure 1.6 Nutrient availability at different pH values for soil cultures (Peterson 1982).

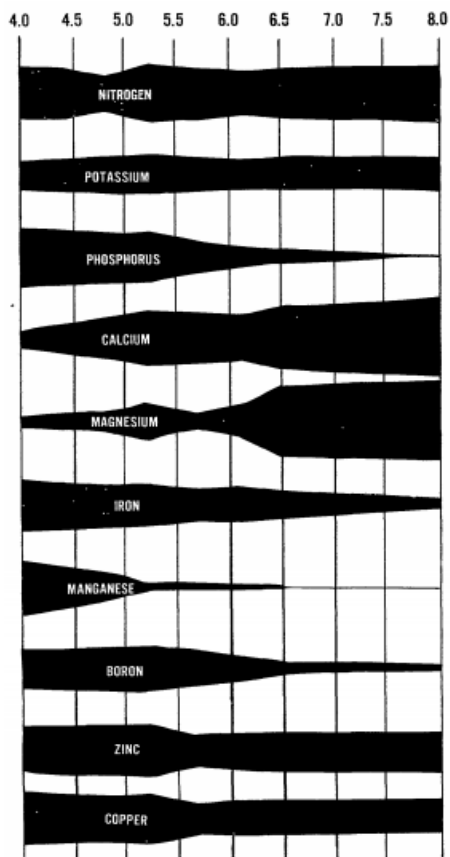


Figure 1.7 Nutrient availability at different pH values for soilless cultures (Peterson 1982).

Temperature

Temperature can affect dissolved oxygen in the water, as increasing temperature will decrease oxygen solubility. A rise in temperature from 20 to 30°C of saturated water will decrease oxygen from 9.62 mg/l to 7.8 mg/l (Stenstrom and Poduska 1980).

Increasing temperature can directly affect plants by increasing respiratory demand for oxygen and indirectly by decreasing dissolved oxygen and thereby affecting plant growth (Jackson 1979, Stenstrom and Poduska 1980, Freeman 2005). High temperatures in the root zone can lead to root disease, Pythium (*Pythium aphanidermatum*) which hampers production (Vandam *et al.* 2017).

Temperature affects fish health and growth by disrupting feed consumption, metabolic rate and energy expenditure (Buentello *et al.* 2000). Different fish require different temperature ranges and therefore is important when selecting species and determining suitability of use in aquaculture.

1.2.8 Aquaculture potential in recovered wastewater

Aquaculture has been mentioned as a potential to increase agricultural productivity by re-using wastewater and this has been put into practice (Pescod 1992, Parkinson and Tayler 2003). Systems that use wastewater for aquaculture can also provide food for the fish due to the high nutrient loads which lead to phytoplankton growth and then zooplankton growth which are both a food source to fish (Pescod 1992). Fish mortality in aquaculture systems fed with wastewater can result from numerous cases; the two most common include oxygen depletion and high ammonia concentration (Pescod 1992, Ip *et al.* 2001, Randall and Tsui 2002).

The east Calcutta sewage fisheries had a large aquaculture system that used wastewater as a water source (Pescod 1992). The fish ponds receive raw sewage from the city in batches and were stored in stagnant ponds. After twelve days the ponds were disturbed manually to oxygenate the water (Olah *et al.* 1986). Fish were stocked 25 days after filling, thereafter wastewater was fed three hours every day for seven days, once a month. This supplied sufficient nutrients and a stable biological environment (Olah *et al.* 1986). This fishery was successful, since it supplied 10-20% of Calcutta's demand, which equates to 10-20 tonnes of fish a day (Pescod 1992).

The yield of fish in wastewater reuse systems can be increased by supplementing energy rich feed (Pescod 1992). In this study, fish were fed with a commercially available feed. Therefore, the only potentially limiting factor was amount of feed provided to fish and water quality. At Ibhayi Brewery (SAB Ltd, Port Elizabeth), fish have been successfully cultured in water recovered from brewery effluent treated in high rate algal ponds (Jones *et al.* 2014 and 2016). The current study will look at using recovered water from different CWs and the effect it has on fish and plant growth in aquaculture.

1.3 Aims and objectives

The overall aim for this study was to compare the efficiency of different constructed wetlands to determine which one provides an environment for improved plant health and growth and downstream use in aquaculture. The study was split up into three experiments to achieve the overall aim.

The objectives were to:

- determine the optimal filling and draining time for tidal wetlands that result in the highest treatment efficiency and crop harvest;

- compare tidal wetlands with optimal filling and draining time to aerated and unaerated horizontal sub-surface wetlands to determine the system that results in highest treatment efficiency and crop harvest; and to
- determine which system results in the fastest fish growth.

Chapter 2: The effect of tidal wetland cycle time on nutrient removal and plant health and growth.

2.1 Introduction

2.1.1 Rationale

Tidal constructed wetlands (CWs) are vertical sub-surface flow wetlands (Section 1.2). These wetlands have multiple flood and drain cycles per day (Stefanakis and Tsihrintzis 2012, Wu *et al.* 2014). Oxygen enters by suction caused when the wastewater flows downwards during the draining phase (Stottmeister *et al.* 2003, Wu *et al.* 2014; Figure 2.1). During this phase, water films around roots and substrate biofilms are exposed to atmospheric oxygen (Figure 2.1), which contributes to filtration bed oxygenation, as compared to horizontal sub-surface flow (HSSF) CWs, where oxygenation occurs through plant aerenchyma (Sun *et al.* 2005, Garcia *et al.* 2010, Jia *et al.* 2010, Vymazal 2014). During the filling phase in tidal CWs, diffusion of atmospheric oxygen is extremely rapid in the root and substrate biofilms and can become saturated in seconds (Jia *et al.* 2010, Wu *et al.* 2011b; Figure 2.1). The transitioning between phases is due to the siphon, which “kicks out” (stops water draining) when the water level reaches the bottom and “kicks in” (starts draining water) when the water level reaches the top (Figure 2.1).

Tidal CWs are more efficient than HSSF CWs as they can treat higher loads of contamination due to increased oxidation (Garcia *et al.* 2010, Vymazal 2010). Increased oxygen enhances the redox ability to oxidise ammoniacal nitrogen, as increased nitrification occurs when sufficient oxygen is present for aerobic bacteria (Jia *et al.* 2010, Wu *et al.* 2011b, Stefanakis and Tsihrintzis 2012). Nitrifying bacteria are autotrophic microorganisms that reproduce slowly and require enough time to establish before effective nitrification can occur (Sun *et al.* 2005).

Ammonia is oxidised into nitrite and then nitrate by nitrifying bacteria in the oxygen rich zones (Jia *et al.* 2010). Tidal CWs have the potential to enhance the lowering of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) via aerobic decomposition (Sun *et al.* 2005, Kadlec and Wallace 2008). The process of lowering BOD, COD and ammonia concentration involves the consumption of oxygen (Sun *et al.* 2005, Kadlec and Wallace 2008). The increase in oxygen into the beds enhances oxidation of organic carbon and effectively removes organic matter (Wu *et al.* 2011a, Stefanakis and Tsihrintzis 2012). Phosphorous removal in CWs remains low unless specific substrates with a high sorption capacity are used (Vymazal 2010).

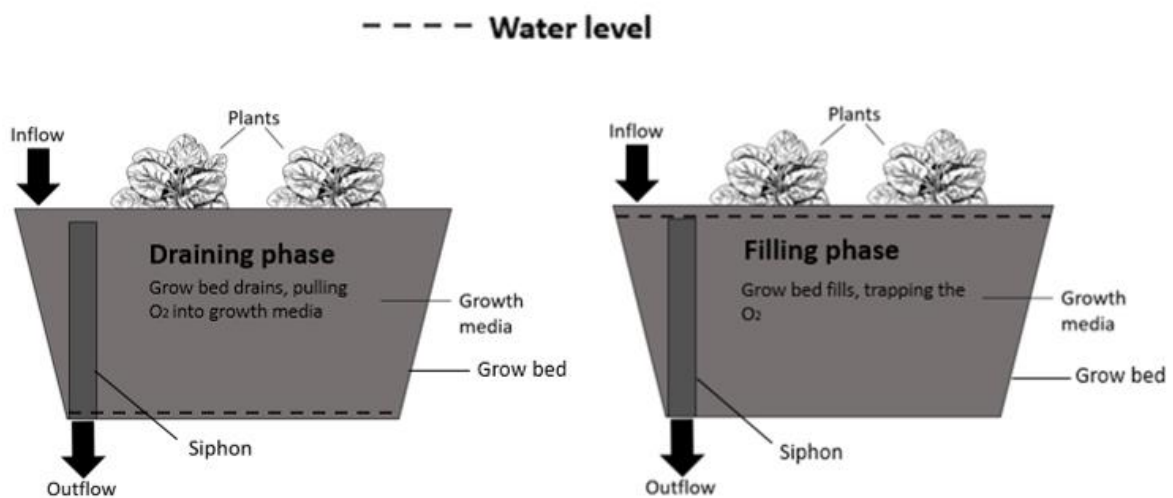


Figure 2.1 Tidal constructed wetland, illustrating the draining and filling phases.

Research on the effect of filling and draining time (FDT) on water treatment has been minimal as only a few studies on tidal CWs have been done and all have been conducted on laboratory-scale (Green *et al.* 1997, Sun *et al.* 1999, Sun *et al.* 2005, Wu *et al.* 2011a). These studies have produced promising results, but are often misleading, as they sometimes provide inaccurate predictions for a full-scale operation (Sun *et al.* 2006). Therefore, experimental trials for

prolonged periods and on a larger-scale are needed to better understand processes and potential problems (Sun *et al.* 2006).

A tidal “cycle” is defined as an event that consists of a complete filling and a complete draining phase. Cui *et al.* (2012) found that increasing the number of cycles per day improved the removal of total nitrogen, phosphorous and ammonia. Kadlec and Wallace (2008) concluded that six filling and draining cycles is the minimum number per day to meet treatment goals. The current experiment focused on further increasing the number of tidal cycles a day and comparing three different cycle times.

Growing a crop of commercial value using brewery effluent has been studied and has shown potential to be successful (Power and Jones 2016). However, pH and salt concentration may impede growth and health of certain crops and are therefore, important to monitor. Constructed wetlands may alter pH and salt and were therefore not adjusted in this experiment (Kadlec and Wallace 2008).

Spinach was selected as a candidate specie due to the plants salt tolerance, fast growth rate, broad temperature range and economic value (Jeavons 2002, Talekar *et al.* 2003, Ors and Suarez 2017). This plant can grow in temperatures as low as 1 °C to a maximum of 30 °C allowing for harvesting throughout the year (Jeavons 2002). Constant harvesting of the plants in CWs is important as it provides insulation during winter in cold and temperate regions as well as acting as a nutrient remover via biomass (Vymazal 2011b). Spinach has relatively high root biomass which is important as many bacterial forms are found here, and it stimulates microbial activity (Vymazal 2011b). Unpublished data also indicates that spinach has been successfully cultured using brewery effluent hydroponically.

2.1.2 Aims and objectives

The aim of the experiment was to determine the best filling and draining time (FDT) that would subsequently be used in Experiments 2 and 3 (Chapters 3 and 4). This was achieved by comparing how the FDT effects nutrient removal and plant harvest and health in the CWs and to identify the FDT which resulted in optimal nutrient removal and plant growth and health.

The objectives of the study were to:

- determine the effect of filling and draining times on the dissolved oxygen in the wetland;
- compare the effect of filling and draining times on brewery effluent treatment efficiency in wetland; and to
- compare the effect of filling and draining times on plant total harvest and health.

2.2 Methods and materials

2.2.1 Experimental species

Two hundred and fifty spinach (*Beta vulgaris*) seedlings, of the same age and size, were purchased from a commercial seedling supplier (Moorland Seedlings (Pty) Ltd, Humansdorp). Of these, 189 were planted and the rest were used for destructive plant health data for the start of the experiment.

Spinach were grown for 19 weeks to observe the effects of filling and draining time (FDT) and water quality on the plant harvest and health. Leaves were harvested from the plants when they were at a harvestable size (± 10 cm long).

2.2.2 Treatment of the brewery effluent prior to the experiment

At the Project Eden experimental facility located at Ibhayi Brewery (SAB Ltd, Port Elizabeth, South Africa), the wastewater is treated using a combination of alternative, sustainable water treatment systems (Figure 2.2). Firstly, the effluent is passed through a drum filter (0.5 mm), where solid wastes are removed. From the drum filter, water is fed into an equalisation basin to regulate pH and the flow of water into an anaerobic digester. The AD is primarily responsible for reducing long-chain molecules in the effluent, into smaller structures that can be utilised in aerobic treatment systems – i.e. by aerobic bacteria, algae and plants (Parkin and Owen 1986, Power and Jones 2016). The water from the AD was used in this project as the brewery effluent source.

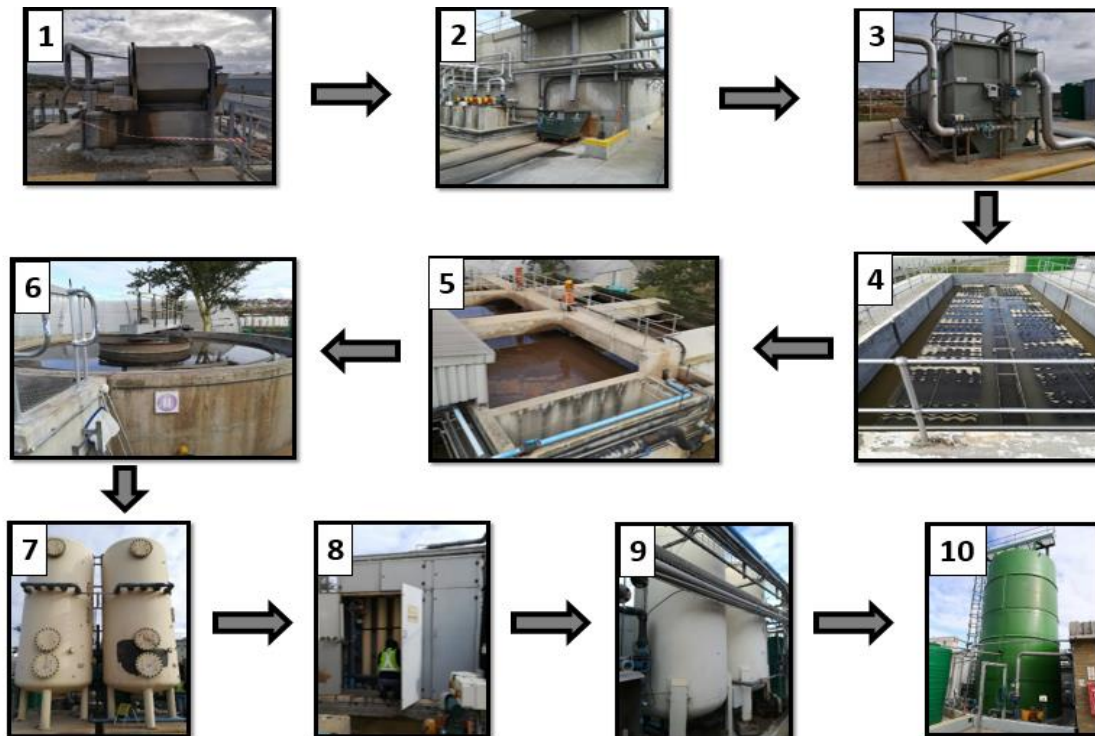


Figure 2.2: Brewery effluent treatment process at Ibhayi brewery. 1-drum filter; 2-equalisation basin; 3-pre-clarifier; 4-anaerobic digester; 5-activated sludge; 6-clarifier; 7-multimedia-filters; 8-ultra-filters; 9-carbon-filters; 10-storage tank. The arrow depicts the flow of water through the treatment process. Brewery effluent used in this experiment was subject to anaerobic digestion; steps 6 to 10 do not form part of the pre-treatment used here.

2.2.3 Experimental system and treatments

The study consisted of nine grow beds (2.0 x 0.8 x 0.4 m) filled with \pm 19 mm crushed gravel (Figure 2.3-2.5). Each grow bed was connected to one 1000 l sump tank that was buried 0.5 m in the ground to allow for gravity return of water to the sump and to minimize the height of the grow beds which stood at 1.2 m (Figure 2.3-2.5). Effluent was pumped from the sump tanks to the beds via submersible pumps (Dophin P-3000, Code CPD0862, KW aquatic supplies Co., Ltd. Malaysia), such that each grow bed and tank could be run as a closed, recirculating system. The beds were separated into fast, medium and slow treatments, where effluent was recirculated to allow for 15 min, 30 min and 60 min drain and fill interval respectively (Table 2.1). The outflow pipes differed between treatments to allow for the siphon to “kick in” and

“kick out” effectively due to differing water flows. The fast and medium treatments had 40 mm PVC piping while the slow treatment had 25 mm PVC pipes.

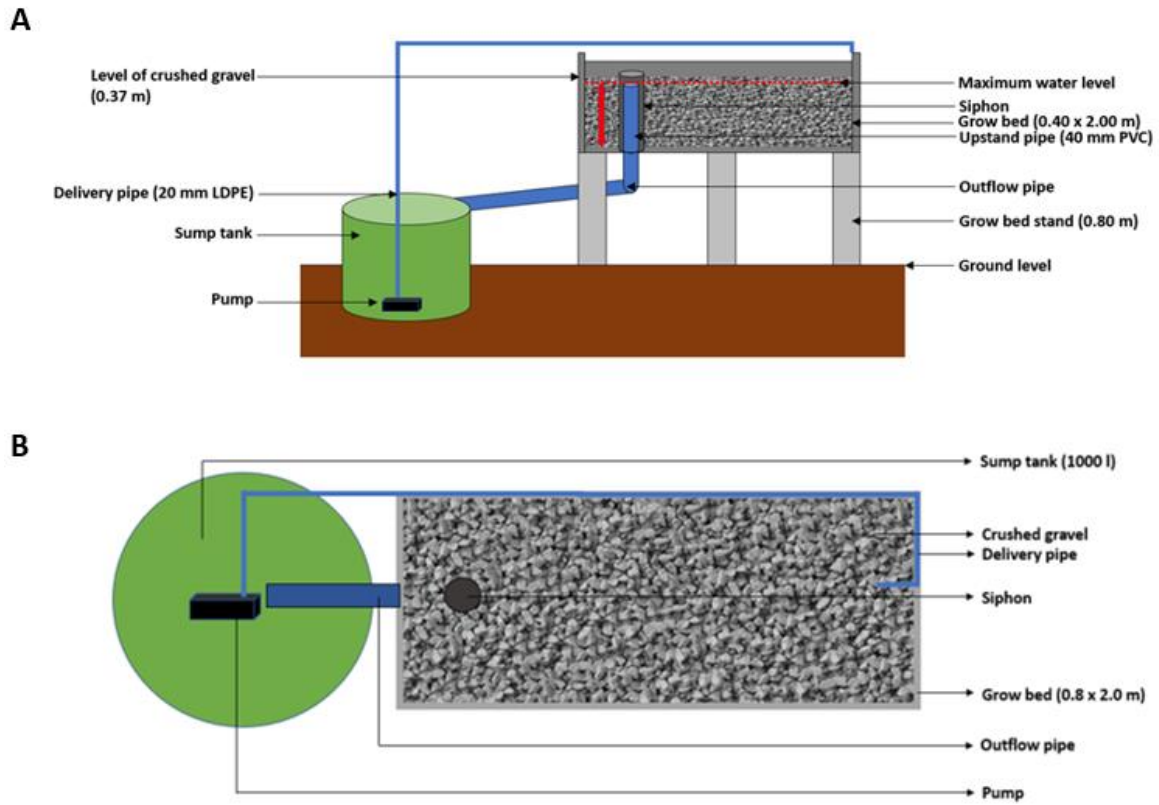


Figure 2.3 A) Side and B) aerial view of the experimental system, the components and dimensions.

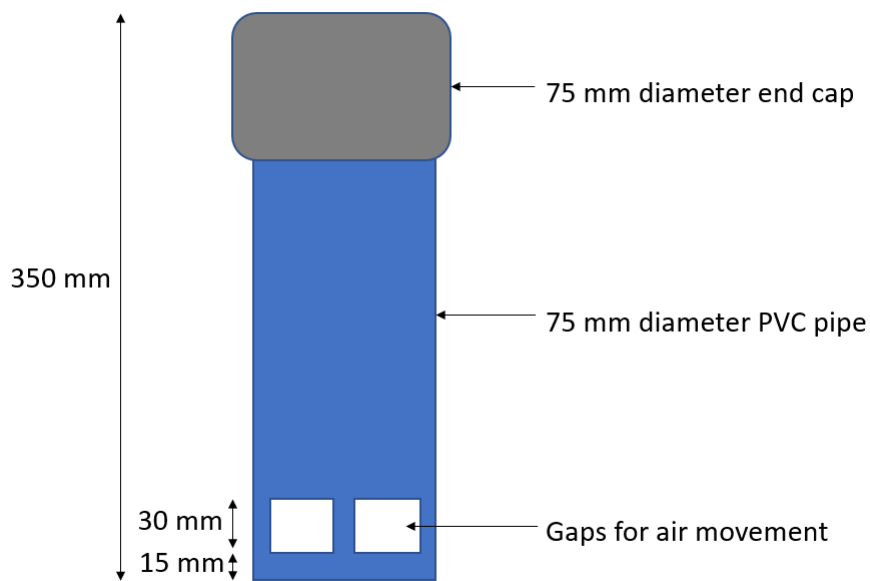


Figure 2.4 The grow bed siphon with dimensions.



Figure 2.5 A photograph of the experimental system and its components.

Three treatments were randomly assigned among nine systems, namely short, medium and long filling and draining times (FDTs; Table 2.1). Therefore, each treatment was replicated three times. Grow bed volume and total volume of grow bed and sump tank were consistent throughout the three treatments (Table 2.1). Plants were thinned out to 21 plants per bed after two weeks. The fast FDT treatment had approximately 96 cycles per day, and the

medium and slow FDT treatments had approximately 48 and 24 cycles per day. A “cycle” is defined as an event where a grow bed completely fills up and drains. Brewery effluent was pumped into each tank via a 40 mm irrigation pipe at the start of each batch cycle. Once the effluent in all treatments were treated to ammonia discharge standards (3 mg/l; DWAF 1998), the grow beds were drained, emptied and sent to the municipal waste tank using another submersible pump which was not part of the closed system (Figure 2.3). Thereafter, the systems were refilled for the next batch cycle. There were 11 five-day batch cycles recorded over a five-month period.

Table 2.1 Treatments with their filling and draining times (FDTs), grow bed volume (L), total volume of grow bed and sump tank (L) and the number of filling and draining cycles per day that was used in the study (T1-T3).

Treatment	FDT duration	Maximum volume of effluent in grow bed (L)	Total volume of effluent in grow bed and sump tank (L)	No. of filling and draining cycle per day
T1	15 min	520	850	± 96
T2	30 min	520	850	± 48
T3	60 min	520	850	± 24

2.2.4 Data collection

Water quality parameters

Temperature (°C) and electrical conductivity (EC; $\mu\text{S}/\text{cm}^2$) was measured using an electrical probe (Lutron conductivity meter, code PCD-432, Lutron Co Inc, USA) and pH was measured using a pH-meter (Crison pH-meter BASIC 20+, code 003-2010, Crison instruments S.A., Spain).

Ammonia ($\text{NH}_3\text{-N}$; mg/l), nitrite ($\text{NO}_2\text{-N}$; mg/l), nitrate ($\text{NO}_3\text{-N}$; mg/l), phosphate ($\text{PO}_4^{3-}\text{-P}$; mg/l) and chloride (Cl^- ; mg/l) concentrations were recorded using a spectrophotometer (Hach DR 2800 spectrophotometer, product number DR2800-01B1, Hach (Pty) Ltd, USA) and commercially available test kits, and standard methods (Hach (Pty) Ltd, USA). Chemical oxygen demand was recorded using a spectrophotometer (Merck Spectroquant Pharo 1000 spectrophotometer, product number 100706, Merck (Pty) Ltd, Germany) and standard methods (Merck (Pty) Ltd, Germany). Water samples were filtered through 8-micron filter paper prior to analysis as filtration removes turbidity which may influence colorimetric analyses (Hach 2013). The following test kits were used:

- High-range ammonia test (product no. 26069-45, Hach (Pty) Ltd, USA)
- Nitrite test (product no. 21075-69, Hach (Pty) Ltd, USA)
- Nitrate test (product no. 21061-69, Hach (Pty) Ltd, USA)
- Phosphate test (product no. 22441-00, Hach (Pty) Ltd, USA)
- Chloride test (product 23198-00, Hach (Pty) Ltd, USA)
- Chemical oxygen demand cell test (product no. 1.14895.0001, Merck (Pty) Ltd, Germany).

All parameters were measured daily for the 11 batch cycles at 09h00 and a mean was generated for each day (day-0 to day-5); except for COD, chloride and phosphate which were measured at the start and end of each batch cycle and a mean was calculated for “start” and “end”. All water samples were collected from the sump tanks.

Plant total harvest and health parameters

At the beginning of the experiment, seedlings from the same population used in the trial that were not re-planted, were randomly selected and their leaves were used for leaf chemical analysis. At the end of the experiment (week-19) leaves from randomly selected plants in each bed were used for the same leaf chemical analysis. This included N, P, Na, Cl, K, Al, Ca, Cu, Fe, Mn, Mg and Zn concentration at a commercial analytical laboratory (Cedara College of Agriculture, Pietermaritzburg). Nitrogen was measured using a carbon, nitrogen and sulphur (CNS) analysing instrument (LECO Truspec CN, Leco corp., USA). The other elements were measured using inductively coupled plasma atomic emission spectroscopy (ICP-OES, Vista MPX, Varian Inc., USA; Sithole, pers. comm., Plant lab manager, Cedara College of Agriculture, Pietermaritzburg, November 2018).

Chlorophyll concentration index (CCI) was recorded at the start of the experiment and at the end of the experiment (week-19) on the upper most leaf of each plant using a chlorophyll content meter (Chlorophyll Content Meter, CCM-200 Plus, Opti-Sciences Inc., USA). Photographs and descriptions were recorded if plants showed evidence of stress during the experiment.

Total harvest was measured by weighing the individual bed's harvests. Each bed was harvested separately when leaves were at a size that were harvestable (± 10 cm) during the experiment and all leaves at the end of the experiment (week-19). Leaves harvested were weighed individually on a balance scale (Radwag precision balance WLC 6/A/A2/C/2, code WL-217-0014, Radwag LLC, Poland). Total harvest was calculated by adding all harvests during the experiment with the harvest at the end of the experiment for the respective treatments.

Statistical analysis

Treatment means were compared using a one-way or multifactor repeated measure analysis of variance (ANOVA). When significant differences were evident, treatment means were further compared using a Tukey multiple range test at $p < 0.05$ ($\alpha = 5\%$). All data were checked for equality of variance and for 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 again for equal variance and normal distribution of residuals. If the assumptions were still not met, a non-parametric Kruskal Wallis ANOVA was used to compare the data between treatments in place of a one-way ANOVA. If the data did not meet the assumptions for repeated measures ANOVA, a Mauchly's sphericity test was included in the analysis, and they were corrected using Greenhouse-Geisser and Huynh-Feldt tests. Multiple regression analysis was used to describe the relationships between variables and a chi-squared test was performed to determine differences between observed and expected frequencies between treatments. All analyses were carried out using statistical software (Statistica, version 10, StatSoft Inc, Tulsa, USA).

The average pH values were converted to H^+ concentrations for statistical comparison using Equation 2.1 where x is the recorded value:

$$H^+ = 10^{-x} \quad (2.1)$$

The mean and standard error from the logged pH values were used for graphical representation.

2.3 Results

2.3.1 Water quality parameters

The dissolved oxygen (DO) concentrations of the effluent were influenced by an interaction between filling and draining time (FDT) and days (Repeated measures ANOVA, $F_{(10, 165)} = 5.09$, $p < 0.001$; Figure 2.6). Dissolved oxygen was low for all treatments on day-0 but increased between day-0 and day-1 in all treatments and levelled off for the remaining days (Figure 2.6). The treatment with fast FDT had higher dissolved oxygen concentration than medium and slow FDT treatments after day-1, and the medium FDT treatment had higher DO than the slow FDT treatment on day-2 and day-5 (Figure 2.6).

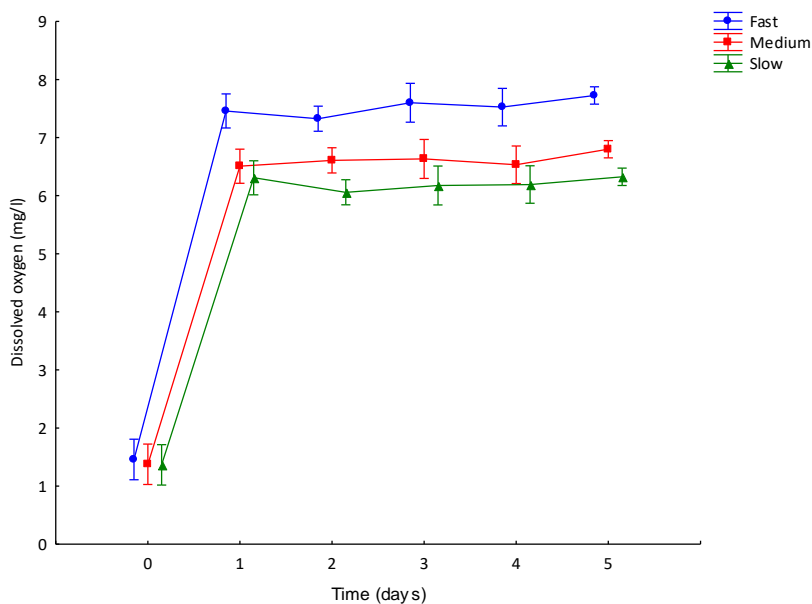


Figure 2.6 The mean (\pm 95% confidence interval) dissolved oxygen concentration in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments over five days (Repeated measures ANOVA, $F_{(10, 165)} = 5.09$, $p < 0.001$).

Dissolved oxygen and ammonia concentration had a significant inversely proportional relationship (Multiple regression analysis, $y = 4.72x + 44.58$, $R^2 = 0.46$, $F_{(1,574)} = 487.10$, $p < 0.001$; Figure 2.7).

Ammonia concentrations in the effluent were influenced by an interaction between FDT and days (Repeated measures ANOVA, $F_{(10, 525)} = 5.59$, $p < 0.001$; Figure 2.8). All FDT treatments treated ammonia to the discharge standard, with the fast and medium treatments lowering ammonia to this standard on day-4 and the slow treatment on day-5 (DWAF 1998; Figure 2.8). From day-1 to day-3 the fast FDT treatment had significantly lower ammonia concentration than the slow treatment; on day-2 and-3 the medium treatment had lower ammonia concentration than the slow FDT treatment but higher concentrations than the fast treatment (Figure 2.8). Fast and medium FDT treatments had significantly lower concentrations than the slow FDT treatment on day-4 and day-5 (Figure 2.8).

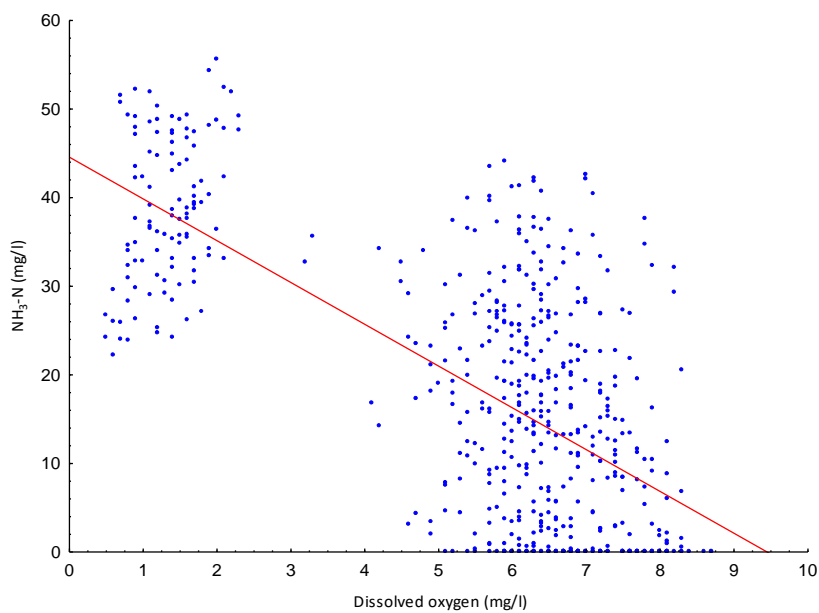


Figure 2.7 Relationship between the effluent ammonia concentration and dissolved oxygen concentration in all treatments (Multiple regression analysis, $y = 4.72x + 44.58$, $R^2 = 0.46$, $F_{(1,574)} = 487.10$, $p < 0.001$).

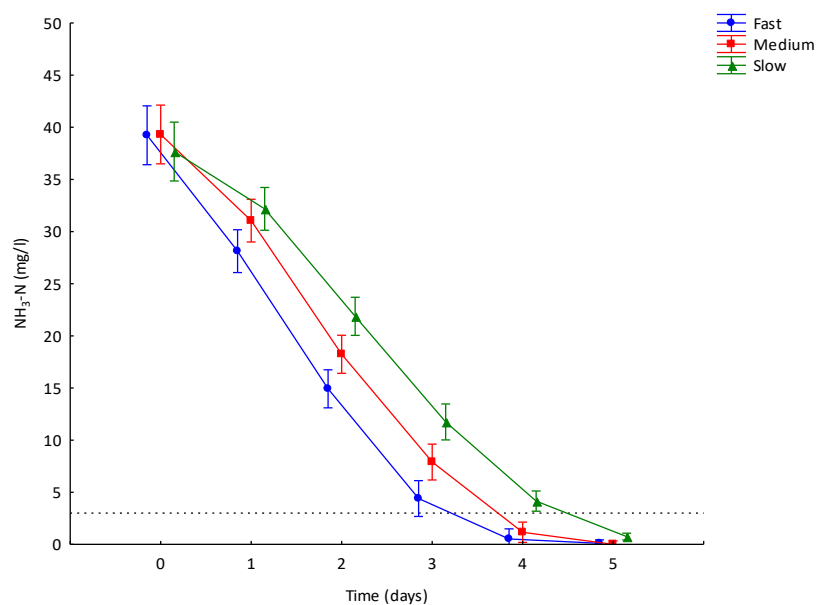


Figure 2.8 The mean (\pm 95% confidence interval) ammonia concentration in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments over five days (Repeated measures ANOVA, $F_{(10, 525)} = 5.59$, $p < 0.001$). The dashed black line represents the discharge standard for ammonia (3 mg/l; DWAF 1998).

Nitrite concentrations were not influenced by an interaction between FDT and days (Repeated measures ANOVA, $F_{(10, 165)} = 1.23$, $p = 0.28$; Figure 2.9). There were no differences between treatments over five days, however all treatments decreased between the start (day-0) and end (day-5) of the batch cycle. Nitrite concentrations were below discharge standards (15 mg/l; DWAF 1998) throughout the trial period (Figure 2.9).

Nitrate concentrations were not influenced by an interaction between FDT and days (Repeated measures ANOVA, $F_{(10, 165)} = 0.75$, $p = 0.68$; Figure 2.10). There was a significant difference between day-0 and day-1, with all treatments decreasing significantly; thereafter nitrate concentration levelled off for the rest of the batch cycle period (Repeated measures ANOVA, $F_{(5, 165)} = 0.75$, $p = 0.68$; Figure 2.10). Nitrate concentrations were below discharge standards (15 mg/l; DWAF 1998) throughout the trial period.

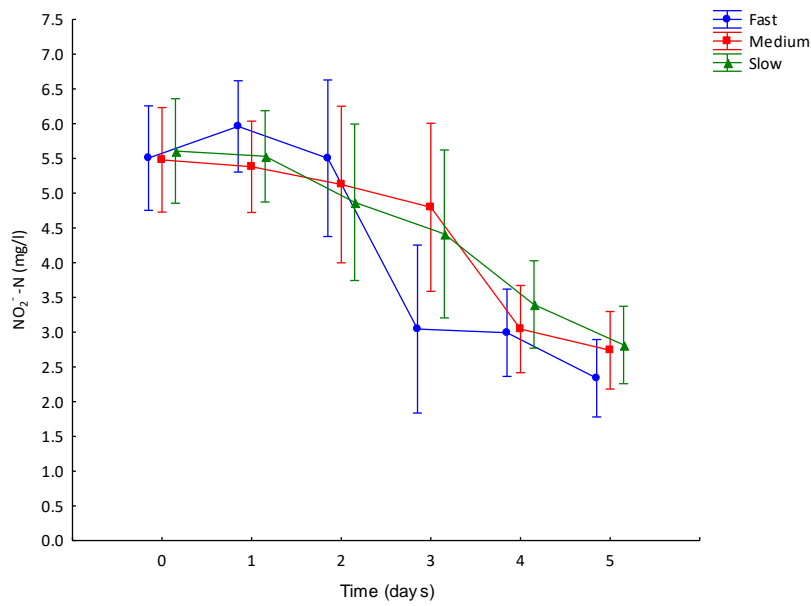


Figure 2.9 The mean (\pm 95% confidence interval) nitrite concentration in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments over five days (Repeated measures ANOVA, $F_{(10, 165)} = 1.23$, $p = 0.28$).

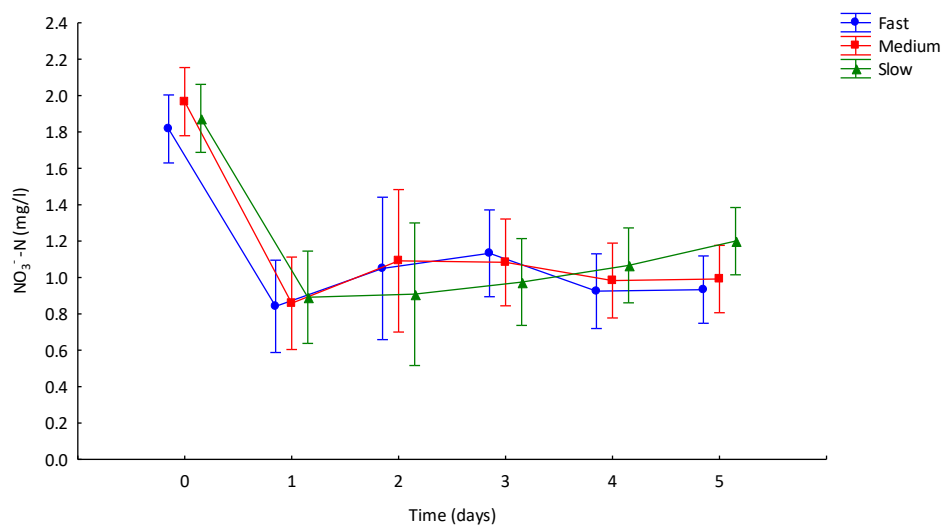


Figure 2.10 The mean (\pm 95% confidence interval) nitrate concentration in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments over five days (Repeated measures ANOVA, $F_{(10, 165)} = 0.75$, $p = 0.68$).

pH values were not influenced by an interaction between FDT and days (Repeated measures

ANOVA, $F_{(10, 165)} = 0.034$, $p = 1.00$; Figure 2.11). All treatments increased significantly

between day-0 and day-1 (Figure 2.11). However, there were no differences between treatments on day-0 and day-1. On day-2, day-3 and day-5 fast FDT treatments had significantly higher pH than the slow FDT treatment. On day-4, pH was higher in the fast FDT treatment than both the medium and slow FDT treatments (Figure 2.11). pH values were within the discharge standard range (5.5 - 9.5; DWAF 1998; Figure 2.11).

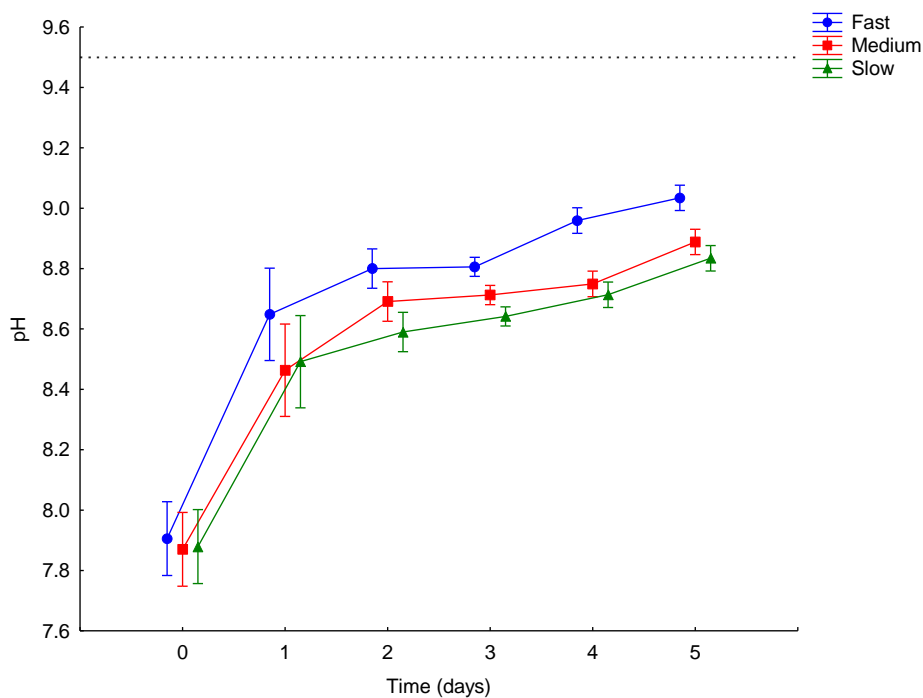


Figure 2.11 The mean (\pm 95% standard error) pH in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments over five days (Repeated measures ANOVA, $F_{(10, 165)} = 0.034$, $p = 1.00$). The dashed black line represents the upper limit of the discharge standard for pH (9.5; DWAF 1998).

Temperature, COD and phosphate were not influenced by an interaction between FDT and the start and end of the batch cycles (Repeated measures ANOVA, $p > 0.05$). There were no significant differences between treatments for these parameters at the start and end of the batch cycles (Repeated Measures ANOVA, $p > 0.05$). However, they all decreased significantly from the start to the end of the batch cycles (Repeated Measures ANOVA, $p < 0.05$; Table 2.2),

with COD being lowered to below discharge standards (75 mg/l; DWAF 1998) with a mean decrease of 63.14 ± 0.81 % across all treatments.

Table 2.2 The mean (\pm standard error) temperature, chemical oxygen demand (COD) and phosphate concentration in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time at the start and end of the batch cycle. Values in the same row represented by a different superscript symbol represent significantly different means (Repeated measures ANOVA, $p < 0.05$).

	Start		End		F	P
	Combined	Fast	Medium	Slow		
Temperature ($^{\circ}$ C)	24.14 ± 0.11^a	17.10 ± 0.14^b	17.03 ± 0.14^b	17.01 ± 0.13^b	$F_{(1, 105)} = 3397.4$	< 0.001
COD (mg/l)	147.33 ± 4.20^a	51.89 ± 2.02^b	56.17 ± 2.64^b	52.06 ± 2.36^b	$F_{(1, 51)} = 797.89$	< 0.001
Phosphate (mg/l)	4.82 ± 0.15^a	4.31 ± 0.26^b	4.48 ± 0.27^b	4.19 ± 0.26^b	$F_{(1, 105)} = 80.68$	< 0.001

Electrical conductivity and chloride were not influenced by an interaction between FDT and the start and end of the batch cycles (Repeated Measures ANOVA, $p > 0.05$; Figures 2.12 and 2.13). Both EC and chloride decreased significantly in all treatments from the start to the end of the batch cycles (Figures 2.12 and 2.13). There was no significant difference between treatments for EC at the start and end of the batch cycles (Figure 2.12). There was however, a significant difference between treatments at the start of the batch cycle for chloride, with the fast FDT treatment having lower chloride concentration than the slow FDT treatment (Figure 2.13).

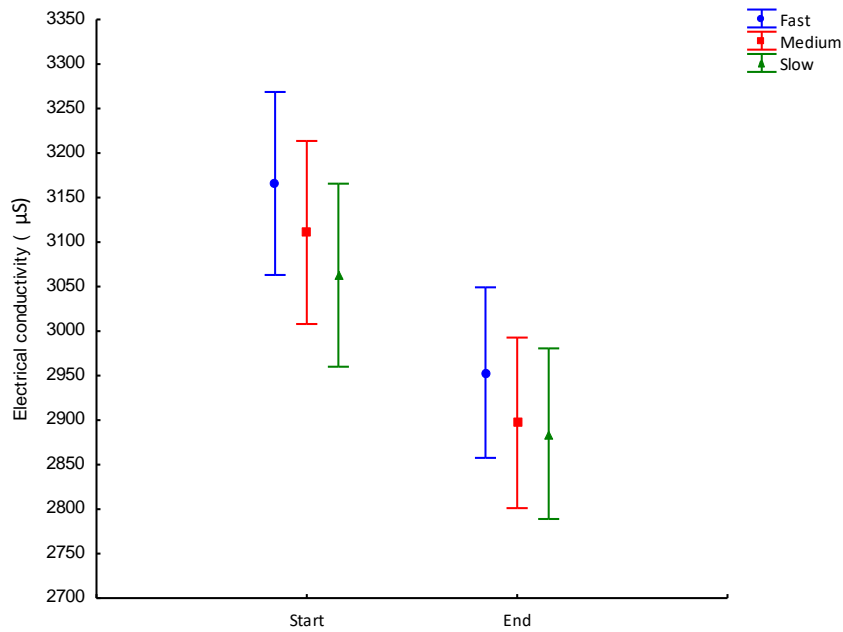


Figure 2.12 The mean (\pm 95% confidence interval) electrical conductivity in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time (FDT) treatments at the start and end of the batch cycles (Repeated measures ANOVA, $F_{(2,105)} = 0.89$, $p = 0.41$).

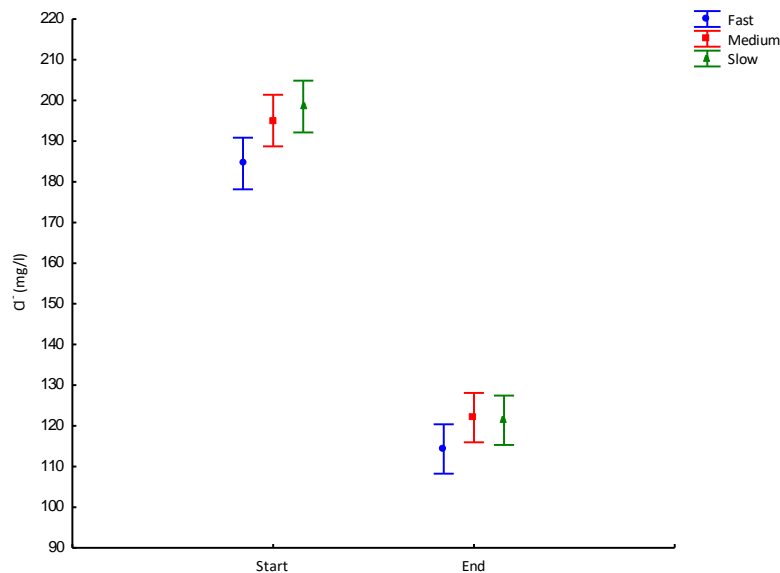


Figure 2.13 The mean (95% confidence interval) chloride concentration in the effluent of constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time at the start and end of the batch cycles (Repeated measures ANOVA, $F_{(2,105)} = 2.73$, $p = 0.07$).

2.3.2 Crop harvest and plant growth

There was a significant difference in total harvest between fast (20.15 ± 1.93 kg) and slow (10.18 ± 0.39 kg) FDT treatments over the trial period, with the medium FDT treatment harvest being intermediate between the two (One-way ANOVA, $F_{(2,6)} = 7.77$, $p = 0.02$). Total harvest increased significantly with faster filling and draining times (Multiple regression analysis, $y = -0.20x + 21.64$, $R^2 = 0.60$, $F_{(1,7)} = 10.59$, $p = 0.014$; Figure 2.14).

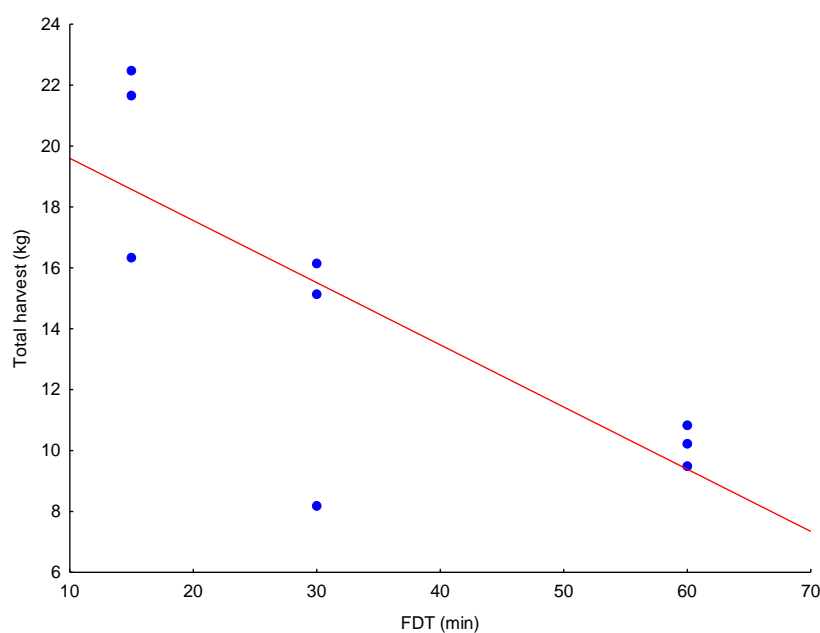


Figure 2.14 Relationship between total harvest and filling and draining time (FDT) in all treatments (Multiple regression analysis, $y = -0.20x + 21.64$, $R^2 = 0.60$, $F_{(1,7)} = 10.59$, $p = 0.014$).

There was an interaction between FDT and chlorophyll concentration index (CCI) (Repeated measures ANOVA, $F_{(2,186)} = 17.07$, $p < 0.001$; Figure 2.15); the CCI in fast and medium FDT treatments increased significantly between the start and end of the experiment, whereas the CCI in the slow treatment did not change significantly over the same period (Figure 2.15). Also, there was a significant difference in CCI between treatments at the end of the trial, where the fast and medium FDT treatments had significantly higher mean CCI values (14.06 ± 0.51 and 13.00 ± 0.47) than the slow FDT treatment (9.85 ± 0.32 ; Figure 2.15).

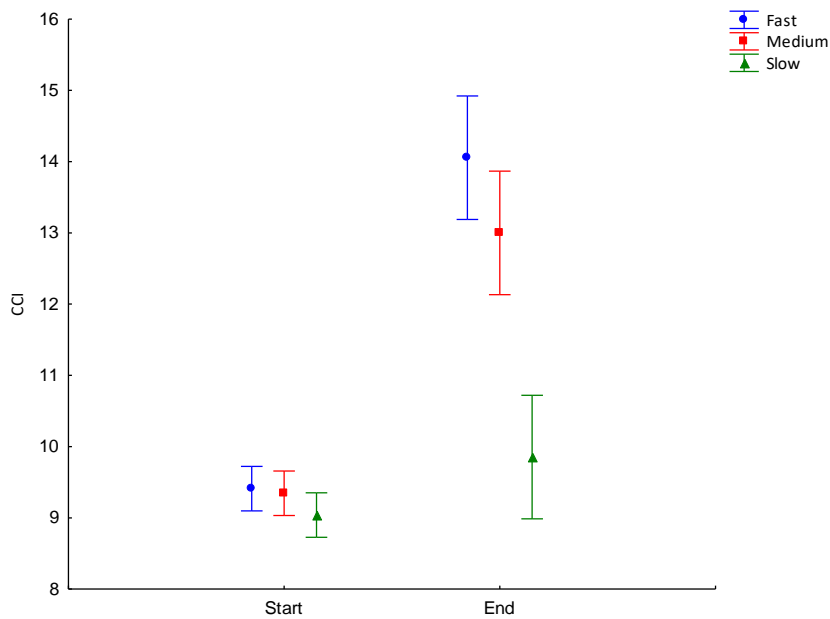


Figure 2.15 The mean (95% confidence interval) chlorophyll concentration index of the leaf tissue in spinach grown in constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments at the start and end (week-19) of the experiment (Repeated measures ANOVA, $F_{(2, 186)} = 17.07$, $p < 0.001$).

Leaf magnesium and sodium concentration increased significantly from seedling to full grown plants at the end of the trial in all treatments, but with no significant difference between treatments (Table 2.3). The leaf potassium, zinc, manganese, iron, phosphorous and aluminium concentrations decreased significantly from seedlings in all treatments, but again, there was no significant difference between treatments (Table 2.3). The leaf nitrogen concentration of plants at the start of the trial did not differ from that at the end of the trial for plants grown in fast and medium FDT (Table 2.3); however, it decreased significantly in those grown in the slow FDT treatment (One-way ANOVA, $F_{(3,8)} = 5.91$, $p = 0.020$; Table 2.3). Leaf calcium concentration remained similar for plants grown in the medium and slow FDT treatments, with the fast treatment showing the only increase from the start to the end of the trial period (Kruskal-Wallis, $H_{(2, 12)} = 8.08$, $p = 0.045$; Table 2.3). Leaf copper concentration decreased significantly from seedlings over the trial period in all treatments, with the slow

FDT treatment (4.77 ± 0.17 mg/kg) having significantly higher copper concentration than the fast FDT treatment (2.40 ± 0.29 mg/kg) at the end of the trial (Kruskal-Wallis, $H_{(3, 12)} = 9.46$, $p = 0.024$; Table 2.3).

Table 2.3 The means (\pm standard error) chemical composition of seedlings (at the start of the trial) and spinach plants grown in constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining times treatments after a 19-week period. Values in the same row represented by a different superscript symbol represent significantly different means (One-way ANOVA/Kruskal Wallis, $p < 0.05$).

Element	Seedlings	Fast	Medium	Slow	F/H	P
Nitrogen (%)	3.87 \pm 0.04 ^a	3.14 \pm 0.24 ^{ab}	3.62 \pm 0.20 ^{ab}	3.03 \pm 0.08 ^b	F _(3, 8) = 5.91	0.020
Calcium (%)	0.76 \pm 0.01 ^a	1.03 \pm 0.04 ^b	0.82 \pm 0.09 ^{ab}	0.97 \pm 0.05 ^{ab}	H _(2, 12) = 8.08	0.045
Magnesium (%)	0.73 \pm 0.01 ^a	0.96 \pm 0.03 ^b	0.88 \pm 0.03 ^b	0.87 \pm 0.03 ^b	F _(3, 8) = 16.11	0.001
Potassium (%)	4.96 \pm 0.05 ^a	3.38 \pm 0.13 ^b	3.29 \pm 0.09 ^b	3.45 \pm 0.26 ^b	H _(3, 12) = 6.59	0.086
Sodium (mg/kg)	17207.1 \pm 116.70 ^a	96252.6 \pm 8469.79 ^b	89199.4 \pm 5639.67 ^b	102763.3 \pm 4511.07 ^b	F _(3, 8) = 51.19	< 0.001
Zinc (mg/kg)	63.79 \pm 1.28 ^a	38.60 \pm 4.28 ^b	27.13 \pm 9.10 ^b	35.34 \pm 3.04 ^b	F _(3, 8) = 8.92	0.006
Manganese (mg/kg)	86.19 \pm 0.38 ^a	17.18 \pm 2.41 ^b	17.26 \pm 1.45 ^b	17.96 \pm 3.49 ^b	F _(3, 8) = 233.82	< 0.001
Copper (mg/kg)	56.63 \pm 0.96 ^a	2.40 \pm 0.29 ^b	2.56 \pm 0.19 ^{bc}	4.77 \pm 0.17 ^c	H _(3, 12) = 9.46	0.024
Iron (mg/kg)	1155.37 \pm 137.87 ^a	68.20 \pm 13.96 ^b	76.40 \pm 16.02 ^b	88.23 \pm 18.27 ^b	F _(3, 8) = 51.47	< 0.001
Phosphorous (%)	0.87 \pm 0.01 ^a	0.15 \pm 0.02 ^b	0.17 \pm 0.01 ^b	0.12 \pm 0.01 ^b	F _(3, 8) = 1296.50	< 0.001
Aluminium (mg/kg)	772.27 \pm 45.75 ^a	170.00 \pm 28.84 ^b	159.58 \pm 28.23 ^b	180.06 \pm 28.22 ^b	F _(3, 8) = 80.38	< 0.001

2.3.3 Plant health and a qualitative record of growth.

At six weeks, plant biomass had the visual appearance of being highest in fast FDT treatments, followed by medium treatments and slow FDT treatments, respectively (Figure 2.16). The occurrence of chlorosis and necrosis was not equally distributed between treatments. It was more prevalent than expected in the slow FDT treatment, whereas there were fewer cases recorded in the fast and medium FDT treatments (Chi-squared analysis, $X^2 = 41.71$, $p < 0.001$; Figure 2.17 and 2.18; Table 2.4). Necrosis was also more prevalent than expected in the slow FDT treatment, whereas there were fewer cases recorded in fast and medium FDT treatments (Chi-square analysis, $X^2 = 16.63$, $p < 0.001$; Figure 2.16 and 2.17; Table 2.4).

A



B



C



Figure 2.16 Spinach plants after six weeks in (A) fast (15 min), (B) medium (30 min) and (C) slow (60 min) filling and draining time treatments.



Figure 2.17 Chlorosis of spinach plants indicated by a decline of green chlorophyll pigmentation (Marschner 1984).



Figure 2.18 Chlorosis and necrosis on spinach plants where chlorosis is indicated by a lack of green chlorophyll pigmentation (Marschner 1984) and necrosis is indicated by spots of dying plant cells forming on leaves (Steddom *et al.* 2005).

Table 2.4 Observed frequencies for chlorosis and necrosis of plants grown in tidal constructed wetlands with fast (15 min), medium (30 min) and slow (60 min) filling and draining time treatments (Chi-square analysis, $p < 0.05$).

	Fast	Medium	Slow	Chi-square (X^2)	p-value
No. of plants with chlorosis	1	4	29	41.71	< 0.001
No. of plants with necrosis	1	2	13	16.63	< 0.001

2.4 Discussion

2.4.1 Water quality parameters

Dissolved oxygen in all three treatments increased over the five-day batch cycle period. The DO concentration in the influent was extremely low due to the effluent source (anaerobic digester) having depleted oxygen levels (Botheju and Bakke 2011). Dissolved oxygen increased due to the constant filling and draining of wastewater, where atmospheric oxygen is pulled into the media due to a pressure gradient and oxygen gets trapped in the media during the filling phase (Stottmeister *et al.* 2003, Wu *et al.* 2014). Dissolved oxygen concentrations were increased in the wastewater in all treatments in the current trial.

Dissolved oxygen concentration increased with faster filling and draining times. The “fast” treatments had the highest dissolved oxygen values over the 5-day period. Oxygen enters the system during each cycle. Thus, more cycles per day will increase oxygen in the system (Cui *et al.* 2012). Therefore, faster cycle rates increase the concentration of dissolved oxygen in the wetland which can affect the performance of nitrifying bacteria in converting ammonia and therefore, treatment efficiency (Kadlec and Wallace 2008, Cui *et al.* 2012). Fast treatments had the highest DO concentrations due to the increased tidal cycles per day.

Fast and medium treatments were able to decrease ammonia in the effluent at a faster rate than slow treatments. Fast and medium treatments were able to treat ammonia to discharge standards of < 3 mg/l (DWA 1998) within 4 days, and to < 0.5 mg/l in 5 days. Ammonia concentration and dissolved oxygen had a significant relationship. As the treatments with higher dissolved oxygen concentration also had faster ammonia treatment rate, and the treatment with the lowest dissolved oxygen concentration had the lowest ammonia treatment rate. This relationship can be explained as the nitrifying bacteria involved are

dependent on oxygen for nitrification and respiration (Hua *et al.* 2017). Li *et al.* (2014) found that aerated wetlands outperform non-aerated wetlands in ammonia treatment performance. Aerated wetlands removed 99.7% ammonia while unaerated wetlands only removed 18.4% ammonia due to aeration improving oxidizing conditions (Li *et al.* 2014). Increasing DO levels improve nitrifying bacteria performance and therefore, ammonia oxidation (Jia *et al.* 2010, Wu *et al.* 2011b).

Ammonia treatment was likely due to nitrification. All treatments had dissolved oxygen concentration means of > 6 mg/l after day 1. These systems can therefore be defined as aerobic systems where nitrification is the reason for ammonia decrease and not denitrification, which is a process that occurs when oxygen is absent from the system. The pH values of the systems also indicate that nitrification was probably the main mechanism by which ammonia was decreased, rather than ammonia volatilization and plant uptake which was unlikely here. Volatilization is a process where ammonia converts to a gaseous form, however this only occurs when the pH value in water is above 9.50 (Park *et al.* 2011) and the current systems ranged from 7.87 to 9.03 in all treatments. Nitrification takes place between pH values 6.45 and 8.95, as values below and above this range inhibit nitrifying bacteria (Ruiz *et al.* 2003). All treatments had pH means between 8.00 and 9.00 after day-0, which is also the optimum range for nitrification (Shammas 1986). This pH range suggests that plant uptake was an unlikely mechanism of ammonia treatment due to nitrate being the preferred form of nitrogen in plant uptake at high pH values (Masclaux-Daubresse *et al.* 2010). Based on dissolved oxygen concentrations and pH values of the treatments, nitrification was probably the main mechanism of nitrogen removal in the wetlands.

Nitrite and nitrate concentrations for all three treatments at the start and end of the batch cycles were below discharge standards of < 15 mg/l (DWAF 1998). The rate of nitrite and nitrate removal was not significantly different between treatments with all filling and draining times following the same trend. Nitrite was stable initially but decreased between the start and end of the batch cycle; the stabilisation was probably due to the conversion from ammonia in the nitrification process (Li *et al.* 2014). Nitrate decreased initially, probably due to plant uptake, and then levelled off, which was likely due to nitrite being converted into nitrate (Kadlec and Wallace 2008, Li *et al.* 2014).

Phosphate removal was low across the different filling and draining times. Treatments showed no differences in phosphate removal. This is expected as wetlands are generally poor in removing phosphate (Vymazal 2010). The decrease that was observed across all treatments were likely due to plant and microbial uptake (Stottmeister *et al.* 2003, Vymazal 2007). In a study by Vymazal (2010), it was found that vertical flow sub-surface wetlands had an incoming phosphate level of 10.3 mg/l and was treated to 4.5 mg/l (i.e. more than 50% reduction). The current study had a lower rate of removal (10%), with a mean incoming and outgoing phosphate concentration of 4.82 ± 0.15 mg/l and 4.33 ± 0.15 mg/l, respectively. Phosphate removal in CWs will remain low, unless substrates with high sorption capacities are used (Vymazal 2010). The current study used gravel, which offers low removal capacity via sorption and precipitation (Vymazal 2007, Vymazal 2010). To improve phosphate removal, substrates such as clay aggregates can be used, but their efficiency decreases over time (Vohla *et al.* 2005). Phosphate concentrations were below discharge standards (DWAF 1998) in the current study and therefore, a substrate change is unnecessary if the only goal is to reach discharge standards.

Chemical oxygen demand (COD) was lowered to below the discharge limit of < 75 mg/l (DWAF 1998) in all treatments. Vertical flow wetlands are known to lower COD effectively; Luederitz *et al.* (2001) found they were able to lower the COD by 94.6%. The efficiency at which COD was lowered improved in systems with a high COD in the influent (Zhu *et al.* 2014). The lower rate at which COD decreased ($63.14 \pm 0.81 \%$) in this experiment was likely due to the low influent COD of means 144.61 ± 7.27 mg/l, 153.78 ± 7.27 mg/l and 143.61 ± 7.27 mg/l for treatments with fast, medium and slow FDT, whereas the incoming COD from Luederitz *et al.* (2001) study was high at 815.7 mg/l.

The pH of effluent increased initially in all treatments before levelling off. The increase in pH was due to the production of CO₂ in anaerobic digestion, where some CO₂ dissolves, thus generating carbonate alkalinity and carbonic acid (van Rensburg *et al.* 2003, Power and Jones 2016). When the effluent is aerated or exposed to the atmosphere, the carbonic acid is removed, leaving the more stable carbonate alkalinity in the water (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003, Power and Jones 2016). This results in the decrease of acidity and an increase in pH in the effluent. This process may explain why the pH increased with faster filling and draining times as higher tidal frequencies result in more draining periods, which increases carbonate alkalinity due to aeration and exposure to the atmosphere from the splashing which occurred at the outflow. The high pH in effluent can negatively affect the availability of nutrients for plant uptake in the effluent (Peterson 1982).

There were no differences in temperature between treatments. Influent temperature from the anaerobic water was relatively high, with means close to 24.0°C. Influent temperatures were similar to Jones *et al.* (2014) mean temperature of $\pm 22.0^\circ\text{C}$ for AD effluent. Water temperatures decreased to around 17.0°C for all treatments when exposed to the atmosphere.

The water temperature means fall into the range needed for effective nitrification, which is 5.0-35.0°C (Shammas 1986). However, the means are below the optimal temperature of 30.0°C (Shammas 1986). Shammas (1986) found that water temperatures of 17.0°C have half the nitrification rate of 30.0°C water bodies. Increasing the wetlands temperature through heating will increase running costs and a trade off with dissolved oxygen will ensue. Higher temperatures will negatively affect the plants in the wetland as the plants respiratory demand for oxygen will increase and the available oxygen in the water body will decrease, thus affecting plant growth (Stenstrom and Poduska 1979, Freeman 2005). The measured temperatures may have decreased the rate of nitrification but was sufficient in keeping desired dissolved oxygen concentrations in the water for plant root respiration and growth.

Chloride concentration was significantly lower in the fast treatment compared to the slow treatment at the start of the batch cycle. This was unexpected as all treatments had the same water source. However, due to the number of systems in each treatment, the time taken to fill all the sump tanks were approximately four hours. Chloride in anaerobically digested effluent is dependent on the volume used as chlorine dioxide during clean-in-place (CIP); which is defined as “the cleaning of complete items of plant or pipeline circuits without dismantling or opening of the equipment and with little or no manual involvement on the part of the operator” (Chen *et al.* 2012). This may vary due to brewing cleaning practices and may explain the difference at the start as concentrations may differ in the anaerobic digester over time due to the inflow of new wastewater from the brewery (Spannenberg, pers. comm., effluent plant operator, Ibhayi Brewery, SAB Ltd., December 2018).

Chloride concentration in the effluent decreased in all FDT treatments. There was no difference between treatments at the end of the batch cycle. Treatments decreased chloride

concentration from 192.69 ± 1.85 mg/l to 119.24 ± 1.77 mg/l. Chloride has numerous biological interactions in a water body, but these do not generally result in concentration changes; and, when concentrations change between inlet to outflow, it presents a challenge for finding the cause (Kadlec and Wallace 2008). Chloride is an essential plant nutrient as plants use chloride in enzyme activation of photosystem II, water-splitting in photosynthesis and aids in water and electrical balance (Epstein and Bloom 2005, Freeman 2005); and concentrations may decrease in wetlands via plant uptake (Xu *et al.* 2004). Therefore, the decrease in concentration in this instance may have been due to plant uptake. A decrease in chloride usually results in a decrease in EC, as chloride is one of the ions that contribute to conductivity (Abyaneh *et al.* 2005). This would explain the EC decrease between the start and end of the batch cycles, with EC concentrations decreasing from an overall means of 3113.15 ± 29.93 $\mu\text{S}/\text{cm}^2$ to 2911.67 ± 27.90 $\mu\text{S}/\text{cm}^2$ in all treatments. High EC values can negatively affect plant growth due to osmotic stress as it decreases osmotic potential between root plasma and water, resulting in lower growth rates due to increased energy expenditure for water uptake (Munns and Termaat 1986). The high EC in brewery wastewater is probably due to addition of sodium hydroxide (NaOH), which is used as a pH buffer of raw brewery effluent prior to anaerobic digestion (Power and Jones 2016). Electrical conductivity removal is generally low in CWs. Kadlec and Wallace (2008) found that their wetland only lowered 2% of wastewater EC. Therefore, the removal of EC in CWs will remain relatively low, which is consistent with the result presented here.

The different drain fill regimes might have been subject to different rates of evapotranspiration, and this could have influenced the result. Evapotranspiration is the sum of evaporation from the wetland surface and transpiration from plants (Giraldi *et al.* 2010)

and it is affected by numerous factors, which include: temperature, humidity, wind speed, plant cover and plant moisture level (Dong *et al.* 2011). This process can concentrate pollutants which may affect water quality readings (Kadlec and Wallace 2008). It was not possible to determine if the rate of evapotranspiration influenced the results in the current work since it was not measured; but this should be considered when interpreting these results and when carrying out further research.

2.4.2 Plant harvest and health

There was a significant increase in total harvest with increasing FDT. This was likely due to faster FDT having higher dissolved oxygen concentrations and fewer waterlogged periods. Increased DO concentrations improve plant respiration and therefore, plant growth (Jackson 1979, Freeman 2005, Marschner 2011). Terrestrial plants have adapted to dry conditions, and the increase in frequency of filling and draining cycles in faster FDT treatments decreased the number of waterlogged periods and increased the frequency of “dry” periods, resulting in increased root exposure to atmospheric oxygen. Increased “dry” periods decrease the time that water and plant roots come into contact and decreased “dry” periods are detrimental to plant growth due to terrestrial plants’ inability to survive water logged conditions (Stottmeister *et al.* 2003). Fast FDT treatments had fewer waterlogged periods due to the faster activation of the siphon in the transition from filling phase to the draining phase. The DO concentration of the fast FDT treatment and increased “dry” periods resulted in improved plant health, higher total harvest and highest chlorophyll concentration increase.

Chlorophyll concentration index (CCI) is an indicator for plant health, since “healthier” plants are known to have a higher chlorophyll concentration (Pavlovic *et al.* 2014). Fast and medium FDT treatments had higher CCI values than slow treatments. This indicates that fast and

medium FDT treatments had healthier plants, likely due to the higher availability of oxygen for the plant roots. In photosynthesis, the energy from sunlight is taken from a chlorophyll-protein complex and transmitted to photosystems I and II which is then converted into chemical energy; the whole photosynthetic process is said to rely on chlorophyll (Pavlovic *et al.* 2014). This may explain why the slow FDT treatments had the lowest total harvest and CCI; since photosynthesis was less efficient.

The pH of water affects the availability of nutrients for plant uptake. The average pH for fast, medium and slow ranged between 8.53 and 8.69. Over the trial period the following elements decreased in the plant leaf tissue in some or all of the treatments: nitrogen, copper, potassium, zinc, manganese, iron, phosphorous and aluminium. A deficiency in these nutrients can result in chlorosis (nitrogen, potassium, manganese, iron), necrotic spots (copper, manganese, phosphorous), and stunted leaves (zinc, phosphorous; Freeman 2005). Manganese, phosphorous and iron have reduced availability at pH > 8.0 (Peterson 1982); and copper, zinc and potassium are found in small quantities in brewery effluent (Alayu and Yirgu 2018). The following elements increased in the plant leaf tissue over the trial period in some or all of the treatments: calcium, magnesium and sodium. Calcium likely increased due to it being available at high pH values (Peterson 1982), and effluent having a high percentage of Ca when compared to other industries such as pulp and paper mills (Alayu and Yirgu 2018). Sodium is readily available due to sodium hydroxide addition in anaerobic digestion (Taylor *et al.* 2018). Magnesium is also available in brewery effluent and increased due to the availability at high pH (Peterson 1982, Alayu and Yirgu 2018). Plant nutrients found in spinach plants were affected by a combination of the availability of nutrients in brewery effluent and nutrient availability at high pH.

Chlorosis is an indication of a deficiency of certain plant nutrients, such as: nitrogen, iron, magnesium, manganese (Epstein and Bloom 2005, Freeman 2005). All these nutrients except for magnesium decreased in the plants during the trial which explains why plants were experiencing chlorosis. Leaf spots could be due to low oxygen and potassium, which results in cell death and “dead spots” (Freeman 2005). Although some plants were void of certain nutrients, the trial was the first of its kind in terms of growing spinach in brewery effluent.

2.5 Conclusion

Filling and draining time influenced the dissolved oxygen concentration and treatment efficiency in the wetlands. The treatment with a FDT of 15 min had higher dissolved oxygen concentration compared to slow (FDT 60 min) treatments. This likely resulted in improved ammonia treatment from the effluent as the fast and medium FDT treatments removed ammonia at a faster rate than slow FDT treatments. The pH increased significantly in all FDTs, with treatments with 15 min FDT occasionally having significantly higher values than treatments with 30 min and 60 min FDT. Nitrate, nitrite, temperature, COD, phosphate, EC and chloride concentrations all decreased significantly from the start and end of the batch cycles, there was however, no significant difference between treatments for these parameters.

Filling and draining time influenced plant harvest and health. Treatments with faster FDT were able to withstand higher pH values and showed the highest total harvest and CCI. Leaf tissue chemical concentration was influenced by FDT for nitrogen (decrease), calcium (increased) and copper (decreased).

The improved ammonia treatment in the fast FDT treatment when compared to the slow FDT treatment and improved plant health and harvest in the faster FDT treatments was most

probably due to increased dissolved oxygen and less waterlogged periods. For these reasons, a fill time of 15 min will be used to compare tidal wetlands to aerated and unaerated subsurface flow wetlands in future experiments (Chapters 3 and 4).

Chapter 3: The influence of aeration and tidal-cycles on nutrient dynamics and crops grown in constructed wetlands.

3.1 Introduction

3.1.1 Rationale

Constructed wetlands (CWs) can be defined according to their flow; these include free water surface (FWS) and sub-surface flow (SSF) (Section 1.2.4, Chapter 1). Sub-surface flow can further be distinguished between horizontal (HSSF) and vertical flow (VSSF) CWs. In HSSF constructed wetlands wastewater flows at a constant depth below the surface of the substrate where emergent vegetation is grown (Garcia *et al.* 2010, Vymazal 2010, Vymazal 2014). In VSSF, which have a flat bed of gravel where plants are grown, the effluent is fed until the bed is flooded (Vymazal 2014). There are four types of vertical sub-surface flow (VSSF) CWs depending on their hydraulic regimes: unsaturated flow, permanent saturated flow, intermittent unsaturated flow and tidal wetlands (Garcia *et al.* 2010; Figure 3.1).

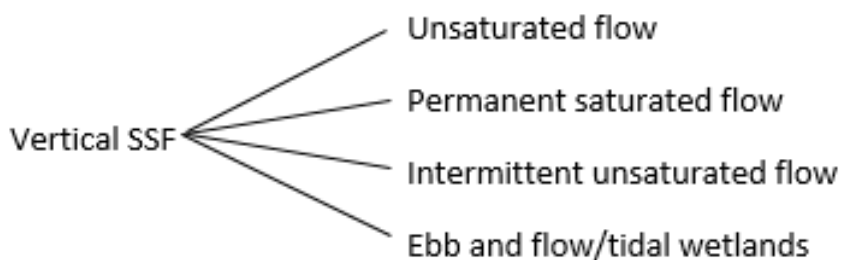


Figure 3.1 Classification of vertical sub-surface flow constructed wetlands (Garcia *et al.* 2010).

These wetlands are efficient in removing organics and suspended solids (Vymazal 2010). Organic compounds are broken down through microbial degradation which occurs in aerobic processes in VSSF CWs while anoxic and aerobic processes prevail in HSSF CWs (Vymazal

2010). Most CWs make use of gravel, which has low sorption capacity, materials with high sorption capacity include iron or aluminium oxides (Vymazal 2014). Phosphorous is removed via ligand reactions when phosphate replaces water or hydroxyls from iron and aluminium oxides (Kadlec and Wallace 2008). All constructed wetlands are poor in phosphorous removal unless substrates with high sorption capacity is used (Vymazal 2014).

Oxygen availability differs between these CWs. In HSSF constructed wetlands anaerobic and anoxic conditions are prevalent, with fewer aerobic zones as they are limited to the upper surfaces where oxygen is supplied through atmospheric diffusion, plant roots and rhizome surfaces (Kadlec and Wallace 2008, Garcia *et al.* 2010, Vymazal 2014). While VSSF constructed wetlands are far more aerobic than HSSF constructed wetlands (Vymazal 2010).

Nitrogen removal is achieved through nitrification and denitrification in HSSF constructed wetlands; and in VSSF constructed wetlands, nitrogen is removed through nitrification only (Vymazal 2010,2014). Nitrification occurs in the small aerobic zones found in HSSF constructed wetlands; after ammonia is converted to nitrate by nitrifying bacteria, it is converted to free nitrogen or nitrous oxide (Kadlec and Wallace 2008, Vymazal 2014). Due to the limited oxygen available in HSSF constructed wetlands, nitrification is limited (Kadlec and Wallace 2008, Vymazal 2014). Therefore, these wetlands rely on denitrification for ammonia removal which requires an anoxic zone and a carbon supply (Kadlec and Wallace 2008).

The lack of oxygen in HSSF constructed wetlands has often restricted treatment efficiency (Wu *et al.* 2014). To enhance oxygen transport capacity in these systems, artificial aeration of HSSF CWs was introduced in the early 2000s (Vymazal 2010, Wu *et al.* 2011b). Aeration of these wetlands improves removal of biochemical oxygen demand (BOD), total suspended solids (TSS) and total Kjeldahl nitrogen (TKN) and has no effect on phosphorous removal

(Wallace *et al.* 2008). However, aeration of HSSF constructed wetlands is costly due to increased operation and maintenance (Kadlec and Wallace 2008). In VSSF constructed wetlands, tidal wetlands improve oxygen transfer in conventional CWs and require only half the power of artificially aerated wetlands (Wu *et al.* 2014). Increased oxygen transfer in tidal wetland applications is advantageous as it increases the removal of nitrogen which has strict disposal limits (Stefanakis and Tsihrintzis 2012).

Wetland and marsh plants have certain characteristics that allow them to grow in extreme rhizosphere conditions which make them suitable for HSSF constructed wetlands (Stottmeister *et al.* 2003). The ability of the root system to supply oxygen from the atmosphere is the reason why these plants survive conditions that are low in oxygen (Stottmeister *et al.* 2003). Marsh plants are limited in terms of economic utilisation as their production is seasonal and often without economic value (Vymazal 2011b).

Land plants (which include most vegetable cash crops) have adapted to dry conditions and many cannot survive waterlogged conditions in horizontal sub-surface wetlands (Stottmeister *et al.* 2003). In tidal wetlands, the draining of the wetland increases the oxygen concentration and artificial aeration in HSSF increases dissolved oxygen concentration which benefits root growth (Jia *et al.* 2010, Vymazal 2011b). The nitrification process in aerobic wetlands (i.e. converting ammonia into nitrate) improves nutrient quality and reduces ammonia toxicity to crops (Kamthunzi 2015).

There has been research comparing the differences of treatment efficiency and macrophyte growth between aerated and unaerated CWs (Vymazal 2010, Wu *et al.* 2014). Tidal wetlands treatment efficiency and macrophyte growth has also been characterised (Abou-Elela and Hellal 2012). However, there is a gap in literature focusing on comparing tidal flow CWs and

aerated and unaerated HSSF using brewery effluent under controlled replicated conditions with the aim of producing crops on a pilot-scale.

3.1.2 Aims and objectives

The aim of this experiment was to determine which constructed wetland resulted in the highest treatment efficiency and crop harvest. This was achieved by comparing tidal wetlands to sub-surface wetlands that are either aerated or unaerated wetlands in terms of nutrient removal from effluent and plant health and harvest.

The objectives of the study were to:

- compare dissolved oxygen in tidal, aerated and unaerated horizontal sub-surface flow wetlands;
- compare effluent treatment efficiency in tidal, aerated and unaerated horizontal sub-surface flow wetlands; and
- to compare total harvest and plant health of the crop in tidal, aerated and unaerated horizontal sub-surface flow wetlands.

3.2 Methods and materials

3.2.1 Experimental species

One hundred and eighty spinach (*Beta vulgaris*) seedlings were purchased from a commercial seedling supplier (Moorland Seedlings (Pty) Ltd, Humansdorp). Of these, 135 were planted and the rest used for destructive plant health analysis.

Spinach (*Beta vulgaris*) were grown for 14 weeks to observe the effects of different CWs and water quality on the plant's growth and health. Leaves from plants were harvested when leaves were at a harvestable size (± 10 cm long).

3.2.2 Experimental system and treatments

The study consisted of nine beds with the same design as the previous experiment (Figure 2.3 to 2.5, Section 2.2.3, Chapter 2). After the first experiment (Chapter 2), the beds were filled and remained flooded for 14 days to exclude aeration, before being drained and the gravel washed for the current trial. The nine beds were separated into three treatments; namely tidal CW, aerated horizontal sub-surface flow (aerated CW) and unaerated horizontal sub-surface (unaerated CW; Table 3.1). Therefore, the treatments were each replicated three times. Grow bed volume and total volume of the grow bed and sump tank were consistent throughout the treatments (Table 3.1). Plants were thinned out to 15 plants per bed after two weeks.

Brewery effluent was pumped into each tank via a 40 mm irrigation pipe at the start of each batch cycle (day-0) and was circulated in each system for a five-day batch cycle period. After the five-day period the grow beds were then drained and emptied using a submersible pump before being refilled for the next batch cycle.

Table 3.1 Treatments with their hydraulic regime, grow bed volume (L) and total volume of effluent in grow bed and sump tank (L), aeration regime and flow rates (l/h) (T1-T3).

Treatment	Hydraulic regime	Maximum volume of effluent in grow bed (L)	Total volume of effluent in grow bed and sump tank (L)	Aeration regime	Flow rate (l/h)
T1	Aerated horizontal sub-surface flow (aerated CW)	520	850	Channel blower	2600
T2	Un-aerated horizontal sub-surface flow (un-aerated CW)	520	850	None	2600
T3	Periods of filling and draining (Tidal CW)	520	850	Fill and drain	2600

The tidal system had the same design as the previous experiment with a filling and draining time of 15 min (Figure 2.3-2.5, Chapter 2). The other beds were converted into horizontal sub-surface flow wetlands by replacing the siphon with a 30 mm polyvinyl chloride (PVC) over-flow pipe, resulting in a constant outflow of effluent into the sump tank that was equal to the inflow of effluent into the bed. The HSSF constructed wetland was aerated through 25 mm PVC pipe that was connected to a 0.55 KW side channel blower (Model ZXB-310, CFW Industries (Pty) Ltd, South Africa) to form the aerated constructed wetland treatment (Figure 3.2), and the HSSF constructed wetland without aeration formed the un-aerated CW (Figure 3.2). The aerated and un-aerated sub-surface flow beds had the same rate of effluent pumped into the bed as the tidal beds.

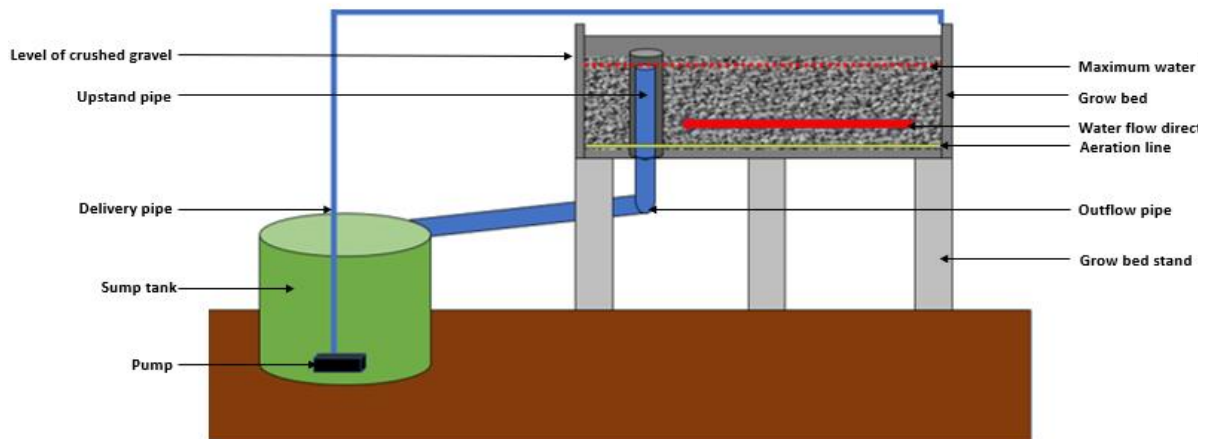


Figure 3.2 Water flow regime and system components in aerated CW (T1). Un-aerated CW (T2) had the same design, except for the aeration line.

3.2.3 Data collection

The same water quality, plant harvest and health parameters, and statistical methods that were used in the previous experiment (Section 2.2, Chapter 2) were applied here. The current trial collected leaves for chemical analysis and measured chlorophyll concentration index at the start and on week-14.

3.3 Results

3.3.1 Water quality parameters

The dissolved oxygen (DO) concentrations of the effluent were influenced by an interaction between wetland design and time (Repeated measures ANOVA, $F_{(10, 345)} = 48.37$, $p < 0.001$; Figure 3.3). It was low for all treatments on day-0 and increased in all treatments on day-1 (Figure 3.3). In the unaerated CW, DO continued to increase to day-2 before it levelled off (Figure 3.3). From day-1 to day-5 the tidal and aerated CWs had significantly higher DO than unaerated CW (Figure 3.3).

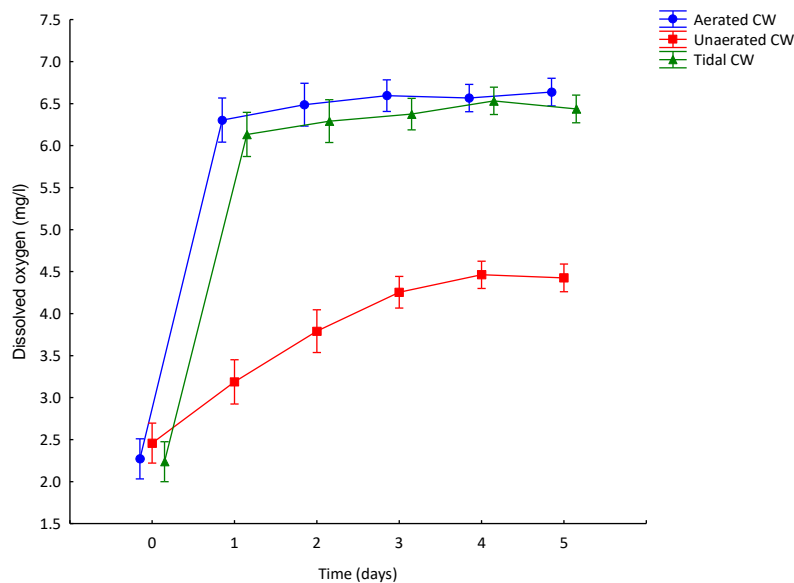


Figure 3.3 The mean (\pm 95% confidence interval) dissolved oxygen concentration in the effluent treated in constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs over five days (Repeated measures ANOVA, $F_{(10, 75)} = 16.73$, $p < 0.001$).

Dissolved oxygen and ammonia concentration had a significant inversely proportional relationship (Multiple regression analysis, $y = -5.85x + 42.22$, $R^2 = 0.66$, $F_{(1, 430)} = 821.98$, $p < 0.001$; Figure 3.4).

Ammonia concentrations in the effluent were influenced by an interaction between wetland design and time (Repeated measures ANOVA, $F_{(10,75)} = 10.78$, $p < 0.001$; Figure 3.5). It decreased in all treatments from day-0 to day-3. Thereafter, tidal and aerated CWs levelled off and the unaerated CW continued to decrease for the rest of the batch cycle (Figure 3.5). The aerated and tidal CWs both lowered ammonia concentration in the effluent to discharge standards within three days while the unaerated CW was unable to do so in the 5-day batch cycle (3 mg/l; DWAF 1998; Figure 3.5). On day-1 and day-2 the tidal CW had the lowest ammonia concentration and the unaerated CW the highest, with the aerated CW being the intermediate (Figure 3.5). Ammonia concentration was lower in the tidal and aerated CWs than the unaerated CW for the remaining days (Figure 3.5).

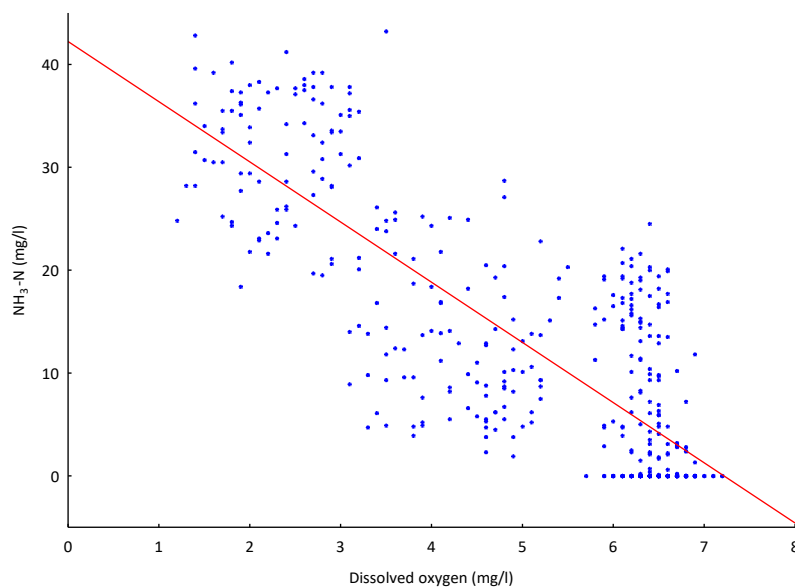


Figure 3.4 Relationship between the effluent ammonia concentration and dissolved oxygen concentration in all treatments (Multiple regression analysis, $y = -5.85x + 42.22$, $R^2 = 0.66$, $F_{(1, 430)} = 821.98$, $p < 0.001$).

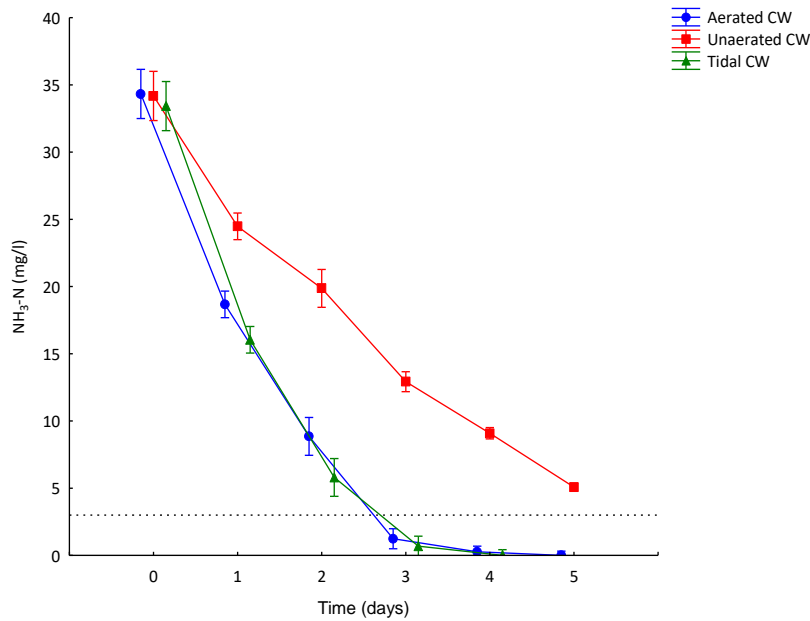


Figure 3.5 The mean (\pm 95% confidence interval) ammonia concentration in the effluent treated in constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs over five days (Repeated measures ANOVA, $F_{(10,75)} = 10.78$, $p < 0.001$). The dashed black line represents the discharge standard for ammonia (3 mg/l; DWAF 1998).

Nitrite concentrations in the effluent were influenced by an interaction between wetland design and time (Repeated measures ANOVA, $F_{(10,345)} = 17.75$, $p < 0.001$; Figure 3.6). It decreased to day-3 in all treatments before levelling off (Figure 3.6). The tidal treatment showed the fastest rate of nitrite decrease, compared with the unaerated CW between day-0 and day-1, and with the aerated CW treatment being the intermediate (Figure 3.6). On day-2 the effluent in the aerated CW had higher nitrite concentration than the tidal CW, and on day-3 and day-4, aerated CW had significantly higher nitrite than both tidal and unaerated CWs (Figure 3.6). The effluent in the tidal CW had the lowest nitrite concentration at the end of the batch cycle (day-5).

Nitrate concentrations in the effluent were also influenced by an interaction between wetland design and time (Repeated measures ANOVA, $F_{(10,345)} = 4.67$, $p < 0.001$; Figure 3.7). It decreased from the start (day-0) to the end (day-5) of the batch cycle for all treatments, with the aerated CW showing the lowest nitrate decrease (Figure 3.7). The unaerated CW and

tidal CW decreased between day-0 and day-1, with no decrease in the aerated CW resulting in higher nitrate than the tidal CW on day-1 (Tukey's HSD, $p < 0.05$; Figure 3.7). The aerated CW had the highest nitrate on day 2, and the unaerated CW the lowest, with the tidal CW being the intermediate (Figure 3.7). Nitrate in the aerated CW was significantly higher than the tidal and unaerated CWs for the remaining days (Figure 3.7).

Nitrite and nitrate concentrations were below discharge standards (15 mg/l; DWAF 1998) throughout the 5-day batch cycles (Figure 3.6 and 3.7).

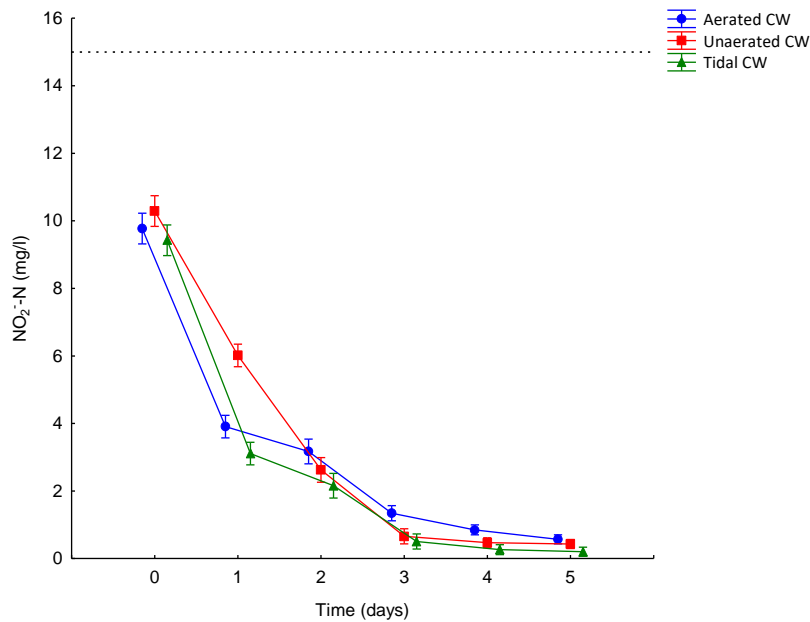


Figure 3.6 The mean (\pm 95% confidence interval) nitrite concentration in the effluent in constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs over five days (Repeated measures ANOVA, $F_{(10,345)} = 17.75$, $p < 0.001$). The dashed black line represents the discharge standard for nitrite (15 mg/l; DWAF 1998).

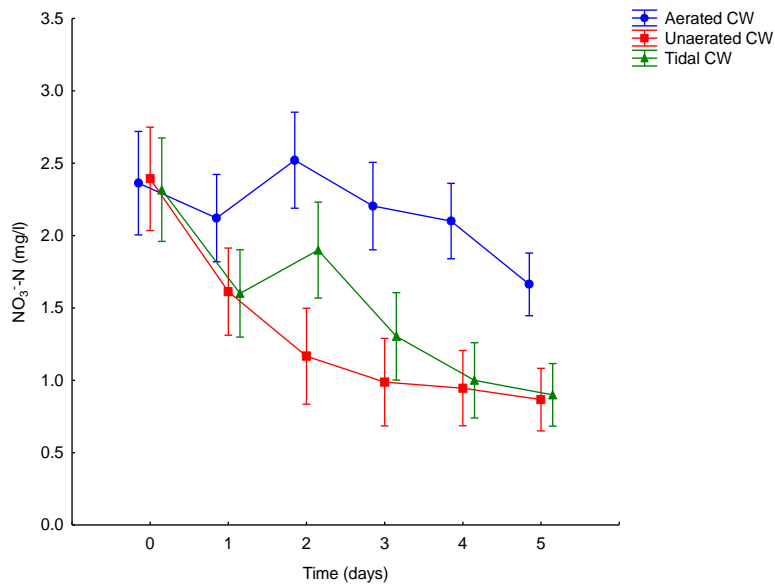


Figure 3.7 The mean (\pm 95% confidence interval) nitrate concentration in the effluent treated in constructed wetlands with aerated and un aerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs over five days (Repeated measures ANOVA, $F_{(10,345)} = 4.67$, $p < 0.001$).

The dissolved oxygen and pH of all treatments had a significant relationship, with pH increasing significantly with increasing dissolved oxygen (Multiple regression analysis, $y = 0.17x + 7.16$, $R^2 = 0.65$, $F_{(1,430)} = 807.62$, $p < 0.001$; Figure 3.8).

The pH values in the effluent were influenced by an interaction between wetland design and time (Repeated measures ANOVA, $F_{(10, 345)} = 7.06$, $p < 0.001$; Figure 3.9). On day-0 all treatments had similar pH values; on day-1 all treatments increased significantly, with the aerated and tidal CWs increasing to a significantly higher pH value than the un aerated CW and remained higher and continued to increase progressively for the rest of the batch cycle (Figure 3.9).

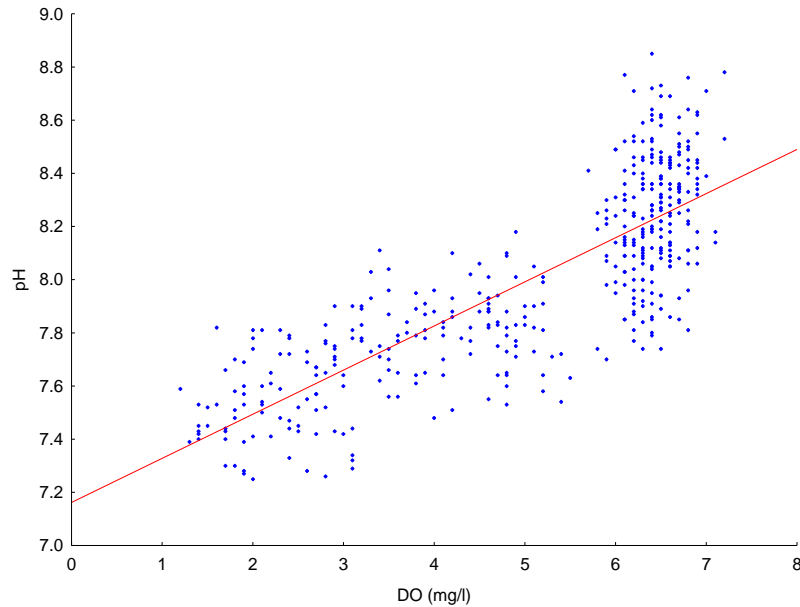


Figure 3.8 Relationship of pH and dissolved oxygen (DO) in all treatments (Multiple regression analysis, $y = 0.17x + 7.16$, $R^2 = 0.65$, $F_{(1,430)} = 807.62$, $p < 0.001$).

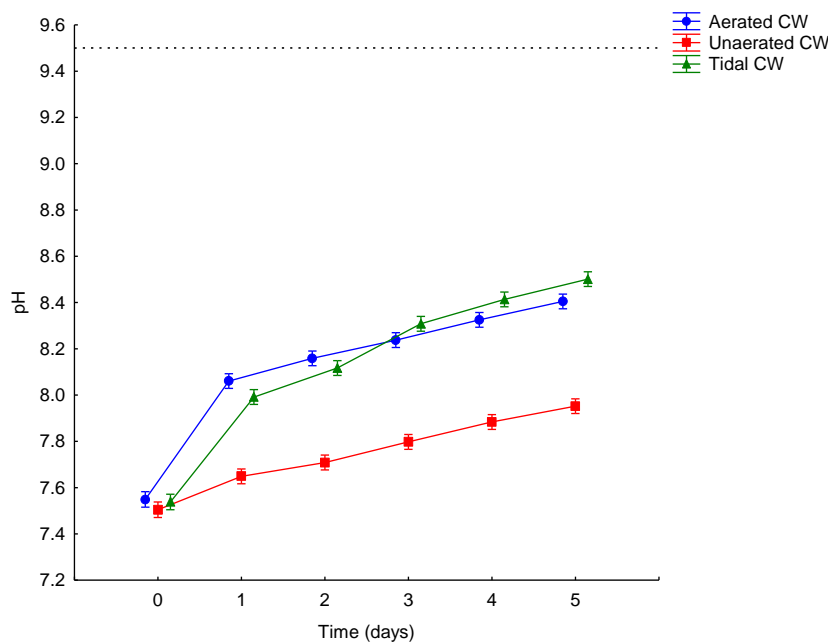


Figure 3.9 The mean (\pm standard error) pH in the effluent in constructed wetlands with aerated and un-aerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs over five days (Repeated measures ANOVA, $F_{(10, 345)} = 7.06$, $p < 0.001$). The dashed black line represents the upper limit of the discharge standard for pH (9.5; DWAF 1998).

Temperature, COD and phosphate in the effluent were not influenced by an interaction between wetland design and time and were similar for the different treatments (Repeated measures ANOVA, $p > 0.05$; Table 3.2). However, all these parameters decreased significantly

from the start to the end of the 5-day batch cycle (Repeated measures ANOVA, $p < 0.05$; Tukey's HSD, $p < 0.05$; Table 3.2). The only difference between treatments in these parameters was the unaerated CW having significantly higher COD than the tidal and aerated CWs at the end of the batch cycle (Tukey's HSD, $p < 0.05$; Table 3.2).

Table 3.2 The mean (\pm standard error) temperature, chemical oxygen demand (COD) and phosphate concentration in constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs. Values in the same row represented by a different superscript symbol represent significantly different means (Repeated measures ANOVA, $p < 0.05$).

	Start		End		F	P
	Combined	Aerated CW	Unaerated CW	Tidal CW		
Temperature ($^{\circ}$ C)	23.05 \pm 0.23 ^a	19.86 \pm 0.47 ^b	19.80 \pm 0.50 ^b	19.82 \pm 0.49 ^b	$F_{(1, 69)} = 125.66$	< 0.001
COD (mg/l)	166.37 \pm 3.21 ^a	51.22 \pm 1.46 ^b	56.67 \pm 1.75 ^c	48.61 \pm 1.44 ^b	$F_{(1, 51)} = 916.84$	< 0.001
Phosphate (mg/l)	4.68 \pm 0.14 ^a	3.89 \pm 0.21 ^b	3.98 \pm 0.14 ^b	3.81 \pm 0.16 ^b	$F_{(1, 69)} = 119.28$	< 0.001

Electrical conductivity and chloride in the effluent were both influenced by an interaction between wetland design and time (Repeated measures ANOVA, $p < 0.05$; Figures 3.10 and 3.11). At the start of the batch cycle, treatments had a similar EC (Figure 3.10). All treatments showed a decrease in EC between the start and end of the 5-day batch cycle with the tidal CW having significantly lower EC than the unaerated CW on day-5 (Tukey's HSD, $p < 0.05$; Figure 3.10). Chloride in the effluent decreased between the start and end of the 5-day batch cycle in all treatments (Figure 3.11). On day-5, the aerated CW had significantly lower chloride than the tidal and unaerated CWs (Figure 3.11).

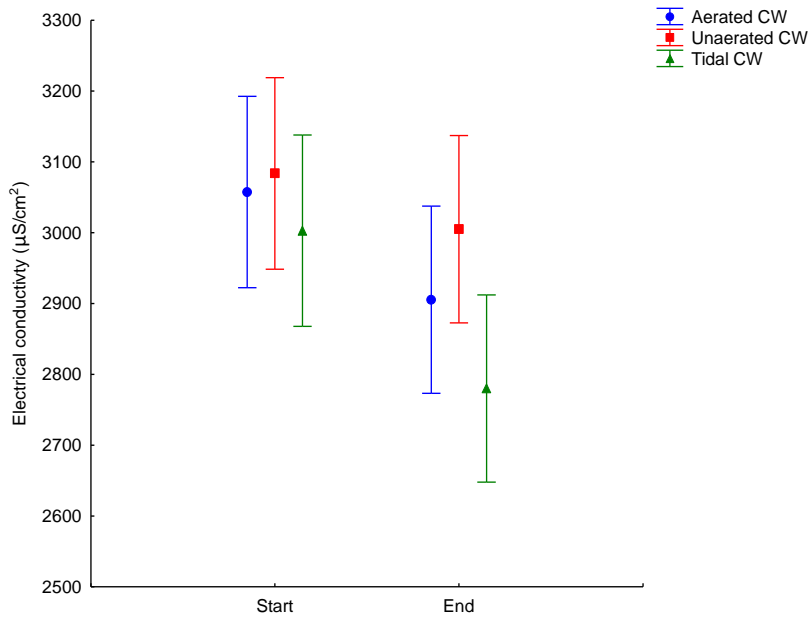


Figure 3.10 The mean (\pm 95% confidence interval) electrical conductivity in the effluent treated in constructed wetlands with aerated and un-aerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs at the start and end of the 5-day batch cycle (Repeated measures ANOVA, $F_{(2, 69)} = 71.03$, $p < 0.001$).

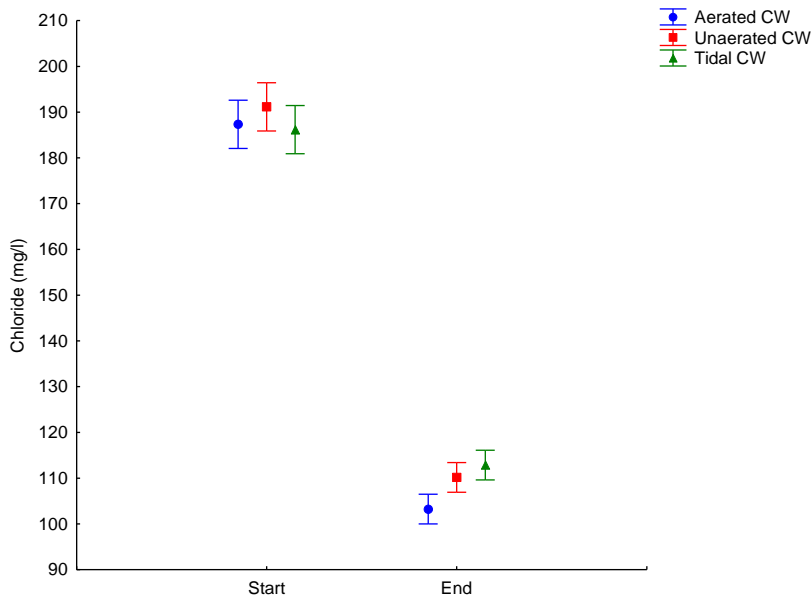


Figure 3.11 The mean (\pm 95% confidence interval) chloride in the effluent treated in constructed wetlands with aerated and un-aerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs at the start and end of the 5-day batch cycle (Repeated measures ANOVA, $F_{(2, 69)} = 3.88$, $p = 0.03$).

3.3.2 Crop harvest and plant health

There was a significant difference in total harvest between the treatments (One-way ANOVA, $F_{(2,6)} = 19.79$, $p = 0.002$; Figure 3.12). The tidal CW (23.97 ± 2.57 kg) had a significantly higher total harvest weight than the aerated (6.41 ± 1.83 kg) and unaerated (6.04 ± 2.44 kg) CWs (Figure 3.12). There was an interaction between wetland design and chlorophyll concentration over time (Repeated measures ANOVA, $F_{(2,118)} = 22.59$, $p < 0.001$; Figure 3.13). The CCI was similar between all treatments at the start (Figure 3.13). The tidal CW (14.51 ± 0.77) showed an increase and had the highest CCI value at the end of the experiment, while the CCI in the unaerated CW treatments was the lowest (6.71 ± 0.89) with the aerated CW treatment being the intermediate and showing no increase or decrease (9.84 ± 0.80 ; Tukey's HSD, $p < 0.05$; Figure 3.13).

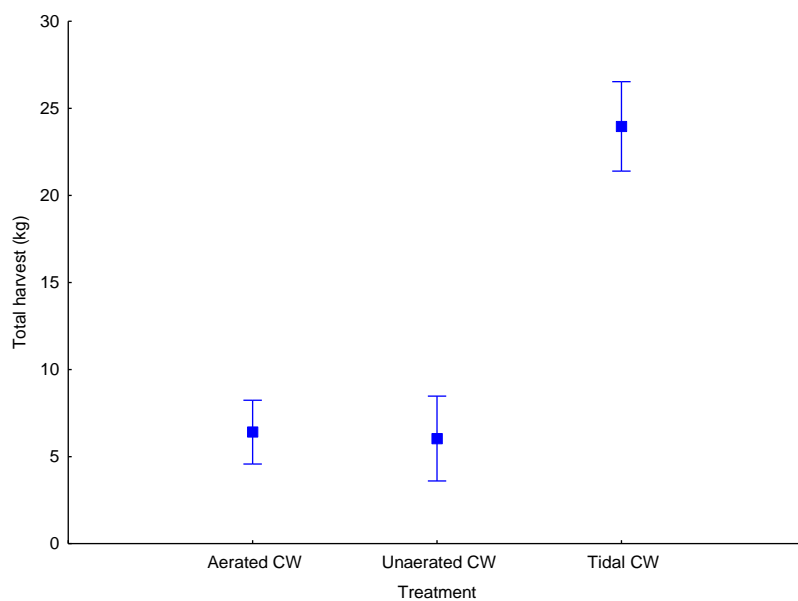


Figure 3.12 The mean (\pm 95% confidence interval) total harvest (kg) of spinach leaves from constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs over a 14-week trial period (One-way ANOVA, $F_{(2,6)} = 19.79$, $p = 0.002$).

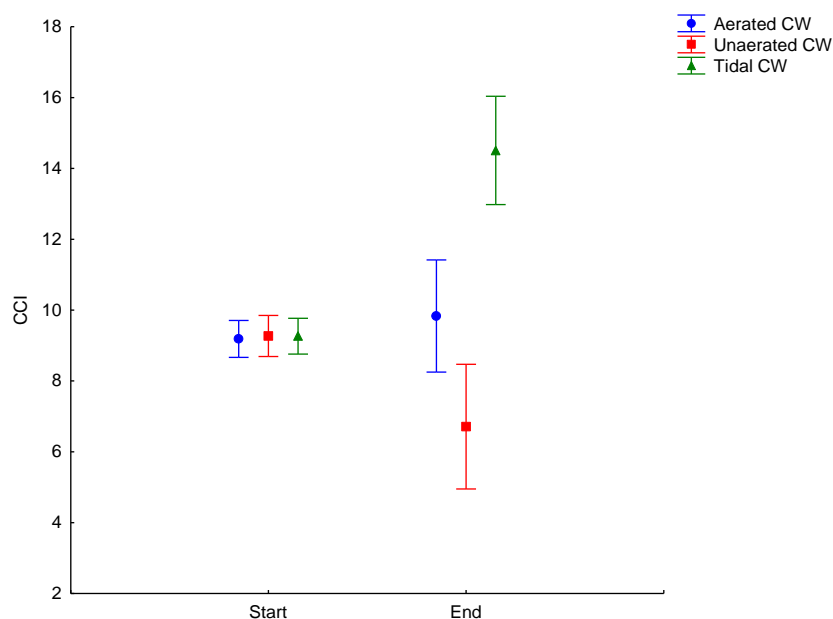


Figure 3.13 The mean (\pm 95% confidence interval) chlorophyll concentration index (CCI) of the leaf tissue in spinach from constructed wetlands with aerated and un aerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs at the start and end of the 14-week trial period (Repeated measures ANOVA, $F_{(2, 118)} = 22.59$, $p < 0.001$).

Leaf nitrogen concentration was similar between treatments at the end of the trial. However, the aerated CW treatment significantly decreased from the seedlings (One-way ANOVA, $F_{(3, 8)} = 8.17$, $p = 0.008$; Table 3.3). At the end of the trial period, leaf sodium concentration in all treatments had significantly increased from the seedlings, with the aerated and tidal CWs treatments increasing to significantly higher concentrations than the un aerated CW treatment (One-way ANOVA, $F_{(3, 8)} = 230.61$, $p < 0.001$; Table 3.3). Leaf zinc concentration decreased significantly from the seedlings in the tidal and un aerated CWs treatments; the aerated CW treatment showed no difference to the seedlings and had a significantly higher zinc concentration than the un aerated CW treatment (One-way ANOVA, $F_{(3, 8)} = 8.37$, $p = 0.008$; Table 3.3). The leaf manganese and iron concentrations significantly decreased from the seedlings in the tidal treatment, but no significant differences were evident between the other CW treatments (Kruskal-Wallis, $H_{(3, N=12)} = 9.46$, $p = 0.024$; Table 3.3). Aluminium concentration decreased significantly from the seedlings in the aerated CW treatment, with

no difference between other CW treatments (Kruskal-Wallis, $H_{(2, N=12)} = 9.67$, $p = 0.022$; Table 3.3). The calcium and magnesium concentrations increased in all treatments from the seedlings but showed no differences between CW treatments (One-way ANOVA, $p < 0.05$; Table 3.3). Leaf copper and phosphorous concentrations decreased significantly from the seedlings in all treatments, with no differences between CW treatments (One-way ANOVA, $p < 0.05$; Table 3.3). Leaf potassium concentration decreased significantly from the seedlings in all treatments, with the unaerated CW decreasing to a significantly lower concentration than the aerated CW (One-way ANOVA, $F_{(3, 8)} = 94.00$, $p < 0.001$; Table 3.3).

Table 3.3 The mean (\pm standard error) chemical composition of seedlings (at the start of the trial) and spinach plants grown in constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs. Values in the same row represented by a different superscript symbol represent significantly different means (One-way ANOVA/Kruskal Wallis, $p < 0.05$).

Element	Seedlings	Aerated CW	Unaerated CW	Tidal CW	F/H	p
Nitrogen (%)	3.83 \pm 0.02 ^a	2.42 \pm 0.19 ^b	3.16 \pm 0.29 ^{ab}	3.07 \pm 0.22 ^{ab}	F _(3, 8) = 8.18	0.008
Calcium (%)	0.73 \pm 0.02 ^a	2.19 \pm 0.08 ^b	1.72 \pm 0.21 ^b	1.93 \pm 0.09 ^b	F _(3, 8) = 26.86	< 0.001
Magnesium (%)	0.73 \pm 0.01 ^a	0.81 \pm 0.01 ^b	0.85 \pm 0.01 ^b	0.83 \pm 0.03 ^b	F _(3, 8) = 13.08	0.002
Potassium (%)	4.90 \pm 0.01 ^a	2.68 \pm 0.09 ^b	1.99 \pm 0.18 ^c	2.49 \pm 0.18 ^{bc}	F _(3, 8) = 94.00	< 0.001
Sodium (mg/kg)	17175.22 \pm 153.77 ^a	83232.30 \pm 2659.57 ^b	69131.42 \pm 2525.53 ^c	82654.60 \pm 1866.39 ^b	F _(3, 8) = 230.61	< 0.001
Zinc (mg/kg)	64.15 \pm 2.94 ^a	61.72 \pm 4.70 ^{ab}	38.89 \pm 5.15 ^c	43.36 \pm 4.54 ^{bc}	F _(3, 8) = 8.37	0.008
Manganese (mg/kg)	86.84 \pm 0.16 ^a	29.01 \pm 4.37 ^{ab}	77.85 \pm 12.91 ^{ab}	17.77 \pm 2.24 ^b	H _(3, N=12) = 9.46	0.024
Copper (mg/kg)	56.04 \pm 0.75 ^a	4.44 \pm 1.81 ^b	3.02 \pm 0.60 ^b	2.87 \pm 0.85 ^b	F _(3, 8) = 565.78	< 0.001
Iron (mg/kg)	1171.86 \pm 190.96 ^a	112.30 \pm 38.26 ^{ab}	206.43 \pm 4.99 ^{ab}	90.41 \pm 9.69 ^b	H _(3, N=12) = 9.46	0.024
Phosphorous (%)	0.87 \pm 0.01 ^a	0.14 \pm 0.01 ^b	0.19 \pm 0.04 ^b	0.20 \pm 0.30 ^b	F _(3, 8) = 198.46	< 0.001
Aluminium (mg/kg)	771.91 \pm 50.74 ^a	229.08 \pm 16.44 ^b	344.97 \pm 2.59 ^{ab}	254.81 \pm 26.56 ^{ab}	H _(3, N=12) = 9.67	0.022

3.3.3 Plant health and a qualitative record of growth

Plant biomass had the visual appearance of being highest in the tidal CW, followed by that in the aerated CW and unaerated CW, after six weeks of growth (Figure 3.14) and this was still clear at week-10 (Figure 3.15). The occurrence of chlorosis and mortalities was not equally distributed between treatments. Chlorosis was more prevalent than expected in the unaerated CW, whereas they were fewer cases recorded in the tidal and aerated CWs ($\chi^2 = 18.5$, $p < 0.001$; Table 3.4; Figure 3.15). Mortalities were also more prevalent than expected in the unaerated CW, whereas there were fewer cases recorded in the aerated CW and no cases recorded in the tidal CW ($\chi^2 = 12.15$, $p < 0.001$; Table 3.4).

A



B



C



Figure 3.14 Spinach plants after six weeks in constructed wetlands with (A) aerated and (B) unaerated horizontal sub-surface flow and (C) tidal flow designs.

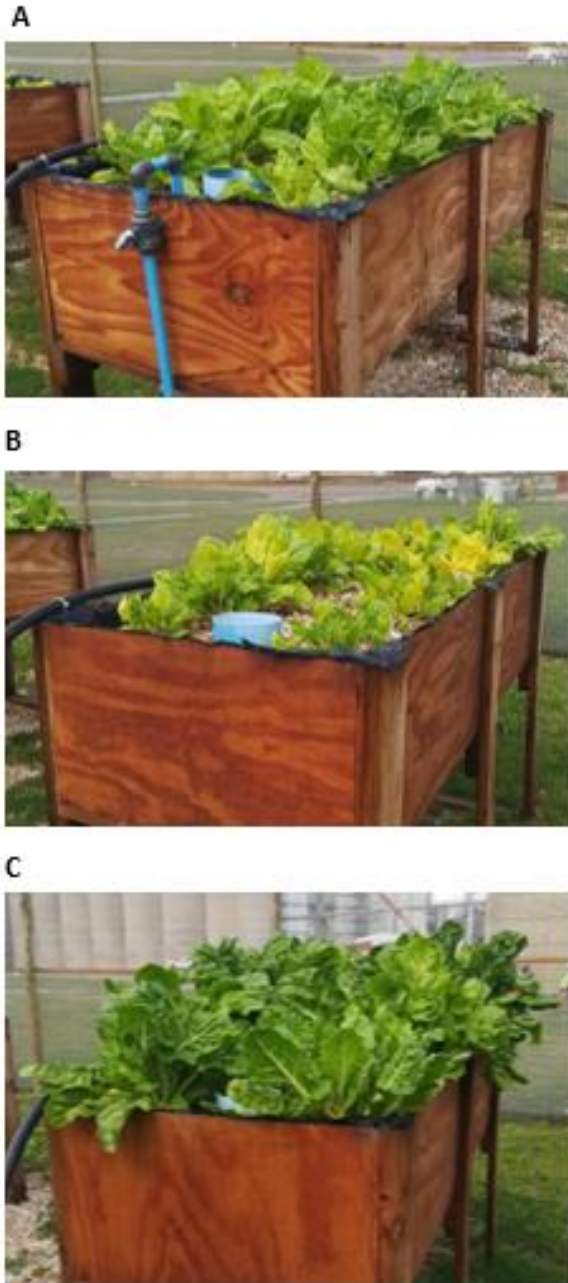


Figure 3.15 Spinach plants after ten weeks in constructed wetlands with (A) aerated and (B) unaerated horizontal sub-surface flow and (C) tidal flow designs.

Table 3.4 Observed frequencies for chlorosis and mortalities in constructed wetlands with aerated and unaerated horizontal sub-surface flow, and tidal flow constructed wetlands (CW) designs (Chi-square analysis, $p < 0.05$).

	Aerated CW	Unaerated CW	Tidal CW	Chi-square (X^2)	p-value
No. of plants with chlorosis	11	23	2	18.50	< 0.001
No. of mortalities	3	10	0	12.15	< 0.001

3.4 Discussion

3.4.1 Water quality parameters

This experiment was the first to compare different CWs in terms of growing spinach and treating effluent. There were differences between the different CWs in both plant production and water quality. Dissolved oxygen levels were low on the day-0, similar to the first experiment (Chapter 2), due to the depleted oxygen from the effluent source (anaerobic digester), that operates at a low DO concentration (Botheju and Bakke 2011; Section 2.3.1, Chapter 2). The lack of oxygen has often restricted treatment efficiency in constructed wetlands, especially ammonia treatment (Vymazal 2010). This is limited due to decreased nitrification which is the oxidation of ammonia to nitrite and nitrate (Vymazal 2010, Wu *et al.* 2011b).

There are numerous ways to improve oxygen transfer in CWs. Two ways are through artificial aeration and applying vertical flow (i.e. tidal) designs (Vymazal 2010, Wu *et al.* 2011b, Wu *et al.* 2014). Constructed wetlands and other wastewater treatment technologies that rely on aerobic microbial activity require oxygen for the bacteria to oxidize carbon and nitrogen-based compounds (Kadlec and Wallace 2008). Studies have shown that artificial aeration and filling and draining of wetlands can increase DO concentrations (Vymazal 2010, Wu *et al.* 2011b). Dissolved oxygen (DO) concentration increased between day-0 and day-1, thereafter the aerated CW and tidal treatments remained the same while the unaerated CW increased to day-3 before levelling off. Aerated horizontal sub-surface flow (aerated CW) and the tidal CW had higher DO concentrations than unaerated horizontal sub-surface flow (unaerated CW). This was due to air being artificially blown into aerated CW treatment and via atmospheric diffusion of oxygen into the grow beds in the tidal CW treatment during the

draining periods (Vymazal 2010, Wu *et al.* 2011b). The unaerated CW treatment had higher DO levels than conventional HSSF wetlands which are mainly anaerobic and have low concentrations or no DO at all (Kadlec and Wallace 2008). There are several reasons for unexpected high levels of DO in HSSF effluents (Kadlec and Wallace 2008). One such reason is reaeration at the outlet due to splashing and contact with the atmosphere which can increase DO concentration (Kadlec and Wallace 2008). The DO measurements were taken from the sump tanks, where splashing and contact with the atmosphere may have occurred from the outflow, which can increase DO (Kadlec and Wallace 2008). This was potentially the reason for the increase in DO from day-0 to day-3 and relatively high DO levels in the unaerated CW.

Despite the higher DO concentration in the aerated and tidal CWs treatments, all treatments were able to provide sufficient oxygen for nitrification which can occur at DO concentrations as low as 1 mg/l (Habermeyer and Sanchez 2005). Regardless of the unexpected increase in concentration of DO in the unaerated CW, it was still lower than the aerated and tidal CWs, which resulted in a slower decrease rate of ammonia. The significant inverse relationship between DO and ammonia, suggests that higher DO improved ammonia oxidation. This was evident in the study as aerated and tidal CWs had faster ammonia treatment rate than unaerated CW. The unaerated CW was unable to treat the ammonia in the effluent to discharge standards (3 mg/l; DWAF 1998), where the aerated CW and tidal wetlands were able to reach discharge standards (3 mg/l; DWAF 1998) within three days.

There was no difference in temperature between treatments. Effluent from all treatments decreased in temperature from the start to the end of the five-day batch cycles and had a mean of $19.83 \pm 0.28^{\circ}\text{C}$, which was higher than $17.04 \pm 0.08^{\circ}\text{C}$ from the first experiment

(Chapter 2). Higher temperatures improve ammonia oxidation through nitrification due to improved aerobic bacteria activity (Shammas 1986, Kadlec and Wallace 2008), which could explain why the tidal CW oxidised ammonia at a faster rate than the similar tidal treatment with the same filling and draining time (FDT) in the first experiment (Section 2.3.1, Chapter 2). Temperature differed between these experiments, and in this instance, it was closer to the optimal range for bacterial activity which is between 25 and 30°C (Kadlec and Wallace 2008). The higher temperatures in the experiment was potentially also the reason for the lower DO concentration in the tidal treatment when compared to the first experiment (Section 2.3.1, Chapter 2), as higher temperatures decrease DO concentration in the effluent (Stenstrom and Poduska 1980). The lower DO concentration did not decrease ammonia oxidation rate in the tidal treatments as the DO levels were sufficient for optimal nitrification (Stenstrom and Poduska 1980).

Nitrite dropped significantly in all treatments over the five-day batch cycle, the aerated and tidal CWs were able to decrease nitrite at a faster rate than the unaerated CW between day-0 and day-1. The tidal CW showed a faster decrease than the aerated CW treatment; these faster decrease rates between day-0 and day-1 were due to the higher DO concentrations. Higher DO concentrations increase the oxidation of nitrite to nitrate through nitrification (Kadlec and Wallace 2008, Garcia *et al.* 2010).

Nitrate was the highest from day-2 onwards in the aerated CW. Nitrate is removed from wastewater through denitrification and plant uptake (Kadlec and Wallace 2008). Denitrification is an anaerobic process that reduces nitrate into N₂ gas (Vymazal 2014). It was unlikely that aerated and tidal CWs had any denitrification taking place due to the high DO concentrations. The nitrate decrease in these treatments was likely due to plant uptake

(Kadlec and Wallace 2008), while it was possible that the unaerated CW treatment had anaerobic zones for denitrification (Vymazal 2010), which would explain the lower nitrate concentrations when comparing the aerated and unaerated treatments. Dissolved oxygen was measured in the sump tanks in the experiment where reaeration through splashing and contact with the atmosphere might have occurred; splashing may have been different between treatments and could have increased DO (Kadlec and Wallace 2008), and might have resulted in misleading DO readings in the current trial. To find out if denitrification could have been a reason for nitrate removal in the unaerated CW, dissolved oxygen readings would have been more accurate if measured in the grow bed at different depths and lengths along the wetland as HSSF wetlands have stratified zones of aerobic and anaerobic zones (Kadlec and Wallace 2008, Vymazal 2014).

Nitrification may have taken place in the experiment, but nitrate, the end product of the process may have been removed via plant uptake, which may explain the low nitrate in the tidal treatment (Kadlec and Wallace 2008). However, this does not explain the difference in nitrate between the aerated and unaerated CWs. If plant uptake was solely responsible for nitrate decreases, the aerated CW treatment should have had lower nitrate concentrations as it had higher plant biomass than the unaerated CW. This was not the case as nitrate concentration was higher in the aerated CW treatment. This may be due to improved nitrification which would increase nitrate production (Kadlec and Wallace 2008); or due to potential denitrification zones in the unaerated CW, which converts nitrate into nitrogen gas, resulting in less nitrate build-up (Kadlec and Wallace 2008). Desorption may have also taken place which removes ammonia through physical means via aeration, surface turbulence or mixing which releases the ammonia molecules into the atmosphere in wastewater with high

pH (Patoczka and Wilson 1984, Monteith *et al.* 2005). The process could have potentially played a role in ammonia oxidation in the experiment as all treatments effluent had aeration (via turbulence and mixing at the outflows) and high pH values. The process can result in less ammonia being converted into nitrite and nitrate due to the decreased nitrification, which could explain the decrease of nitrite and nitrate in the experiment. Ammonia desorption and nitrification may occur simultaneously (Srinath and Loehr 1974). It is possible that a combination of desorption and nitrification were responsible for ammonia decrease in all treatments, and the denitrification process may have been present in the unaerated CW treatment.

The pH range was 7.53 - 8.29 for all treatments in the experiment, which is within the optimal range for ammonia oxidation through nitrification of 6.45 - 8.95 (Ruiz *et al.* 2003). Values increased in all treatments, with the unaerated CW treatment having the lowest pH values throughout the five-day batch cycle. The increases in the treatments were due to the dissolved CO₂ in the anaerobic digester producing carbonate alkalinity and carbonic acid (van Rensburg *et al.* 2003, Power and Jones 2016). When the effluent is exposed to the atmosphere or aerated, carbonic acid is removed and the more stable carbonate alkalinity remains, which increases the pH of the effluent until an equilibrium is reached between air and the effluent (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003, Power and Jones 2016). The higher pH values in the aerated and tidal CWs were likely due to higher aeration rate as pH and dissolved oxygen had a significantly direct relationship. With increased oxygen the incoming effluents carbonic acid is removed at a faster rate, leaving the carbonate alkalinity in the effluent. Thus, the lower pH in the unaerated CW treatment may have been due less

aeration, which resulted in slower removal of carbonic acid which will result in slower rate of pH increase.

The COD was lowered to that of discharge standards (75 mg/l; DWAF 1998) in all treatments, with the unaerated CW showing the smallest decrease. The unaerated CW may have had anoxic or anaerobic zones, which may explain it having the highest COD at the end of the batch cycle due to the aerated and tidal CWs having aerobic zones which results in higher level of COD decrease as aerobic microorganisms are more efficient than anaerobic microorganisms in decreasing COD (Kadlec and Wallace 2008). Similar to these findings, the unaerated CW had 70.3 % COD decrease compared to 90.1 % COD decrease in the aerated CW used to treat sewage wastewater (Li *et al.* 2014). This result is not consistent with a study compared the effect of macrophytes, loading and aeration in fish farm wastewater, where the aeration of CWs had no effect on lowering COD (Maltais-landry *et al.* 2007). The incoming COD of 166.37 ± 3.2 for all treatments here is relatively low, as some studies had influent COD of 815.7 mg/l (Luederitz *et al.* 2001). Higher COD decrease is seen in systems with high COD in the effluent (Zhu *et al.* 2014). Therefore, the relatively low COD levels in this experiment may have accounted for the COD decrease being similar between tidal and aerated CWs.

All treatments had a decrease in phosphate concentration between the start and end of the five-day batch cycles, with phosphate concentrations being below discharge standards throughout the study. There was no difference between treatments for phosphate removal. The decrease may be due to plant and microbial uptake (Stottmeister *et al.* 2003, Vymazal 2007). Constructed wetlands are known to have low phosphate removal (Vymazal 2010). Phosphate removal will remain low unless substrates with a high sorption capacity are used,

unlike gravel (Vymazal 2010; Section 2.4, Chapter 2); and all the treatments made use of the same substrate and therefore, had similar phosphate removal.

Electrical conductivity decreased in all treatments, with the tidal CW showing a higher decrease than the unaerated CW. Chloride, among other ions, affects EC (Abyaneh *et al.* 2005). The decrease in EC was likely due to the decrease in chloride, as other ions (NO_2^- , NO_3^- and PO_4^{3-}) in unaerated and tidal CW treatments mostly had similar concentrations throughout the batch cycle period (Abyaneh *et al.* 2005). Chloride has numerous biological interactions in a water body, but these do not generally result in concentration changes and when concentrations change between inlet to outflow, it presents a challenge for finding the cause (Kadlec and Wallace 2008). Chloride is an essential plant nutrient and can decrease in concentration in the wetland via plant uptake (Xu *et al.* 2004, Freeman 2005). Plants use chloride in enzyme activation of photosystem II, water-splitting in photosynthesis and aides water and electrical balance (Freeman 2005, Epstein and Bloom 2005). The decrease in chloride concentrations between the start and end of the 5-day batch cycle, that was observed in the treatments here, most likely due to plant uptake. The aerated CW treatment had a lower chloride concentration than the tidal and unaerated CWs at the end of the batch cycle. Lower chloride should reflect lower EC for the aerated CW due to their close relationship (Abyaneh *et al.* 2005), but this was not the case which may be due to other ions which were not measured, influencing EC, being different in the aerated CW when compared to the tidal CW and this could explain why aerated CW did not have a lower EC (Abyaneh *et al.* 2005).

3.4.2 Plant harvest and health

The tidal CW's mean total harvest was almost four times higher than the unaerated and aerated CWs over the 14-week period. This was likely due to the unaerated and aerated CWs treatments being constantly flooded which resulted in the spinach roots being constantly submerged in the effluent. Aeration around plant roots is essential for root respiration (Chun and Takakura 1994). Terrestrial plants cannot survive waterlogged conditions in HSSF (Stottmeister *et al.* 2003), and this was evident in the experiment as the unaerated and aerated CWs had higher frequencies of chlorosis and mortalities as well as significantly lower total harvest, compared to those grown in the tidal wetlands. Spinach is a terrestrial plant and has adapted to dry conditions and a lack of oxygen for root respiration can affect plant health and growth (Jackson 1979, Freeman 2005, Marschner 2011). Oxygen diffused at a slower rate into the root cells from the aerated HSSF when compared to the tidal wetland; as the rate of diffusion of gasses from water into a cell is 10000 lower than from air into a cell (Armstrong *et al.* 1994). This could have resulted in the roots being depleted in oxygen, resulting in lower yields. The low DO level in the effluent for the unaerated CW, and the waterlogged conditions in the unaerated and aerated CWs suggests that the spinach in these treatments were not able to maximize respiration, and therefore growth and health was affected. Chlorosis and mortality can occur when plants have insufficient aeration (Bergman 1959). The aerated CW had lower occurrence of chlorosis and mortality than the unaerated CW, which was most probably due to the higher dissolved oxygen in the effluent (Bergman 1959). The aerated CW had similar DO concentrations in the effluent than the tidal CW, but plants were exposed to atmospheric oxygen which can diffuse more readily into plant roots than dissolved oxygen in

the water. This would explain why the tidal CW had significantly higher total harvest and CCI than the aerated CW and unaerated CWs.

All essential plant nutrients measured in this experiment have been reported in brewery effluent (Ajmal and Khan 1984, Kanagachandran and Jayaratne 2006, Ipeaiyeda and Onianwa 2009, Aneke and Onukwuli 2012, Power and Jones 2016). The uptake of nutrients from water is affected by pH and salinity (Peterson 1982, Adams 1992, Tyson *et al.* 2007). Brewery effluent in this experiment had relatively high pH of 7.53 ± 0.02 to 8.29 ± 0.02 and an EC of 2896.81 ± 38.27 to $3048.06 \pm 39.13 \mu\text{S}/\text{cm}^2$. The high pH resulted in leaf potassium, copper and phosphorous concentration decreasing significantly from the seedlings in all the treatments and copper and manganese decreasing significantly in the tidal CW; as all these elements are less soluble at high pH values (Peterson 1982). Leaf calcium, magnesium and sodium leaf concentration were more concentrated in the larger plants at the end of the trial; these were all less concentrated in the seedlings at the beginning. This was probably due to the high solubility of these elements at high pH values in all treatments (Peterson 1982). High salinity may have also played a role in lowering nitrogen, potassium and phosphorous concentrations, since increasing salinity directly affects plant uptake of these elements (Adams 1992). Adams (1992) also found that high pH and salinity had a direct and negative affect on cucumber (*Cucumis sativus*) weights (Adams 1992). Furthermore, Power and Jones (2016) found that adjusting pH significantly improved the growth and yield of tomatoes (*Lycopersicum escolentum*) and Taylor (2018) found conductivity to be a major inhibitor for cabbage (*Brassica oleracea*) growth. The adjustment of pH may help increasing the total harvest and plant health in CWs that use brewery effluent. Maintaining the pH may improve all treatments plant health, by increasing the availability of plant nutrients at an optimal range

of 6.0 – 6.5 (Power and Jones 2016). This may improve health and total harvest in all treatments, but it may be possible that the plants being constantly submerged in the unaerated and aerated CWs will still be limited by oxygen deprived roots.

3.5 Conclusion

Wetland design affected the dissolved oxygen (DO) and treatment efficiency in the system. The aerated and tidal CWs had significantly higher DO concentrations than the unaerated CW which resulted in higher ammonia treatment efficiency as the aerated and tidal CWs were able to treat ammonia to discharge standards while the unaerated CW was unable to do so (< 3 mg/l; DWAF 1998). There were no differences in temperature between treatments, however temperature was higher than the first experiment (Chapter 2) which likely resulted in improved ammonia treatment in the tidal CW. This suggests the importance of testing the influence of seasonality in future research.

Chemical oxygen demand, phosphate and EC decreased in all treatments. The aerated and tidal CWs outperformed unaerated CW in reducing COD and the tidal CW had a higher decrease than the unaerated CW. There were no differences between treatments for phosphate removal due to the similar substrate used in the wetlands.

Chloride concentration in all treatments decreased between the start and end of the batch cycle, with the aerated CW having the lower chloride concentration than the tidal and unaerated CWs on day-5. The pH increased in all treatments, with aeration and pH indicating a possible trade-off situation as increased aeration increases pH. The unaerated CW had the lowest aeration and pH over the five-day batch cycles. The lower pH in unaerated CW treatments did not improve plant health and growth. As the unaerated CW had significantly lower total harvest and CCI than the tidal CW. Total harvest was significantly higher in the

tidal CW compared to unaerated and aerated CWs, this was due to the tidal CW providing atmospheric oxygen, which diffuses into plant roots at a much faster rate, while the unaerated and aerated CWs only provided dissolved oxygen, which does not diffuse as easily and leads to poor plant performance due to respiration being affected.

Due to the unaerated CW being unable to treat ammonia to discharge limits and showing the smallest decrease in COD and EC suggests it had the lowest treatment efficiency. It also produced the lowest plant harvest, CCI and it had the highest plant mortality rate; and this indicates it had the worst crop performance. Therefore, it was not selected as a treatment in the water source for aquaculture work that follows (Chapter 4). Instead, treated effluent from the aerated and tidal CWs will be used as a water source in the next experiment (Chapter 4).

Chapter 4: Wetland effluent as a water source in aquaculture.

4.1 Introduction

The access to fresh water supply will become the largest environmental and economic concern in both developing and developed countries in the future (Watanabe *et al.* 2002). Rapid population growth and the increased demand for natural resources by industry has resulted in overexploitation of these resources which has led to increased pollution of land, air and water (Pokhrel and Viraraghavan 2004). The decline of the ocean's fish stocks and the need to feed a fast-growing population provides incentive for expanding aquaculture production (Piedrahita 2003, Crab *et al.* 2007). The demands for fresh water in agriculture, industry and domestic use are increasing and limit the available water for aquaculture purposes (Suresh and Lin 1992, Piedrahita 2003). The growth of freshwater aquaculture production could be severely limited by the scarcity of fresh water (Naylor *et al.* 2000). Water shortages have resulted in countries, such as Egypt, prohibiting the use of fresh water and drainage water for aquaculture, and instead focus has been placed on developing alternative water resources through water reuse and recycling (Khalil and Hussein 1997). Wastes have been referred to as "resources out of place" as the ever-growing production of waste is continually underutilized (Jana 1998). Using wastewater for aquaculture production has been practiced using effluent arising from sewage in Kolkata (formerly Calcutta), India (Furedy and Ghosh 1984, Jana 1998) and has been gaining scientific attention (Edwards 1993). However, the wetlands used in Kolkata cover a large area and the sustainability has been questioned due to the competition of space required for rapid urbanization (Furedy and Ghosh 1984). This will result in the wetland area decreasing, which will bare negative impacts in drainage, sewage treatment and food production for the city (Furedy and Ghosh 1984). Therefore, there

is a need to develop aquaculture systems that produce more fish, while using less water (and space), food and time to reduce environmental impact and production costs (Watanabe *et al.* 2002). Brewery effluent has been used as a water source in aquaculture (Jones *et al.* 2016, Jones *et al.* 2018). Tilapia were used in a trial that investigated their use in removing algae from recovered high rate algal pond water (Jones *et al.* 2018) and ornamentals were also successfully grown in this effluent (Jones *et al.* 2018). Both studies suggest that there is potential for aquaculture using recovered brewery effluent treated using algal ponding (Jones *et al.* 2016, Jones *et al.* 2018). The lack of literature on using constructed wetlands (CWs) to treat brewery effluent for aquaculture suggests that the idea is novel.

It is important to take environmental tolerances into consideration when assessing a candidate aquaculture species, especially since recovered wastewater might not fall within the optimal range for fish culture. Environmental parameters that should be considered include temperature, oxygen, salinity, pH and ammonia. Brewery effluent recovered from algal ponds has high salinity and high pH due to evaporation and photosynthesis of algae respectively (Power and Jones 2016), and therefore a species selected would need to tolerate these conditions. A summary of the environmental tolerance of *Oreochromis mossambicus* is provided in Table 4.1.

Table 4.1 Summary of *O. mossambicus* tolerance to certain environmental variables.

Environmental variable	Tolerance	Reference
Temperature (°C)	15 - 37	Philippart and Ruwet (1982)
Salinity (ppt)	0 - 120	Whitfield and Blaber (1979)
pH	3.7 - 10.3	Swingle (1961)
Dissolved oxygen (mg/l)	> 0.3	Maruyama (1958)
Ammonia (mg/l)	< 14.0	Sampath <i>et al.</i> (1991)

Findings from the previous experiment (Chapter 3), and a desktop analysis of *O. mossambicus* tolerance of various water quality parameters (Maruyama 1958, Swingle 1961, Whitfield and Blaber 1979, Lobel 1980, Philippart and Ruwet 1982, Murthy *et al.* 1984, Sampath *et al.* 1991, Senguttuvan and Sivakumar 2002, Kamal and Mair 2005, Jones *et al.* 2018) suggest that tidal and aerated horizontal sub-surface flow CWs can treat the brewery effluent to suitable levels for use in aquaculture. Due to unaerated sub-surface flow wetlands inefficiency to treat brewery effluent and low plant harvest and health, it was eliminated as a candidate water source. Therefore, tidal wetlands and aerated HSSF wetlands were compared to a municipal water control.

4.1.1 Aims and objectives

The aim of this experiment was to determine if the tidal or aerated constructed wetlands can be used to treat brewery effluent for downstream use in aquaculture and to compare these water sources to a municipal water control. This was achieved by rearing *O. mossambicus* in tanks with water supplied by the tidal CW, aerated CW and the municipal water and measuring water quality parameters and fish growth.

The objectives of this study were:

- to compare water quality parameters in systems supplied with effluent from the tidal CW, aerated horizontal sub-surface flow CW and municipally supplied water; and
- to compare fish growth in systems supplied with water from the tidal CW, aerated horizontal sub-surface flow CW and municipally supplied water.

4.2 Methods and materials

4.2.1 Experimental system and treatments

The system included six isolated tanks, with two tanks allocated to one of the three treatments (Table 4.2). That is, two tanks were filled using water from tidal CW, two from the aerated horizontal sub-surface flow (aerated CW) and two filled with municipal water (Table 4.2). Each tank had oxygen supply from a 0.55 KW side channel blower (Model ZXB-310, CFW Industries (Pty) Ltd, South Africa) blowing air through a zeolite filter to minimize increases in ammonia concentration. Water was replaced in each tank on week-5 due to water loss from evaporation and increasing ammonia levels, from the same water sources (Table 4.2). Incoming water qualities were similar between all treatments for ammonia, nitrite, DO and temperature; and were different in the tidal and aerated CWs treatment which had higher nitrate, EC and pH than the municipal treatment (Table 4.2).

Table 4.2 A description of the three treatments (T1-T3), including the tidal constructed wetlands (CW), aerated horizontal sub-surface flow CW and municipal water sources for aquaculture. The incoming water quality values include the mean, followed by the range in brackets.

	T1	T2	T3
Water source	Tidal CW	Aerated CW	Municipal
Ammonia (mg/l)	0	0	0
Nitrite (mg/l)	0.38 (0.30 - 0.61)	0.38 (0.30 - 0.61)	0.38 (0.30 - 0.61)
Nitrate (mg/l)	1.30 (0.90 - 1.8)	1.80 (1.60 - 2.00)	0.18 (0.10 - 0.20)
EC* ($\mu\text{S}/\text{cm}^2$)	2908 (2550 - 3210)	3043 (2860 - 3140)	783 (760 - 800)
DO*	6.30 (6.10 - 6.50)	6.18 (6.10 - 6.30)	6.53 (6.40 - 6.60)
pH	8.52 (8.41 - 8.72)	8.29 (8.22 - 8.35)	7.21 (7.18 - 7.24)
Temperature ($^{\circ}\text{C}$)	21.83 (21.20 - 22.40)	21.87 (21.10 - 22.60)	21.65 (20.90 - 22.20)
No. tanks	2	2	2
No. fish per tank	20	20	20
Tank volume (l)	350	350	350
Stocking density (no. of fish/ m^3)	56	56	56

*EC – Electrical conductivity; DO – Dissolved oxygen

4.2.2 Experimental species

One hundred and fifty Mozambique tilapia (*Oreochromis mossambicus*) were supplied by a private aquaculture farm outside Port Elizabeth (Innovative Bay Solutions CC, South Africa). They were transported to the project site in a water tank that had a constant oxygen supply and were acclimated to the experimental system fish tanks for four months prior to the start of the experiment in the same tanks used for the experiment. At the start of the trial, 120 randomly selected fish were divided evenly between the six tanks at 20 fish per tank; i.e. a stocking density equivalent to 56 fish/ m^3 (Table 4.2). The mean fish length was 105.23 mm and mean weight was 18.34 g at the start.

4.2.3 Data collection

Water quality parameters

Temperature (°C) and electrical conductivity (EC; $\mu\text{S}/\text{cm}^2$) were measured using an electrical probe (Lutron conductivity meter, code PCD-432, Lutron Co Inc, USA) and pH was measured using a pH-meter (Crison pH-meter BASIC 20+, code 003-2010, Crison instruments S.A., Spain).

Ammonia ($\text{NH}_3\text{-N}$; mg/l), nitrite ($\text{NO}_2\text{-N}$; mg/l), nitrate ($\text{NO}_3\text{-N}$; mg/l) concentrations were recorded using a spectrophotometer (Hach DR 2800 spectrophotometer, product number DR2800-01B1, Hach (Pty) Ltd, USA) and commercially available test kits, and standard methods (Hach (Pty) Ltd, USA). Water samples were filtered through 8-micron filter paper prior to analysis as filtration removes turbidity which may influence colorimetric analyses (Hach 2013). The following test kits were used:

- High-range ammonia test (product no. 26069-45, Hach (Pty) Ltd, USA)
- Nitrite test (product no. 21075-69, Hach (Pty) Ltd, USA)
- Nitrate test (product no. 21061-69, Hach (Pty) Ltd, USA)

All parameters were measured at the start of the experiment and again weekly for eight weeks at 09h00 on the first day of the week.

Fish growth

Fish were fed once a day at 09h00 for eight weeks (Tilapia grower 3 mm pellets, Avi-Products (Pty) Ltd, South Africa; Table 4.3) at 8 % body weight per day (DeLong *et al.* 2009).

The fish were anaesthetized (0.2ml/L 2-phenoxyethanol) prior to handling and were weighed (0.01 g) and measured (1 mm) at the start and end of the experiment. Fish growth was calculated as percentage weight gain and specific growth (SGR) using Equations 4.1 and 4.2. Condition factor (CF), feed conversion ratio (FCR) and mortality (M) were calculated using equations 4.3 to 4.5.

$$\text{Percent weight gain} = ((\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight (g)}) \times 100 \quad (4.1)$$

$$\text{SGR} = [\ln (\text{final weight (g)}) - \ln (\text{initial weight (g)})] \times 100 / \text{time (days)} \quad (4.2)$$

$$\text{CF} = [\text{weight (g)} / (\text{length (mm)}^3)] \times 100000 \quad (4.3)$$

$$\text{FCR} = \text{dry feed supplied (g)} / \text{wet weight (g)} \quad (4.4)$$

$$\text{M} = (\text{initial number of fish} - \text{final number of fish} / \text{initial number of fish} \times 100) \quad (4.5)$$

Table 4.3 Nutritional value of the fish feed (Tilapia grower 3mm pellets, Avi-Products (Pty) Ltd, South Africa).

Nutrient	Ratio
Metabolisable Energy (MJ/kg)	12.7
Digestible Energy (MJ/kg)	14.7
Crude Protein (g/kg)	350
Lysine (g/kg)	18.6
Methionine (g/kg)	6.1
*T.S.A.A. (g/kg)	12.4
Isoleucine (g/kg)	15.4
Tryptophan (g/kg)	3.7
Threonine (g/kg)	14.1
Valine (g/kg)	18.3
Arginine (g/kg)	23.4
Fat (g/kg)	74
Fibre (g/kg)	32
Ash (g/kg)	62
Calcium (g/kg)	10.6
Total Phosphorus (g/kg)	9.4
Sodium (g/kg)	2.4

*T.S.A.A. – Total sulphur amino acids.

4.2.4 Statistical analysis

Treatment means were compared using a one-way or multifactor repeated measure analysis of variance (ANOVA). When significant differences were evident, treatment means were further compared using a Tukey multiple range test at $p < 0.05$ ($\alpha = 5\%$). All data were checked for equality of variance and for 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 again for equal variance and normal distribution of residuals. If the assumptions were still not met, a non-parametric Kruskal Wallis ANOVA was used to compare the data between treatments in place of a one-way ANOVA. If the data did not meet the assumptions for repeated measures ANOVA, a Mauchly's

sphericity test was included in the analysis, and they were corrected using Greenhouse-Geisser and Huynh-Feldt tests.

The average pH values were converted to H⁺ concentrations for statistical comparison using Equation 2.1 (Section 2.2.4, Chapter 2). The mean and standard error from the logged pH values were used for graphical representation.

4.3 Results

4.3.1 Water quality parameters

Ammonia concentrations in the fish tanks were not influenced by an interaction between water source and time (Repeated measures ANOVA, $F_{(16, 24)} = 0.91$, $p = 0.57$; Figure 4.1). There were no differences between treatments over eight weeks, except for week-1, where the municipal treatment had higher ammonia concentration than the tidal and aerated CWs (Figure 4.1). All treatments showed an increase in ammonia from week-0 to week-1, before levelling off until week-2 and increasing again to week-3 (Figure 4.1). Ammonia decreased in all treatments between week-4 and week-5 (after the water change; Figure 4.1), with the tidal treatment showing the only increase between week-5 and week-6 (Figure 4.1). There was another increase in all treatments between week-6 and week-7 before levelling off between week-7 and week-8 (Figure 4.1)

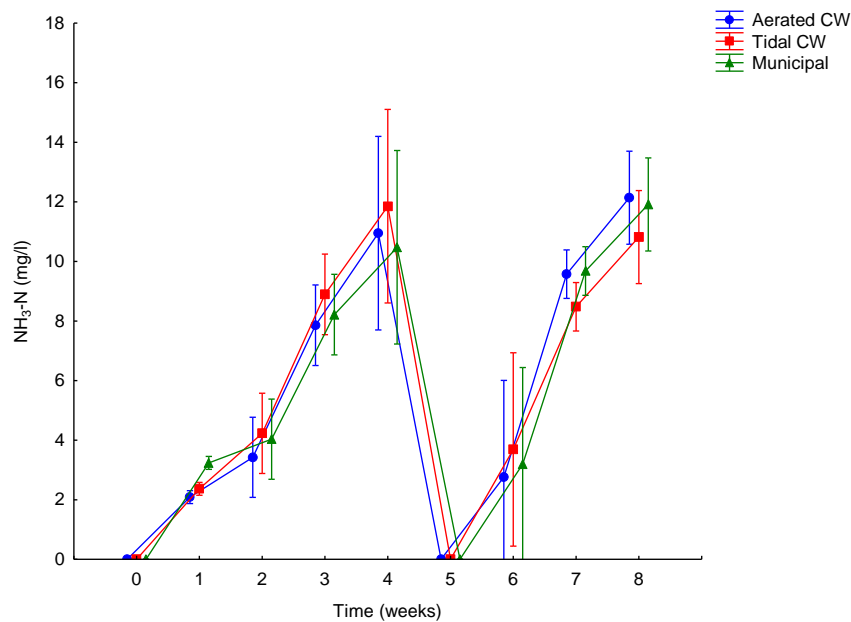


Figure 4.1 The mean (\pm 95% confidence interval) ammonia concentration in the tanks supplied with aerated horizontal sub-surface flow (aerated CW) and tidal flow (tidal CW) constructed wetlands and municipally supplied water over eight weeks (Repeated measures ANOVA, $F_{(16, 24)} = 0.91$, $p = 0.57$).

Nitrate concentrations in the fish tanks were not influenced by an interaction between water source and time (Repeated measures ANOVA, $F_{(16, 24)} = 1.33$, $p = 0.26$; Figure 4.2). All treatments showed an increase between week-0 and week-1, before levelling off to week-3 (Figure 4.2). Thereafter, treatments increased between week-3 and week-4 before decreasing on week-5 (water change) and then increasing again to week-6 with the aerated CW having the only increase between week-6 and week-7, with no increase in week-8 in all treatments (Figure 4.2). At the start of the trial (week-0), municipal treatments had significantly lower nitrate concentrations than the tidal and aerated CWs and remained lower until week-3 before rising to similar concentrations in week-4 (Figure 4.2). Thereafter (week-5 to week-8), the municipal treatment remained lower than tidal and aerated CWs (Figure 4.2).

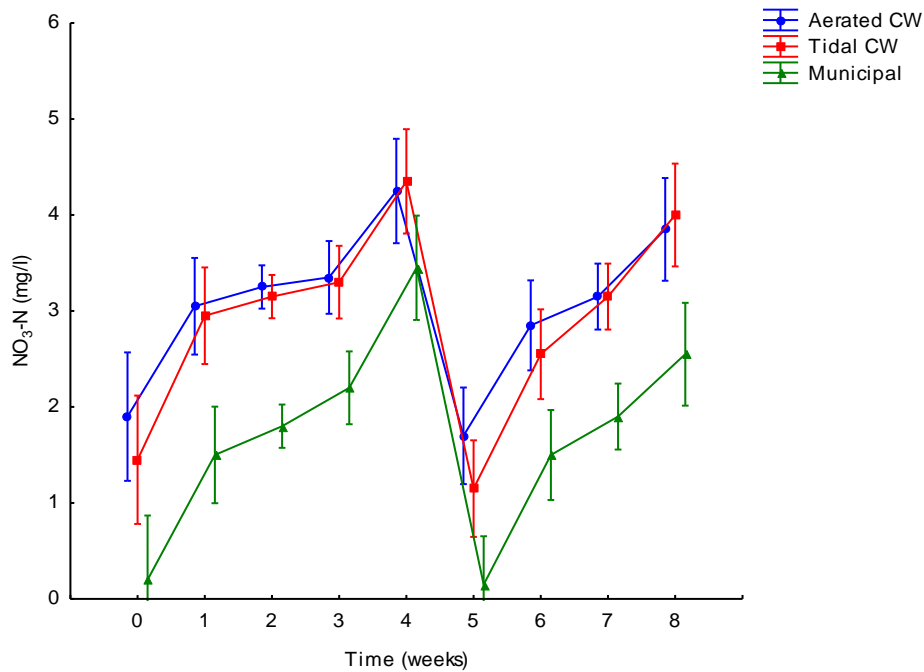


Figure 4.2 The mean (\pm 95% confidence interval) nitrate concentration in the tanks supplied with aerated horizontal sub-surface flow (aerated CW) and tidal flow (tidal CW) constructed wetlands and municipally supplied water over eight weeks (Repeated measures ANOVA, $F_{(16, 24)} = 1.33$, $p = 0.27$).

There were no significant differences between treatments for nitrite, dissolved oxygen and temperature (Repeated measures ANOVA, $p > 0.05$; Table 4.4). However, there were significant differences between treatments for electrical conductivity and pH which showed no differences over time (Repeated measures ANOVA, $p < 0.05$, Table 4.4). Electrical conductivity was significantly lower in the municipal treatment when compared to the tidal and aerated CWs (Repeated measures ANOVA, $F_{(2, 3)} = 91.27$, $p = 0.002$; Table 4.4). The same trend was observed for pH, with the municipal treatment having significantly lower values than tidal and aerated CWs (Repeated measures ANOVA, $F_{(2, 3)} = 1933.10$, $p = 0.002$; Table 4.4)

Table 4.4 The mean (\pm 95% standard error) water quality parameters in fish tanks supplied with treated aerated horizontal sub-surface flow (aerated CW) and tidal flow (tidal CW) effluent; and municipal water as a water source. Values in the same row with different superscript symbols represent significantly different means (Repeated measures ANOVA, $p < 0.05$).

	Aerated CW	Tidal CW	Municipal	F _(2, 3)	p value
NO ₂ ⁻ -N (mg/L)	0.86 \pm 0.02	0.85 \pm 0.07	0.79 \pm 0.05	0.508	0.646
Dissolved oxygen (mg/L)	4.63 \pm 0.01	4.70 \pm 0.04	4.76 \pm 0.01	5.353	0.102
Electrical conductivity (μ S/cm ²)	3059.44 \pm 43.89 ^a	2946.67 \pm 225.56 ^a	810.00 \pm 1.11 ^b	91.265	0.002
pH	8.20 \pm 0.03 ^a	8.43 \pm 0.09 ^a	7.18 \pm 0.01 ^b	1933.1	<0.001
Temperature ($^{\circ}$ C)	20.01 \pm 0.01	19.98 \pm 0.03	19.89 \pm 0.03	5.400	0.101

4.3.2 Fish growth

There were no significant differences in treatment means for starting length and weight, end length and weight, percentage weight gain, specific growth rate, condition factor, feed conversion ratio and mortality (Kruskal-Wallis ANOVA, $p > 0.05$; Table 4.5).

Table 4.5 The mean (\pm 95% standard error) starting length and weight, end length and weight, percentage weight gain, condition factor (CF), specific growth rate (SGR), food conversion ratio (FCR) and percentage mortality for *O. mossambicus* in fish tanks supplied with water from aerated horizontal sub-surface flow (aerated CW), tidal flow (tidal CW) and municipal water (One-way ANOVA/Kruskal-Wallis ANOVA, $p < 0.05$).

	Aerated CW	Tidal CW	Municipal	F/H	p-value
Starting length (mm)	104.68 \pm 0.38	105.30 \pm 2.75	105.73 \pm 6.48	H _(2, N=6) = 0.00	1.000
End Length (mm)	138.38 \pm 1.25	135.67 \pm 1.96	140.80 \pm 4.08	F _(2, 3) = 0.90	0.493
Starting weight (g)	17.92 \pm 0.32	18.01 \pm 0.76	19.09 \pm 2.25	F _(2, 3) = 0.20	0.828
End weight (g)	45.54 \pm 1.23	42.34 \pm 0.71	48.26 \pm 5.09	H _(2, N=6) = 2.43	0.180
Weight gain (%)	154.06 \pm 2.41	135.67 \pm 13.95	153.14 \pm 3.22	H _(2, N=6) = 3.71	0.156
SGR	1.66 \pm 0.02	1.52 \pm 0.12	1.66 \pm 0.02	H _(2, N=6) = 3.71	0.156
CF	1.64 \pm 0.01	1.62 \pm 0.01	1.67 \pm 0.04	F _(2, 3) = 1.31	0.391
FCR	3.04 \pm 0.10	3.46 \pm 0.21	2.91 \pm 0.28	H _(2, N=6) = 3.43	0.180
Mortality (%)	15.00 \pm 10.00	17.50 \pm 2.5	12.50 \pm 2.5	F _(2, 3) = 0.17	0.854

4.4 Discussion

Water quality in aquaculture is extremely important to monitor, as it may determine the success or failure of an aquaculture venture (Piper *et al.* 1982). The pH and electrical

conductivity (EC) were higher in aerated and tidal CWs. This was due to these treatments having source water with high pH and EC from the end of the five-day batch cycles in the previous experiment (Section 3.3.1; Chapter 3). Although pH means were higher in the aerated and tidal CWs than those of the control system where municipal water was used to culture the fish, all treatments pH means were within *O. mossambicus* survival range between 3.7 and 10.3 as well as the optimal growth range between 6 and 9 (Swingle 1961, Murthy *et al.* 1984, Ross 2000, DeLong *et al.* 2009).

Electrical conductivity was approximately three times lower in the municipal treatment when compared to the aerated and tidal CWs. *Oreochromis mossambicus* is known to be able to survive salinities similar to seawater (Whitfield and Blaber 1979, Lobel 1980, Kamal and Mair 2005). The high salinity tolerance of *O. mossambicus* is due to the fish's ability to develop chloride cells in the gills and opercular membrane that are used to secrete salt (Uchida *et al.* 2000). Mortality is known to increase at high salinities when the water temperature is around 15°C (Sardella *et al.* 2004). The mean temperature in this experiment was approximately 20°C, which is within an acceptable range for this fish (Philippart and Ruwet 1982). The higher EC values 3059.44 ± 43.87 and $2946.67 \pm 225.56 \mu\text{S}/\text{cm}^2$ in aerated and tidal CWs respectively, were therefore, unlikely to affect fish health and growth when compared to the municipal treatment.

Mean water temperatures for all treatments were in the species thermal range of 15 – 37°C (Philippart and Ruwet 1982), but not into the optimal range of 25 – 27°C (Makori *et al.* 2017). Although the temperature was within the tolerance range of *O. mossambicus*, feeding is reduced at temperatures of 16 – 17°C (Lim and Webster 2006). Temperature can fluctuate daily, and it is possible that water temperatures may have varied between weekly

measurements and lower temperatures may have reduced feeding, and thus growth. Therefore, temperature should have been measured more frequently (daily) in this trial to gain a better understanding on its effect on feeding and growth.

The mean dissolved oxygen concentration was well within the range for *O. mossambicus* survival, as these fish can tolerate low dissolved oxygen concentrations down to 0.3 mg/l (Maruyama 1958). Fish require oxygen for respiration which influences feed consumption, metabolic rate and energy expenditure, and therefore, their survival, growth and health (Buentello *et al.* 2000). Fish growth rate and health is generally greater in waters with optimal levels of dissolved oxygen (Makori *et al.* 2017). The concentrations of dissolved oxygen in all treatments were limited from 4.63 ± 0.01 to 4.78 ± 0.01 mg/l (i.e. below the optimum level of > 5 mg/l for tilapia; Pescod 1992) due to the channel blower being divided between the grow beds used in the previous experiment (Chapter 3) and the tanks in the present study. Therefore, the amount of air being blown into the fish tanks would have been improved were it not for the division between the two experiments.

Zeolite is a silicate mineral that makes use of ion exchange to decrease ammonia concentration in the water which can lower it by 90 – 97 % (Piper *et al.* 1982). The decrease in air flow through the zeolite filters, may have decreased the water flow through the zeolite filters. Thereby decreasing the potential for ammonia to bind to the zeolite material (Chiayvareesajja and Boyd 1993). The high EC may have also decreased the efficiency of the zeolite in ammonia removal. Higher EC results from dissolved ions in a water, some which are cations that also bind to zeolite (Chiayvareesajja and Boyd 1993). These compete with ammonia for a fixed number of exchange sites on the zeolite, which may decrease ammonia removal (Chiayvareesajja and Boyd 1993). The mean ammonia in the experiment

(5.55 ± 0.08 mg/l) was within the survival range of 3 – 14 mg/l for *O. mossambicus* (Sampath *et al.* 1991). However, growth and health may have improved if the ammonia concentration was maintained at a lower concentration, because feeding can be affected in tilapia at ammonia levels above 1.0 mg/l (Popma and Masser 1999, Lim and Webster 2006). This suggests that the ammonia concentrations may have affected feeding and growth of *O. mossambicus* in the present study. A way to have mitigated this problem would have been to employ a partial flow through system where 10 to 20 % of the volume of water was replaced every day or two as this occurs in commercial systems (Broders *et al.* 2005), and it would have provided a better test of the incoming water source. Previous studies employed this technique and water changes were done every two days (Sardella *et al.* 2004).

The increase of nitrite and nitrate in the experiment suggests that nitrification was occurring in the zeolite filters and on the walls of the tank, to some extent. As nitrification is a process that oxidizes ammonia into nitrite and then nitrate via nitrifying bacteria which will result in an increase in nitrite and nitrate in the fish tanks (DWAF 1996, El-Sayed 2006). Nitrite toxicity occurs mainly in closed systems where fish are exposed to high nitrite concentrations due to malfunctioning filtration systems and can result in brown blood disease (Durborow *et al.* 1997). It enters the blood through the gills and combines with hemoglobin and forms methemoglobin which results in the loss of oxygen transport in the blood which can cause anoxia and therefore reduce growth and health (DWAF 1996, Durborow *et al.* 1997, Çağiltay 2007). Nitrate toxicity is uncommon for tilapia, but high levels of nitrate over a long time may affect immunity and cause mortality (Plumb 1997). The LC50 nitrite-nitrogen for *O. mossambicus* is between 10-15 mg/l and the target aquaculture for nitrate-nitrogen is < 300 mg/l for most fish (DWAF 1996). The nitrite and nitrate concentrations in the fish tanks

in this study fell well below these levels; therefore, the mean concentrations of nitrite and nitrate were unlikely to have influenced fish health and growth.

Water quality parameters may fluctuate daily as well as throughout the day (Alam and Al-Hafedh 2006). These include pH, DO, ammonia, nitrite and nitrate (Alam and Al-Hafedh 2006). Therefore, the water quality parameter means for all treatments may not be truly representative of the actual water quality parameters that were present in the study, since these parameters were only measured once every seven days here. Water quality is important as it is known to affect fish health and growth (Piper *et al.* 1982) and should be measured daily to ensure a better understanding of the relationship of these parameters and fish health and growth. If this study is ever repeated, water quality parameters will be measured more frequently.

Percentage weight gain, specific growth rate (SGR) and food conversion ratio (FCR) in the experiment was lower than reported by Wang *et al.* (2000) over an 8-week study, which were 600 %, 3.55 %/day and 1.81 respectively. The high increase in body weight and high SGR was likely due to the fish being fed to satiation twice a day and the water having a mean temperature of 25.6°C where the present study fish were fed once a day at 8.0 % initial mean mass per tank and had a mean temperature of 19.8 ± 0.28 °C respectively. Higher weight gain percentages have been reported; Olvera-Novoa *et al.* (2002) presented weight gains of around 900 %, where the high weight gain was likely due to optimal water temperatures of 28°C and feeding rate of three times a day at 6.0 % body weight. The amount of feed provided (8% body weight per day) to fish did not increase over time in the present study; this method was implemented because DeLong *et al.* (2009) found that, as fish grow, they require less food as they attain larger sizes. At a size class of 5 - 18 g, feed of 5 – 10 % of body weight

should be provided; at a size class of 18 – 75 g, feed of 1.5 – 3 % of body weight should be provided (DeLong *et al.* 2009). Increasing the feeding rate (which was limited to once a day in the trial) may have improved fish growth in the present study, since previous studies where fish feeding was reliant on phytoplankton and little artificial feed, fish had a SGR of 1.41 %/day (Khalil and Hussein 1997).

Condition factor for all treatments were low compared to those reported previously, where experimental fish had condition factors around 3.0 (Riley *et al.* 2005). However, that experiment was also conducted at a higher temperature of 25 °C, and tilapia grow better and utilize feed more efficiently at higher temperatures (Popma and Masser 1999).

Dissolved oxygen concentration is known to affect SGR and FCR (Tsadik and Kutty 1987, Bergheim 2007, Makori *et al.* 2017). Specific growth rates in tilapia in water with DO concentrations between 3 and 4 mg/l are known to have half the SGR of DO concentrations > 7 mg/l (Tsadik and Kutty 1987). As mentioned before, the DO concentration in all treatments was limited due to the divided air supply of the channel blower to the CWs in Chapter 3. The treatments' relatively low growth parameters were probably due to limited feeding, lower temperatures, lower dissolved oxygen and higher ammonia levels, since all of these reduce fish growth (Pescod 1992, Ip *et al.* 2001, Randall and Tsui 2002). The mortality rates in the treatments were similar and did not differ from those reported elsewhere (Dverdal *et al.* 2018). Handling stress may have severe consequences to tilapia survival due to bacterial and fungal infections which can lead to fish mortality (Fernanda Deriggi *et al.* 2006). Mortalities may have occurred due to fish handling which can increase the chance of infectious disease. The lack of differences in fish growth between treatments, was likely due to the water quality parameters being similar, except for pH, EC and nitrate and for which *O. mossambicus* have

broad tolerances ranges (Murthy *et al.* 1984, DWAF 1996, Kamal and Mair 2005). Although no water quality parameters were in the lethal range for *O. mossambicus*, the parameters could be improved to optimise fish growth and health in future studies.

Condition factor provides an indication of health, and low CF values may indicate potential health issues (McPherson *et al.* 2010, Jin *et al.* 2015). However, analyzing fish health could be enhanced in future studies as it could have added considerable value in this study. Health parameters that probably would provide a better understanding of the effects of different water quality parameters on fish health in this study include: macroscopic analysis of fins, eyes, mouth, scales and gills; histopathological analysis of gills, liver, kidney and skin; blood samples and hepatosomatic index. Gill morphology is sensitive to salinity and is a good indicator of stress since gills have the largest fraction of total body surface area that is in contact with water (Sardella *et al.* 2004, Ahmed *et al.* 2013). Salinity can affect plasma osmolality and apoptotic chloride cells which are also good indicators of osmoregulation stress (Sardella *et al.* 2004). This may have been useful in this study to determine fish health differences due to the CWs having higher EC concentrations than the municipal control. Blood samples indicate stress in fish; ceruloplasmin, glucose and haematocrit levels in the blood vary and indicate level of stress in fish (Cnaani *et al.* 2004). These parameters are known to be good fish health indicators and they provide a useful indicator of water quality and environmental stress (Cnaani *et al.* 2004, Rašković *et al.* 2013).

It has been proposed that the use of treated wastewater for aquaculture demands thorough examination due to potential implications on public health (Khalil and Hussein 1997, Jana 1998). Fish grown in wastewater-based cultures should be pathogen free and should have chemicals at reasonable levels that comply with the World Health Organization guidelines

(Khalil and Hussein 1997). Examples of these include; chemical and bacterial analyses (on fish skin, gills, intestines and muscles) and heavy metal accumulation analyses (muscles, gills, liver and intestine; Buras *et al.* 1987, Khalil and Hussein 1997, Liang *et al.* 1999). This is unlikely to be a problem in recovered brewery effluent as studies done on fish grown in raw sewage with high levels of pathogenic organisms and toxic metals have shown acceptable levels of contaminants (Khalil and Hussein 1997); and trace amounts of toxic metals and almost no pathogenic organisms were found in brewery effluent (Stocks *et al.* 2002). However, it is important to verify these claims through future research as water quality may vary at different breweries, and consumers will require verification.

Deteriorating water quality (increase of ammonia over time) in the study produces a potential trade-off between production cost and food production. If treated brewery effluent for aquaculture is proposed, it is essentially taking treated water and polluting it with additional nutrients that are produced by the fish (e.g. ammonia); i.e., the same elements that were initially treated from the brewery effluent in the CWs. Therefore, post-aquaculture effluent treatment is needed, and this carries its own cost, but this could be dealt with by removing these nutrients with cash crops. Aquaponics is an integrated system where nutrients from fish are recovered by high value vegetables (Watanabe *et al.* 2002). Since the wetlands are used to produce vegetables, this is essentially the addition of an aquaponics loop in the process. A two-stage ebb and flow may be suitable, where the first stage treats water from the anaerobic digester and sends treated water to the second stage where fish are added, and more vegetables are grown. This will increase crop and fish growth and health, and at the same time, minimise the amount of water and area required per kilogram biomass produced.

4.5 Conclusion

The water quality in fish tanks that drew treated effluent from both CWs were similar. There were some differences in these parameters between those treatments and the municipal control; EC, pH and nitrate were higher in fish tanks that drew from both CWs in Chapter 3, compared with the same parameters measured in the control. These parameters were still within the tolerance of *O. mossambicus* and did not affect growth performance, which remained similar among all treatments.

The lack of literature of using recovered wastewater from aerated sub-surface flow and tidal CWs suggests that the application of this technology is novel, therefore this study is a first step in optimising the use of recovered water from CWs in aquaculture. Although fish growth parameters were not optimal, probably due to high ammonia levels resulting from an inadequately designed and/or managed recirculating aquaculture system, this was similar among treatments which demonstrates potential for treated brewery effluent reuse from CWs in aquaculture.

Chapter 5: Discussion.

Constructed wetlands have the potential to be used as an integrated system that includes brewery effluent treatment, crop growth and aquaculture for further reuse and recycling of brewery wastewater (Chapters 2, 3 and 4). Effluent remediation and crop growth were demonstrated in Chapters 2 and 3, where all experimental treatments lowered ammonia concentration and chemical oxygen demand (COD); and maintained pH, nitrite and nitrate below discharge standards (DWAF 1998). The effluent was treated suitably to be sent to the municipality without incurring penalties or it could be further reused in the brewery for cleaning or irrigation, for example etc.). Filling and draining time (FDT) in tidal wetlands had an effect on treatment efficiency and crop growth, with improved effluent treatment efficiency with increasing FDT due to higher dissolved oxygen.

The faster FDT had the highest total harvest when compared to the slower treatments, due to the plant roots being exposed to atmospheric oxygen and being submerged in the effluent for shorter periods. The type of CW affected plant health and growth and treatment efficiency. Aerated and unaerated horizontal sub-surface flow (aerated CW, unaerated CW) resulted in plant mortality and low overall harvest; where the tidal CW showed the highest potential for crop growth due to the absence mortalities, highest overall harvest and showed improved effluent treatment efficiency when compared to the unaerated CW. Tidal and aerated CWs, along with municipal water as a source, have potential for use in aquaculture as their differences in water quality (e.g. nitrate, EC and pH) when compared to municipal water did not affect fish growth and mortality between treatments. The unaerated CW was not considered for the aquaculture experiment due to its inferior treatment efficiency and plant health and growth.

Brewery effluent had certain properties, which are all directly related to effluent treatment efficiencies and crop growth; and the most significant of these were: (5.1) oxygen, (5.2) pH and (5.3) EC. The following discussions will provide a synthesis of the conclusions that relate to these properties, that were drawn from the different chapters in this thesis. Finally, (5.4) a comparison has been drawn between tidal wetlands to the current wastewater treatment technology at Ibhayi Brewery (SAB Ltd, Port Elizabeth). The tidal wetland was used in this comparison due to this treatment's high potential for crop production and to its superior treatment efficiency compared with the other CW tested in this work. This will provide the first opportunity to better understand the feasibility of tidal wetlands in terms of treatment capabilities and environmental sustainability in treating brewery effluent, and to draw comparisons based on real data.

5.1 Oxygen – The most probable reason for improved effluent treatment and plant growth

A faster filling and draining time enhanced the removal of ammonia in tidal CWs. This was due to higher dissolved oxygen concentrations as nitrification is an aerobic process which is enhanced in environments where higher DO concentrations are available for aerobic bacteria (Jia *et al.* 2010, Wu *et al.* 2011b, Stefanakis and Tsihrintzis 2012). Oxygen can enter the wetland through plant roots, filling and draining, splashing and artificial aeration (Kadlec and Wallace 2008, Dong *et al.* 2011).

Plants provide oxygen near the rhizosphere through active or passive oxygen transport from the atmosphere to the roots which results in higher nitrogen removal in planted wetlands (Dong *et al.* 2011). In the draining phase of tidal wetlands, the draining water passively pumps air into the wetland media which leads to improved nutrient removal (Babatunde *et al.* 2008,

Wu *et al.* 2014). Therefore, faster filling and draining times (FDT) result in more “draining” phases per day, and therefore increase the dissolved oxygen in the wetland. Increasing the number of cycles per day improves ammonia oxidation (Cui *et al.* 2012). Results from Chapter 2 support this claim as the fastest FDT had the highest number of cycles per day, and therefore, the highest DO concentration and the fastest ammonia treatment rate. Splashing at the outflow of wetlands may also aerate the water due to exposure to atmospheric oxygen (Kadlec and Wallace 2008); so, faster FDTs have more cycles a day and oxygen may have been higher due to increased splashing from the outflow. Artificial aeration can greatly enhance dissolved oxygen in horizontal sub-surface flow CWs (Fan *et al.* 2013). In Chapter 3, the aerated CW outperformed the unaerated CW in terms of treatment efficiency due to higher DO concentrations. Tidal and aerated CWs had similar DO concentrations and treatment efficiencies while the unaerated CW had the lowest DO concentration and lowest ammonia treatment which indicates that low oxygen availability limits ammonia oxidation (Dong *et al.* 2011).

Plants require oxygen for respiration which affects growth and health (Freeman 2005, Marschner 2011). Chapter 2 results suggested that higher DO concentrations in the effluent led to higher total harvests in faster FDT tidal CWs. However, in Chapter 3, the aerated CW had higher DO concentrations than unaerated CW but there were no differences in their total harvests, while the tidal CW had almost four times higher total harvest. Crop growth in floating hydroponics were not affected by different DO concentrations in the water (Goto *et al.* 1996) which suggests that the DO concentration in the effluent was unlikely to explain the differences in total harvests. The differences were probably due to the tidal CW spinach plants being exposed to atmospheric oxygen, as water has high aqueous resistance to dissolved

oxygen, making it harder for oxygen to diffuse into the plant root cells from water when compared to air (Armstrong *et al.* 1994).

Dissolved oxygen in aquaculture is essential for aquaculture as it influences fish feed consumption, weight gain and growth rate (Buentello *et al.* 2000). There were no differences in DO concentration and fish health and growth between treatments in Chapter 4. However, low aeration through the zeolite filter may have decreased water flow in the tanks which can lead to the filter not functioning properly and the build-up of potentially harmful substances such as faeces, uneaten feeds and other metabolites which can lower water quality and therefore health and growth (El-Sayed 2006). An increase in DO concentrations may have improved fish health and growth by minimising ammonia build-up in Chapter 4.

Therefore, dissolved oxygen concentration in CWs affect treatment efficiency, especially lowering ammonia concentration, but plant health and growth was influenced by exposure time to atmospheric oxygen due to faster diffusion rates from air when compared to water.

5.2 High pH – A possible limit to crop health and growth in constructed wetlands

The pH of water affects the solubility and availability of essential plant nutrients in plants (Tyson *et al.* 2008). At high pH (> 7) certain plant nutrients precipitate into insoluble and unavailable salts (Trejo-Téllez and Gómez-Merino 2012). The ideal pH for crop growth in soilless culture ranges between 5.5 and 6.5 (Trejo-Téllez and Gómez-Merino 2012). Incoming brewery effluent pH was above 7.0 in Chapters 2 and 3 and increased over the five-day batch-cycles. The reason for high pH in brewery effluent is due to the buffering of raw brewery effluent by the addition of sodium hydroxide (NaOH) in the anaerobic digestion phase of treatment (Power and Jones 2016). In anaerobic digestion, carbon dioxide is produced, and a portion is dissolved in the digester liquor which produces carbonate alkalinity and carbonic

acid (van Rensburg *et al.* 2003, Power and Jones 2016). When the effluent for the anaerobic digester is exposed to the atmosphere, the volatile carbonic acid is released, and the carbonate alkalinity remains, thus increasing pH (van Rensburg *et al.* 2003, Power and Jones 2016). This explains the pH increases in Chapters 2 and 3; pH increased faster in CWs that had increased aeration and therefore, exposure to atmospheric partial pressure for the stripping of carbonic acid. The aeration of CWs for plant health and growth may present a trade off with pH as increasing the oxygen for improved health and growth in plants, results in a pH increase, making less plant nutrients available for uptake. The high pH in the study did not affect growth as the tidal CW had high pH values but also had the highest crop harvest; however, it did affect plant health as many nutrients in the plant tissue at the end of the trial, in the CWs, were lower than those in the seedlings at the start of the trial.

A way to combat the pH increases is through pH adjustment with the addition of chemicals, such as phosphoric, nitric or sulphuric acid (Trejo-Téllez and Gómez-Merino 2012, Power and Jones 2016). Using phosphoric acid can maintain pH in the effluent between 6.0 and 6.5, which increased growth and yield of crops due to the increased availability of plant nutrients at that pH level (Power and Jones 2016). However, adjusting pH for nutrient uptake may decrease treatment efficiency. Nitrification rates are decreased at lower pH values, as nitrification rates decrease by 13 % for every pH unit decrease from 9 to 5; in some instances, nitrification can slow significantly or even terminate at pH 6 (Brunty 1995, Tyson *et al.* 2008). Nitrification is essential in brewery effluent treatment and filtration in aquaculture. Fish stocking density is related to the performance of the biofilter in aquaculture, therefore a low pH may decrease nitrification and increase ammonia build up (Tyson *et al.* 2008). Adjusting pH may therefore, increase plant productivity but reduce treatment efficiency of brewery

effluent and filter efficiency in aquaculture. The balance between the two should be tested in future research.

5.3 High electrical conductivity – A possible limit to nutrient and water uptake in crops

The relatively high electrical conductivity (EC) in brewery effluent is a concern when using brewery effluent for crop growth and health. Electrical conductivity is an index of salt concentration in solution (Trejo-Téllez and Gómez-Merino 2012). High salts in water exerts osmotic pressure on the plants, which decreases nutrient and water uptake (Dalton *et al.* 1997, Samarakoon *et al.* 2006). The high EC originates from the addition of sodium hydroxide in the anaerobic digestion process where carbonate is formed as well as the use of chemicals such as caustic soda, calcium sulphate, potassium meta bisulphate, ortho phosphoric acid, lactic acid and chlorine used in manufacturing of beer (Senthilraja *et al.* 2013). The EC decreased slightly in all CWs but remained above the recommended EC of 1500 to 2500 $\mu\text{S}/\text{cm}^2$ for soilless cultures (Trejo-Téllez and Gómez-Merino 2012). However, the EC tolerance range for crops differs between species and is related to environmental conditions (Sonneveld and Voogt 2009). High conductivity may decrease growth and health of plants (Senthilraja *et al.* 2013). Spinach (*Beta vulgaris*) has high salt tolerance but high salinity may still reduce yields (Shannon *et al.* 2000). Therefore, the high EC of brewery effluent may have inhibited optimal plant growth and health.

The high EC did not affect tilapia (*Oreochromis mossambicus*) growth in Chapter 4. Fish growth was similar between treated effluent (with high EC) and municipal water (low EC). This was likely due to *O. mossambicus* tolerance for saline conditions (0-120 ppt) and ability to adapt their chloride cells for salt excretion (Hwang 1987). It has been reported that it can

grow equally well and sometimes, even better in saline waters (Kamal and Mair 2005). Therefore, the high EC in treated brewery effluent may be suitable for *O. mossambicus* culture but carries a potential “red flag” for crop growth and health or for other fishes.

5.4 Desk top comparison of tidal constructed wetlands to the brewery’s current method of effluent treatment

All CWs were able to treat the water to below, or close to discharge standards (DWAF 1998). However, the differences in plant growth and health indicates that tidal wetlands are most suitable for cash crop cultivation due to the exposure of plant roots to atmospheric oxygen. It is important to compare tidal wetlands to the conventional treatment facilities at Ibhayi brewery in terms of water quality and cost (SAB Ltd, Port Elizabeth). Activated sludge (AS) effluent treatment systems currently treat 880 m³ of Ibhayi Brewery effluent per day (Spannenberg, pers. comm., effluent plant operator, Ibhayi Brewery, SAB Ltd., October 2018). This process involves a system in which organic waste is continually circulated in the presence of oxygen and digested by aerobic bacteria (Piper *et al.* 1982, Appels *et al.* 2008). Conventional treatment technologies such as activated sludge are expensive due to their high construction, operation and energy costs (Mo and Zhang 2013); while CWs are land intensive, cost effective, easily operated and maintained and have potential for other applications (Wu *et al.* 2015). Constructed wetlands have less global warming potential (CO₂ emissions) and less energy use during operation (Fuchs *et al.* 2011).

Brewery effluent treatment efficiency was similar between post-tidal CWs in this trial and the commercial scale post AS (Table 5.1). The post-tidal CW had a higher DO concentration and pH (Table 5.1), whereas post-AS had higher temperature, ammonia and nitrate (Table 5.1). Due to similar water quality parameters post-treatment in tidal CWs and AS, it appears that

CWs may offer an alternative, more environmentally sustainable method of treating brewery effluent when compared to the current AS method. However, it is important to note that incoming effluent from anaerobic digestion may vary due to seasonality and unforeseen circumstances such as effluent treatment plant malfunction. Also, there are limitations in comparing and scaling up laboratory scale work (presented in this thesis) and large-scale work. Therefore, a more comprehensive comparison carried out on a pilot- or semi-commercial-scale is required to support the claim that the wetland could replace AS. A comparison of the operation cost, energy consumption and reuse potential are required on a pilot- or semi-commercial scale in future work (Section 5.5).

Table 5.1 Average water quality parameters of effluent treated in tidal CWs and activated sludge (AS) with associated discharge limits (DWAF 1998).

Parameter	Post-tidal CW	Post-AS	Discharge limits
pH	8.77	8.41	5.5 - 9.5
Temperature (°C)	18.46	26.38	N/A
DO (mg/l)	7.08	1.07	N/A
EC ($\mu\text{S}/\text{cm}^2$)	2867	< 3000	1500
COD (mg/l)	50.25	± 50.00	75
NH ₃ -N (mg/l)	0.06	0.28	3
NO ₂ -N (mg/l)	2.53	0.08	15
NO ₃ -N (mg/l)	0.92	9.00	15

Dissolved oxygen (DO), Chemical oxygen demand (COD), Anaerobic digestion (AD), Primary facultative pond (PFP), CW (constructed wetland) * Post-tidal CW values calculated as an average from Chapters 2 and 3. * Post-AS values provide by Spannenberg, pers. comm., effluent plant operator, Ibhayi Brewery, SAB Ltd., October 2018)

Although this study was not designed to compare CWs to current treatment technologies, it was included with the purpose to highlight the potential for future research to compare these systems on a full-scale.

5.5 Future recommendations and research

Constructed wetlands have potential to be used in brewery effluent treatment. However, future studies should research ways to optimise plant and fish health and growth. One way of improving plant health and growth is through pH adjustment. Research should study the difference between pH adjustment and no pH adjustment in CWs similar to the approach used by Power and Jones (2016); and should also determine the effect of lower pH on nitrification rates and treatment efficiency.

Seasonality is also an important factor to consider, as treatment efficiencies varies over seasons in CWs (Newman *et al.* 2000, Song *et al.* 2006). Plant health and growth may also have seasonality differences as *Beta vulgaris* has shown greater yields in spring and winter as they prefer cooler months (Zhang *et al.* 2009). Similarly, the production of *O. mossambicus* may also be subject to seasonal differences as changing temperature may affect growth rates (Lim and Webster 2006). Future studies on aquaculture production should also increase stocking density and longevity and a far greater and in-depth investigation of fish health and growth performance in constructed wetlands by measuring water quality parameters daily and incorporating health parameters such as macroscopic analysis of fins, eyes, mouth, scales and gills; histopathological analysis of gills, liver, kidney and skin; blood samples and hepatosomatic index. Mitigating ammonia build up in future studies can be achieved through a management routine that includes a partial flow through system where water changes occur more frequently or by improving the design of the biological filtration system.

Public conceptions are a major consideration when using wastewater to produce food and it may affect the economic viability of growing and selling crops (Toze 2006). Future studies should investigate public perceptions towards this issue.

Life cycle analysis (LCA) has been applied since 1990 to compare different wastewater treatment technologies (Fuchs *et al.* 2011, Corominas *et al.* 2013). This is a technique that aims to quantify a product, process or service's environmental impacts that includes process from mineral extraction to manufacturing to use and recycling or final disposal (Racoviceanu *et al.* 2007, Fuchs *et al.* 2011, Corominas *et al.* 2013). Studies on the use of tidal CWs in wastewater treatment have been done on pilot or laboratory scale and very few full-scale studies over a long period (Jing and Lin 2004, Song *et al.* 2006). When laboratory scale data is used it limits the applicability of the results, especially in the construction phase (Corominas *et al.* 2013). The current experimental-scale provides sufficient motivation to invest a pilot-scale facility. Future studies must test the claims made here on a larger-scale, to gain a better understanding of the efficiency of effluent treatment, crop production and aquaculture production and their associated impacts at a larger scale. This will increase credibility of LCA between tidal CWs and other conventional water treatment technologies, such as activated sludge.

5.6 Conclusion

Brewery effluent has potential to be used as a water and nutrient source for the growth of crops with economic value and for downstream use as a water source for aquaculture. The effluent can be treated by CWs, with faster filling and draining tidal CWs and aerated horizontal sub-surface flow CWs showing the highest treatment efficiency due to increased dissolved oxygen in the wetland and exhibiting similar aquaculture potential. However, the low crop harvest in aerated CW indicates that the wetland may not be ideal for crop production and are probably only suitable for the growth of wetland macrophytes. Tidal CWs were the only systems tested here that had no crop mortality and had significantly higher

total harvests, which was probably due to the exposure of plant roots to atmospheric oxygen. Therefore, for the integration of effluent treatment, crop and aquaculture production, tidal CWs showed the greatest potential. Although tidal and current methods of effluent treatment used at the brewery (i.e. activated sludge) had similar water quality parameters post-treatment, it is imperative to study tidal CWs on a large-scale to comprehensively compare them to other water treatment technologies in terms of environmental impacts in construction, process, maintenance and reuse.

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