The use of aeration in constructed wetlands and the potential for earthworm and crop production

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Abstract

Constructed wetlands (CWs) are used in a variety of industries to treat effluent for safe reuse or discharge. They can however take up large areas of land. Adding oxygen can increase treatment efficiency, as it is vital to many nutrient removal pathways, such as nitrification and aerobic respiration. Increasing efficiency can decrease the cost of treatment and reduce land area needed to operate a CW. The study aimed to find the best method of aerating wetlands treating brewery effluent, the potential production of worms and plants in the system and their effects on water treatment. The first experiment tested a hybrid constructed wetland, using small (1.0 m^3) ebb and flow filtration additions to a horizontal subsurface flow constructed wetland against nanobubble aeration in a similar horizontal flow wetland cell. It was not possible to add replicates to the study, but findings prompted further investigation into the hybrid setup. Experiment two focussed on the ebb and flow filters within the hybrid system comparing the presence of plants and worms. The ebb and flow filters increased dissolved oxygen (DO) from an average inflow of 3.89 mg/L to an outflow of 5.70 mg/L and decreased the ammonia content of the effluent from 14.8 mg/L to 11.2 mg/L. Swiss chard (*Beta vulgaris*) was successfully grown in the system and was found not to affect DO. The addition of both Swiss chard and earthworms (*Eisenia fetida*) was found to decrease PO_4^{3-} by 17.69 ± 1.36 %. Experiment 3 compared the previous ebb and flow filters to trickle filters with the addition of celery (*Apium graveolens*) to both systems to determine which filter treated the effluent more effectively. The ebb and flow filters performed better than the trickle filters, increasing the DO by 37.54 ± 0.06 % compared to 19.22 ± 0.06 %. Celery increased this change in both systems, to 44.16 ± 0.06 % in the ebb and flow filters and 30.96 ± 0.06 % in the trickle filters. The ebb and flow filters were also able to decrease the NH_3-N concentration by 36.1 ± 0.2 %. This effluent could sustain plant growth; Swiss chard grew at a rate of 0.04 ± 0.02 g/g/d and celery at 0.2 ± 0.1 g/g/d. Ebb and flow filters are better at aerating brewery effluent and decreasing nutrient load than nanobubble pumps and trickle filters. The addition of ebb and flow filters to the CW treating brewery effluent has the potential to improve nutrient removal and provide an edible crop.

Table of contents

List of Figures

List of Tables

List of abbreviations

BOD	Biological oxygen demand
COD	Chemical oxygen demand
CW	Constructed wetland
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
HRT	Hydraulic retention time
NB	Nanobubble pump
NOB	Nitrite oxidising bacteria
PFP	Post-facultative pond
PVC	Polyvinyl chloride
NTU	Nephelometric turbidity units
RGR	Relative growth rate
SAB Ltd	South African Breweries Limited
TDS	Total dissolved solids
TKN	Total Kjeldahl nitrogen
TSS	Total suspended solids
TWQR	Target water quality range

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Chapter 1: Introduction

South Africa, with increasing frequency of years-long droughts such as in the Western Cape from 2015 - 2018 and in the Eastern Cape from 2015 - 2020, faces severe pressure on its water systems (Mahlalela *et al.* 2020, Archer *et al.* 2022). The growth of industry has led to increasing quantities of wastewater and less available clean water (Hanjra & Qureshi 2010). Wastewater is a liability to companies as it poses an environmental threat, and must be treated before entering the municipal sewer system (Braeken *et al.* 2004). Major producers of nutrient-rich wastewater are breweries (Braeken *et al.* 2004). Ibhayi Brewery (SAB Ltd) treats wastewater created on-site before being released to the municipality. South African Breweries is committed to decreasing water use to 2.50 L of water per litre of beer packaged (SAB 2021). One strategy to achieve this goal is reusing treated wastewater in the brewery (SAB 2021).

1.1 Problem identification

Breweries use large quantities of water and produce nutrient-rich wastewater (Braeken *et al*. 2004). Companies in South Africa are charged for water resource management, water resource infrastructure, waste mitigation, and a water research levy (DWS 2022). To decrease costs, wastewater can be treated on-site before being released into the municipal sewer system. However, the treatment process is expensive and energy-demanding (Simate *et al*. 2011).

Constructed wetlands (CWs) are a low-cost, energy-efficient method of treating many forms of wastewater, even those with high nutrient loads (Zhang *et al*. 2011, Wu *et al.* 2014). Wetlands have been employed to treat pharmaceutical, agricultural, brewery, winery and heavy metal wastewater (Khan *et al.* 2009, Serrano *et al.* 2011, Dordio and Carvahlo 2013, Auvinen *et al.* 2017).

Treating high-nutrient loads can, however, take up large areas of land. Increasing the efficiency of wetlands so that more water can be treated without increasing land is important to our environment, and human and animal health (Choudhary and Kumar 2020). Constructed wetlands can be adapted to handle high nutrient loads by adjusting one or more of the physical, biochemical, or external factors (Lee *et al.* 2009). There have been many examples of these adjustments such as increasing the hydraulic retention time or comparing the type of media used in the CWs in a study by Chen *et al.* 2006. Using a 5.0 d retention time and ceramic bioballs they achieve an NH_3-N removal of 56 % (Chen *et al.* 2006). Other studies have looked at combinations of different types of CWs, effluent recirculation, media, and plant use (Vymazal 2007, Lee *et al.* 2009). One found that using an organic media led to higher total nitrogen removal, however when the effluent was recycled in the same system the removal rates were lower (Saeed *et al.* 2018). The same study investigated using recycled brick media which increased the reduction of phosphorus (Saeed *et al.* 2018). Constructed wetlands can be adapted for various levels of nutrient load using low-technology adaptations, without increasing land use.

In partnership, Ibhayi Brewery (SAB Ltd), Rhodes University and the Water Research Commission have formed Project Eden, which is working on wastewater treatment strategies for the effluent from the brewery (Jones *et al.* 2014). There are currently six wetland cells in operation at the brewery and Project Eden is now focusing on increasing the efficiency of the wetlands. This presents the opportunity to explore methods of improving wetlands that can be applied worldwide to various effluent streams and wetland sizes.

1.2 Literature review

This is a comprehensive review highlighting critical aspects of CW technology for effluent treatment. It will discuss how CWs remove nutrients, and how different media, aeration and bacteria can influence their functioning.

Constructed wetlands are used worldwide to treat various forms of wastewater, from agriculture and aquaculture to industrial effluent such as mines and breweries (Poach *et al*. 2003, Lin *et al*. 2005, Babatunde *et al*. 2008, Jones *et al*. 2014). These wetlands vary greatly in scale, from small, experimental sites treating 2.0 m^3 of effluent per day to large industrial wetlands treating more than 350 m^3/d (Fu *et al*. 2018). Constructed wetlands improve water quality by removing excess nutrients such as nitrogen, phosphorus, and organic matter (Greenway 2005, Sindilariu *et al*. 2007, Kadlec 2016). This is done through three main components:

- plants, which do not always occur in CWs but have been shown to improve the removal of nutrients through root uptake and by releasing oxygen into the system (Naylor *et al*. 2003, Zhai *et al*. 2013, Wang *et al*. 2017). Plants provide support for the biofilm (Button *et al*. 2015);
- media or substrate that creates a matrix allowing aerobic and anaerobic zones to exist in a wetland (Li *et al*. 2014, Saeed and Sun 2012). Substrates can also physically filter particulates and adsorb pollutants as well as providing structure for plant roots (García *et al*. 2010);
- bacteria which form a biofilm on the plant roots and the media, ad- and ab-sorb pollutants as well as convert the pollutants into biologically available forms for the plants to take up or to be released into the atmosphere (Saeed and Sun 2012).

1.2.1 Nutrient removal

Conversion by bacteria is the main mechanism of pollutant removal from wastewater (Zhai *et al*. 2013, Button *et al*. 2015). One of the main nutrients to remove from wastewater is nitrogen. The conversion of nitrogen is carried out by a host of different bacteria and can be done in various ways. Ammonification, the conversion of organic-N to ammonia-N can occur under aerobic conditions by heterotrophic bacteria or when oxygen is limited or unavailable (Ready *et al*. 1984). Anaerobic bacteria and facultative anaerobic bacteria can convert organic-N to ammonia-N, however, the reaction time is slower (Ready *et al*. 1984, Vymazal 2007).

Nitrification forms part of the main pathway of ammonia-nitrogen removal; it is an aerobic conversion of ammonia-N to nitrite-N by *Nitrosomonas, Nitrosococcus and Nitrosospira* bacteria (Bernhard 2010, Saeed and Sun 2012). Nitrite-N is then oxidised to nitrate-N by *Nitrobacter* and *Nitrospira* bacteria. After this conversion, nitrate-N can follow three paths; a) nitrate-N uptake where NO_3-N is taken up by the plants within the wetland and leaves the system, however, plant uptake can be limited (Button *et al.* 2015, Vymazal 2007); b) nitrate-ammonification where by nitrate-N is reduced to ammonia-N, this occurs in anoxic environments (Vymazal 2007); and c) denitrification, the major removal pathway of nitrogen (Vymazal 2007). Nitrogen can then leave the system when facultative anaerobic bacteria such as *Pseudomonas, Bacillus* and *Enterobacter* reduce nitrate-N to nitrogen gas which can exit the wetland into the atmosphere (Kadlec and Knight 1996). Nitrogen fixation, the conversion of nitrogen gas to ammonia-N can also occur within the system but is very low when nitrogen is freely available (Kadlec and Knight 1996).

Another way to remove nitrogen from wastewater is through ammonia-N adsorption to the media. This is dependent on the concentration of ammonia-N in the water and adsorbed ammonia-N will be released when the concentration in the water is low (Vymazal 2007). Ammonia volatilization is the transfer of ammonia gas from the water to the atmosphere. It is dependent on the pH of the wastewater as a high pH (> 9.3) allows ammonium ions to convert to ammonia gas (Saeed and Sun 2012).

Anaerobic ammonium oxidation (anammox) is the oxidation of ammonium to nitrogen gas by *planctomycete* bacteria. While this is a faster reaction than the traditional nitrification/denitrification process, *planctomycete* is slow-growing and ammonium is only oxidised under specific conditions (Cho *et al*. 2019, Saeed and Sun 2012). Nitrogen has many paths out of wastewater, but all rely on specific conditions (Vymazal 2007).

Another nutrient to reduce in wastewater is phosphorus (Button *et al*. 2015). Excess phosphorus in water sources can lead to harmful algal blooms and hypoxia, therefore phosphorus levels in wastewater must be reduced before release or reuse (Blaas and Kroeze 2016). Phosphate in wetlands is controlled in three ways (Button *et al*. 2015). Phosphate adsorbs to media such as limestone, but this depends on the size and texture of the media (Naylor *et al*. 2003, Button *et al*. 2015). Phosphate can leave the system through plant uptake, however, the amount of phosphate taken up from wetlands is highly variable between systems (Button *et al*. 2015). Bacteria, such as *Candidatus phosphoribacter and Candidatus Accumlibacter phosphatis* can store large amounts of phosphate, however, because it is stored not processed it is a temporary mechanism and when the bacteria die the phosphate is released back into the system. (Button *et al*. 2015, Dorofeev *et al*. 2020, Singleton *et al*. 2022). Phosphate can be difficult to remove from wastewater and often must be specifically targeted with substrate choice (Naylor *et al*. 2003, Button *et al*. 2015).

1.2.2 Improving wetland efficiency

Wetland efficiency is impacted by a variety of factors. Firstly, the type of wetland affects functioning and efficiency. Wetlands are split into two categories: free water surface and subsurface flow. Subsurface flow wetlands are further split into vertical flow wetlands and horizontal flow (Figure 1.1). Free water surface wetlands are the most like natural wetlands. In these wetlands, water flows over the substrate and oxygen naturally diffuses into the wetland at the air-water interface (Saeed and Sun 2012, Vymazal 2007). In subsurface flow wetlands, water is contained beneath the surface, entering at the surface of the substrate, exiting below in vertical subsurface flow and entering and exiting below in horizontal subsurface flow (Saeed and Sun 2012; Figure 1.2).

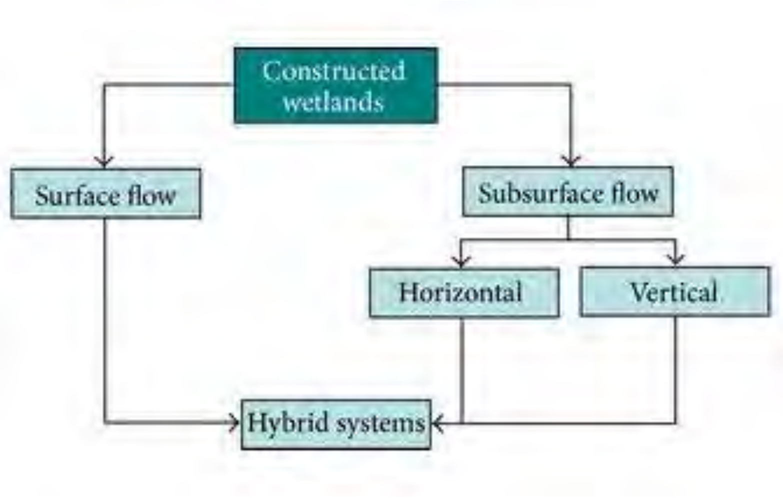

Figure 1.1 Types of constructed wetlands (Vymazal 2007, Vymazal 2010).

Each of these designs affects nutrient removal differently. Water in a free water surface is constantly exposed to the atmosphere and therefore oxygen can enter the wetland freely allowing for nitrification in the top layer of water and denitrification below as the oxygen content decreases (Hamilton *et al*. 1993, Wang *et al*. 2017). Subsurface flow wetlands are excellent at removing solids however this can lead to clogging in the matrix (Saeed and Sun 2012). Vertical subsurface flow wetlands allow more oxygen into the system than horizontal subsurface flow, causing vertical wetlands to have improved aerobic metabolic pathways such as nitrification and aerobic oxidation of dissolved organic matter. They can lead to decreased denitrification due to the anaerobic nature of denitrification (Vymazal 2010). Hybrid systems incorporating both vertical and horizontal subsurface flow are often used due to their improved nitrogen removal (Langergarber *et al.* 2011, Vymazal and Kröpfelová 2015, Saeed *et al.* 2019). Vertical wetlands allow nitrification to take place in an oxygenated environment followed by horizontal flow wetlands which allow sufficient denitrification to take place in an anaerobic environment (Wang *et al*. 2017).

Bacterial degradation, metabolism and uptake are the main pathways out of wastewater for nutrients. Bioaugmentation is the introduction of bacteria specific to the nutrients and the conditions found in the wastewater (Wu *et al*. 2014). This ensures faster and more established biofilms within the wetland that can degrade specific nutrients and pollutants faster (Wu *et al*. 2014). Bioaugmentation has been used in CWs to combat salt stress by adding salt-tolerant denitrifying bacteria and to improve nitrogen removal in cold climates by inoculating the wetland with a variety of cold-tolerant nitrifying and denitrifying bacteria (Zhao *et al*. 2016, Wang *et al*. 2020).

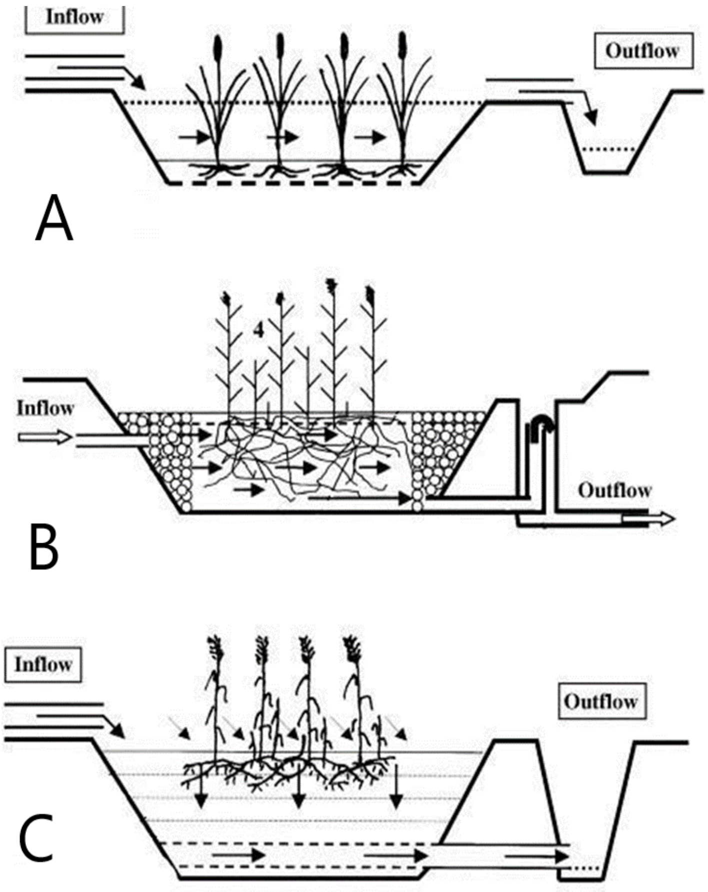

Figure 1.2 Types of constructed wetlands (A) free surface water wetland; (B) horizontal subsurface flow wetland; (C) vertical subsurface flow wetland (Vymazal 2007).

Substrate type plays a role in the treatment capabilities of a wetland. Carbon is used by bacteria during denitrification certain substrates can be used as a carbon source (Saeed and Sun 2012, Zhai *et al*. 2013). Substrates with high cation-exchange capabilities facilitate ammonium adsorption leading to increased nitrogen removal and are the main aid for phosphorus removal (Saeed and Sun 2012, Button *et al.* 2015).

Effluent recirculation involves redirecting a portion of the already filtered wastewater back into the wetland; this gives the wastewater more contact time and interaction with bacteria. It has been shown to work in conjunction with aeration with a 54.8 mg/L decrease in total N (Foladori *et al.* 2013). However, when the organic load is high in wastewater nutrient removal is more efficient, and as recirculating dilutes the organic load it can decrease the overall nutrient removal (Stefanakis and Tsihrintzis 2009, Saeed and Sun 2012).

Hydraulic retention time (HRT), the amount of time a pollutant is retained in the system can be adjusted to increase the interaction between the microbes and nutrients. Increasing the HRT from 1 to 4 days has led to 90 % removal of NO_3-N and complete removal or NH_4-N (Ghosh and Gopal 2010).

Nutrient removal can be improved in a variety of ways, but wetland and wastewater conditions can impact the efficiency of these improvements.

1.2.3 Oxygenation to improve wetland functioning

Adding oxygen to CWs is a well-documented method of increasing the efficiency and intensity of water treatment (Sun *et al*. 2005, Vymazal 2007, Sindilariu *et al*. 2009, Nivala *et al*. 2013, Wu *et al*. 2014). Many nutrient removal pathways in wetlands rely on oxygen. Nitrification cannot take place without oxygen (Vymazal 2007). Without nitrification, denitrification is affected as it uses the NO_2-N formed during nitrification (Vymazal 2007). Aeration of these wetlands improves the removal of biological oxygen demand (BOD), total suspended solids (TSS) and total Kjeldahl nitrogen (TKN). Even with additional aeration, oxygen availability can be rate-limiting (Nivala *et al*. 2013). This can be seen in the nitrification process which is limited by dissolved organic matter removal when oxygen is available. The bacteria responsible for organic matter removal are faster growing than nitrifying bacteria and consume most of the oxygen needed for nitrification (Saeed and Sun 2012). Oxygen increases microbe activity and changes the structure and diversity of microbial communities. With enough oxygen nitrifying bacteria are given an advantage and can better establish their population (Saeed and Sun 2012).

Adding oxygen to a wetland can have adverse effects on anaerobic bacteria populations. Aerobic populations use the available resources now that oxygen is available, however, these bacteria can still form in anaerobic and anoxic zones within the roots and substrate (EPA 2000).

Overall, the addition of oxygen aids in nutrient removal as aerobic conditions reduce BOD, DOM, and nitrogen (Ouellet-Plamondon *et al*. 2006, Zhang *et al*. 2011, Li *et al*. 2014, Ilyas and Masih 2017). Creating ways for oxygen to enter a CW system is an easy and often cheap way to improve the functioning of the wetland.

1.2.4 Technologies for oxygenation

The type of wetland can influence the amount of oxygen that is present in the system; vertical subsurface flow and free water surface systems allow more oxygen than horizontal subsurface wetlands. This is due to the contact time the water in the wetland has with air allowing oxygen to diffuse into the water (Vymazal 2010). Water in horizontal subsurface flow wetlands constantly flows through the system with no exposure to the outside environment. This allows no external oxygen into the water (Vymazal 2010, Nivala *et al.* 2013). Vertical flow wetlands allow oxygen into the system through batch or drip feeding. (Vymazal 2010, Nivala *et al.* 2013). Drip-fed vertical wetlands have a constant low flow of water into the system which is why they are also known as trickle systems. Oxygen enters the gaps in the media and this can diffuse into the wastewater as it flows down different paths in the media and enters these gaps (Vayenas 2011, Bulter and Boltz 2014). In batch-fed systems, wastewater floods the system and as it drains air is passively drawn into the system due to the pressure gradient created. Oxygen then diffuses into the wastewater when the system is next flooded (Stottmeister *et al*. 2003, Stefanakis and Tsihrintzis 2012, Wu *et al*. 2014).

Free water surface systems allow oxygen to enter the water on the surface, however, an oxygen gradient forms and the system is still predominately anaerobic (Vymazal 2010).

Wetlands can also use drop aeration to increase oxygen in the water. This is a type of vertical flow system where wastewater is released into the system from a height allowing the water to interact with the air and oxygen is transferred between the two. A drop of 2.5 m can increase DO levels by 2.7 mg/L (Nivala *et al.* 2013).

Artificial aeration uses a blower to distribute air bubbles into the water column, any bubbles that do not diffuse burst at the water surface and the oxygen is released into the atmosphere. Diffusers and air stones are two forms of artificial aeration. Air diffusers which use an attached hose for even distribution of bubbles and air stones which have air forced through a stone instead of a hose which creates different-sized bubbles. Air diffusers and stones do oxygenate the water but it is inefficient with only between 3.0 and 5.0 % of the oxygen being released into the water (Wu *et al*. 2016).

Nanobubble technology is a more efficient method of artificial aeration (85% oxygen released into the system) whereby nanobubbles are created mechanically and injected into the water body. Fans or microporous aerators are used to create microscopic bubbles with high surface tension enabling them to stay in the water column for longer and disperse over a larger area (Xiao and Xu 2020). When comparing aeration strategies between standard artificial aeration and nanobubble aeration a 17 % higher removal of ammonia was measured using a nanobubble pump running (Lyu *et al.* 2023). This resulted in a 65 % removal of ammonia while running the pump for 10 min/d (Lyu *et al.* 2023).

Aerating a wetland using these methods has had different effects depending on the type of wastewater and the conditions of the system. More than one of these solutions can be employed together in one system or having multiple stages in use depending on the constraints of land, the environment and budget.

1.2.5 Addition of plants for improved wetland functioning

Adding plants to a CW is one of the most common ways to improve effluent treatment since it is easy and can be done cheaply (Naylor *et al*. 2003, Wang *et al*. 2017). Plant roots provide a surface area for microbes, which are the main removal mechanism of nitrogen, to attach (Button *et al*. 2015). Plants transport oxygen from the external environment into the system and remove nutrients from the effluent through root uptake (Zhai *et al*. 2013, Wang *et al*. 2017). Plants contributed 7.0 – 9.0 % of total Kjeldahl nitrogen removal in CWs treating agricultural wastewater (Gotschall *et al.* 2007). Plants also provide oxygen to their surroundings as oxygen travels from their roots into the rhizosphere via diffusion, this is however a small amount of oxygen that only affects the roots' immediate surrounds (Rehman *et al*. 2016). Plants lead to overall positive effects in wetlands not only through nutrient uptake and microbe attachment but also through aesthetic improvement and a possible source of income or food from crops.

1.2.6 Addition of worms for improved wetland functioning

Worms have been shown to improve effluent treatment in many studies with a variety of wetland types (Nuengjamnong *et al*. 2011, Xu *et al*. 2013, Wu *et al*. 2014, Singh *et al*. 2021). They use nutrients from the wastewater to sustain themselves and can take in oxygen through their skin from air or water (Brown and Doube 2004). Earthworms can survive up to two hours in anaerobic environments (Brown and Doube 2004). Earthworms mineralise organic-N during feeding (Xu *et al*. 2013). This increases nitrification and denitrification potentials in the effluent as there is more inorganic nitrogen available for nitrifying and denitrifying bacteria (Xu *et al*. 2013, Wu *et al*. 2014, Singh *et al*. 2021). Nitrification potentials were increased by 236 % in the presence of earthworms, denitrification potentials were also increased but to a lesser extent at only 8.0 % (Xu *et al.* 2013). The mineralisation of organic-N y worms also makes this more available to plants, this can increase growth which increases the effects plants have on the wetland functioning (Singh *et al*. 2021, Xu *et al*. 2013). Plant height has been shown to increase by 15 - 61 % depending on the plant species when earthworms were included in the substrate (Xu *et al.* 2013).

1.2.7 Treating brewery effluent

Treated wastewater in South Africa must be within the guidelines set by the Water Act for release into a water resource or safe reuse in the Ibhayi Brewery (Table 1.1, DWAF 1996, DWAF 2004, Jones *et al.* 2014). Nutrients in brewery effluent come from various sources, sugars, starch and ethanol (SAB 2022). These contribute to the organic load of the effluent. Average nutrient values from brewery effluent are presented in Table 1.1.

The wastewater enters the Ibhayi Brewery wetlands from an anaerobic digester where organic matter is broken down (Jones *et al.* 2014). The effluent from the digester still contains nutrients essential to plant growth and health, making it ideal for further treatment through a wetland and has the potential to sustain viable commercial crops (Jones *et al*. 2014).

The crops grown in the wetland must be able to deal with high conductivity and pH effluent from the anaerobic digester (Jones *et al*. 2016). At the start of this project, the wetland was planted with Calla lilies (*Zantedeschia*) and Cattails (*Typha*), popular wetland plants*,* but Swiss chard (*Beta vulgaris*)*,* lettuce *(Lactuca sativa*) and tomatoes (*Lycopersicon esculentum*) *have* been successfully grown in the Ibhayi brewery effluent (Jones *et al*. 2014, Power and Jones 2016, de Jong 2019).

The wetland system alone could not filter the brewery effluent to reuse standards. Extra efficiency was required; however, wetlands can be improved in many ways, but the effects can vary between climates, seasons and the type of wastewater.

Table 1.1 Guidelines for wastewater discharge, target water quality range (TWQR) for industrial reuse and the average concentration of nutrients in brewery effluent (DWAF 1996, DWAF 2004, Hultberg and Bodin 2017).

Parameter	Discharge limit	TWQR for reuse	Brewery effluent
Chemical oxygen demand	75	0 - 30	2000 - 6000
pH	5.5 - 9.5	6.5 - 8.0	N/A
Total nitrogen (mg/L)	N/A	N/A	25 - 100
Ammonia as nitrogen (mg/L)	3	N/A	N/A
Nitrate/nitrite as nitrogen (mg/L)	15	N/A	N/A
Phosphate	10	N/A	10 - 60
Electrical conductivity (mS/m)	70 - 150 above intake	0 - 70	1 - 70

1.3 Overall aims and research questions

This study aimed to find the best method of aerating wetlands, thus increasing the efficiency of brewery effluent treatment and whether these methods could sustain crop and earthworm production, leading to more efficient space use.

The objectives were to:

1. Determine the best aerator between ebb and flow wetlands and nanobubble pumps;

2. Establish whether ebb and flow filters can support a healthy food crop and if this affects effluent treatment efficiency; and
3. Determine the filter with the highest treatment efficiency between ebb and flow and trickle bioreactors and compare crop growth in the two bioreactors.

Chapter 2: The effect of ebb and flow filters versus nanobubble pumps on the treatment of brewery effluent

2.1 Introduction

Constructed wetlands (CW) are often classed by the way water flows through the system (Vymazal 2010). Horizontal subsurface flow CWs are mainly anaerobic or anoxic environments (Kadlec and Wallace 2008, Vymazal 2010). Anaerobic systems limit nitrification (Kadlec and Wallace 2008). The main mechanism of nitrogen removal is denitrification in anaerobic systems (Kadlec and Wallace 2008). Very low nitrification takes place in horizontal subsurface flow wetlands due to the anaerobic nature of the system (Kadlec and Wallace 2008, Vymazal 2010). In contrast, vertical subsurface flow CWs are aerobic (Kadlec and Wallace 2008, Vymazal 2010). The aerobic conditions provided by the vertical water flow allow nitrification to take place (Vymazal 2013). Combinations of different CWs, known as hybrid CWs have been used to increase overall nutrient removal; these utilise the different conditions provided by different CWs to increase performance (Vera *et al*. 2010, Serrano *et al*. 2011, Vymazal 2013). In these systems, effluent is first passed through vertical flow CWs, where nitrification is optimised (Vymazal 2013). This is then followed by a horizontal flow CW where nitrates are removed through denitrification (Vymazal 2013).

The CW at Ibhayi Brewery was not treating the designed effluent capacity (350 m^3/d) to reuse standards (Chapter 1; Section 1.2.7; Table 1.1). In an attempt to address this, the wetland cells were artificially aerated using nanobubble pumps, which added air to the effluent at a rate of up to 170 L/min (MK1 Nanobubbler, Fine Bubble Technologies, South Africa). Nanobubbles could aerate the effluent in a biofilm reactor to 10.01 mg/L (Xaio and Xu 2020). This was not reflected in the dissolved oxygen (DO) levels, which did not rise above 0.9 mg/L. A minimum DO concentration of 2 mg/L is needed for the efficient operation of the CW (Gerardi 2006, Taylor 2020). A new system was designed to replace the pumps at Ibhayi Brewery without increasing the cost of running the CW. The system designed was a recirculating hybrid CW. The CW at Ibhayi Brewery was already built and operational, any change to the wetland cells would be costly. Building additions onto the cells was a cheaper and easier way to potentially improve nutrient removal. Twenty smaller (1.0 $m^{2)}$ vertical flow CWs were built alongside the wetland cell. These were units attached to the wetland cell with piping and a pump. This addition created a hybrid CW. The effluent would pass through the vertical additions and the pre-existing horizontal flow wetland cell.

The design of the vertical flow CW was based on tidal CWs which flood with water and then drain completely before filling again. The cycle causes air to be passively drawn into the system due to the pressure gradient caused by the draining of the CW (Stottmeister *et al*. 2003, Stefanakis and Tsihrintzis 2012, Wu *et al*. 2014). These CWs are known for their effective removal of organics, suspended solids and enhanced nitrification (Vymazal 2010). A hybrid system such as this, utilising multiple small CWs recirculating into a larger CW has not been studied in South Africa.

2.2 Aims and objectives

This study aimed to describe effluent treatment in an ebb and flow filter system and in a system that used nanobubble pumps to aerate and treat brewery effluent in a horizontal subsurface flow CW and to determine if further research into the use of ebb and flow filter should be considered in optimising brewery effluent treatment in constructed wetlands.

The objectives were to:

2.1 Describe the change in water quality parameters in constructed wetlands treating effluent using the following aeration systems:

  2.1.1 ebb and flow filters;

  2.1.2 nanobubble pumps; and

2.2 Describe the water treatment efficiency of the wetlands using the different aeration systems.

2.3 Materials and methods

2.3.1 Effluent source and pre-treatment

The source of the effluent in the CW was wort boiling, an essential part of the brewing process, and cleaning-in-place and bottle washing that occurred at Ibhayi Brewery (Cilliers 2014, Taylor 2019). The effluent passed through a 0.5 mm drum filter, an equalisation basin, a pre-clarifier, and an anaerobic digester (de Jong 2019). After the anaerobic digester, the effluent entered a primary-facultative pond (PFP). From the PFP the effluent followed two paths into the CW; cell path A and cell path B (Figure 2.1). Each path flowed through two cells (1 & 2) with nanobubble pumps (NB) and a final unaerated cell before the recycled effluent was returned to the brewery to be used for cleaning.

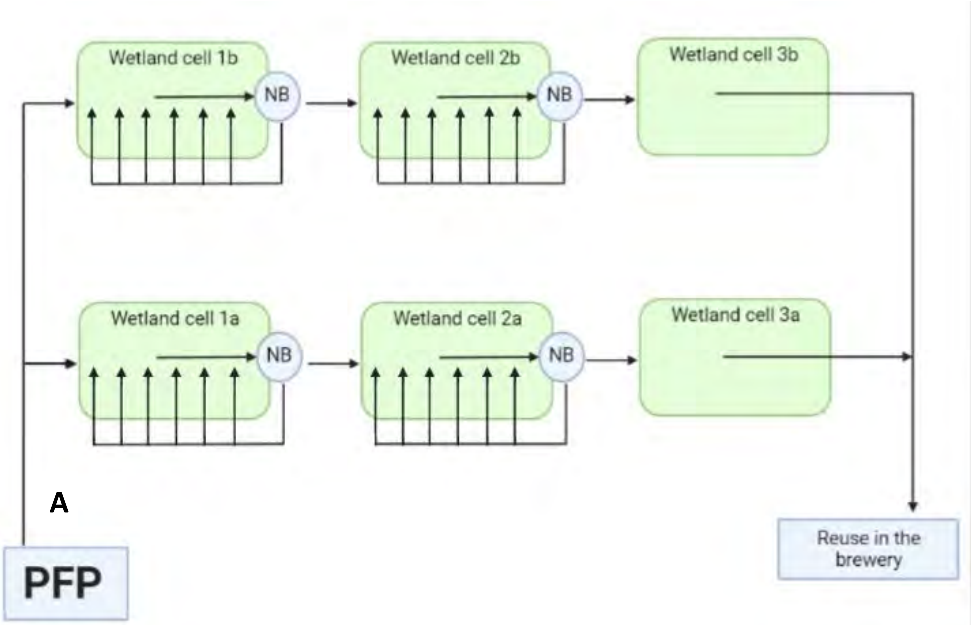

Figure 2.1 The path of wastewater in constructed wetland (CW) at Ibhayi Brewery. The effluent exits the primary facultative pond (PFP) at point A and enters the CW. Effluent follows two cell paths, each with two cells aerated by nanobubble bubbles and a third unaerated cell before flowing back to the brewery.

2.3.2 Experimental systems

The CW was made up of six wetland cells, each 18 m x 24 m (Figure 2.2). Four of the cells were equipped with nanobubble pumps (MK1 Nanobubbler, Fine Bubble Technologies, South Africa) and planted with Arum lilies (*Zantedeschia aethiopica*) before the start of the experiment (Figure 2.1 and Figure 2.2). All plants were removed from the CW before the trial began. Only the first wetland cell in each line was used in the study, Cells 1a and 1b (Figures 2.1 and 2.3). Both lines received brewery effluent from the same source (Section 2.2.1) but varied in aeration strategies: Treatment 1 was aerated using nanobubble air blowers at the base of the wetland, and this cell was not altered in any way from the original design (Figure 2.3). It received effluent from the pre-treatment which flowed through the cell until it reached the nanobubble pump where it was infused with air and pumped into the base of the wetland at six points (Figure 2.3). Treatment 2 included an alteration where the nanobubble pump was replaced with a normal pump (Dophin P-3000, Code CPD0862, KW Aquatic Supplies Co., Ltd. Malaysia). Twenty tanks were placed alongside Cell 2a (Figure 2.3). These tanks were filled with 19 mm gravel and fitted with a bell siphon protected by a gravel guard (Figure 2.4 and Figure 2.5). The effluent filtered through Cell 1b until it reached the pump housed at the end of the cell, where it is then pumped to the ebb and flow filters. Each ebb and flow filter drained back into Cell 1b (Figure 2.1).

Figure 2.2 Constructed wetland Cell 1a prior to the start of this trial.

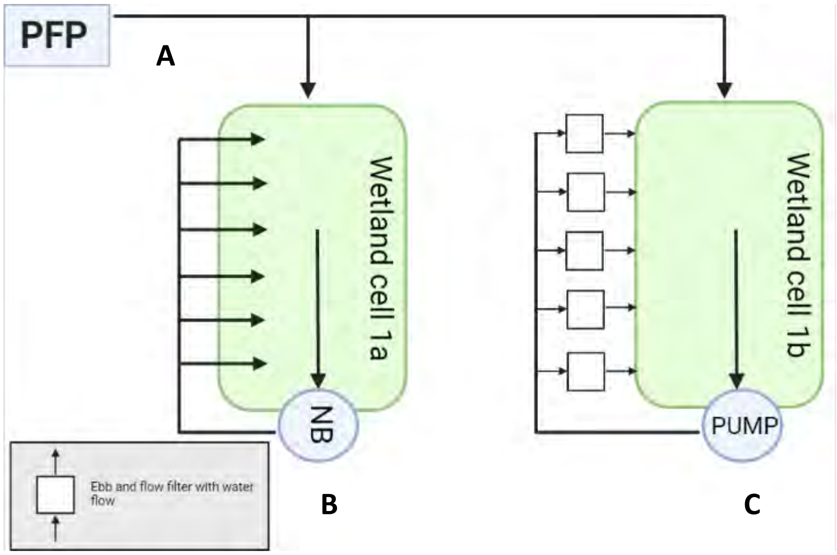

Figure 2.3 A closer view of wetland cells 1a and 1b which were used in the experiment. Effluent flowed from the post-facultative pond (PFP; point A) to Cell 1a and Cell 1b. Effluent in Cell 1a was aerated with a nanobubble pump (NB) and pumped back into Cell 1a. Effluent in Cell 1b was pumped to 20 ebb and flow filters (not all of which are pictured in the schematic) which drained back into Cell 1b. Effluent exited the cells at points B and C and flowed to the next cell in the path.

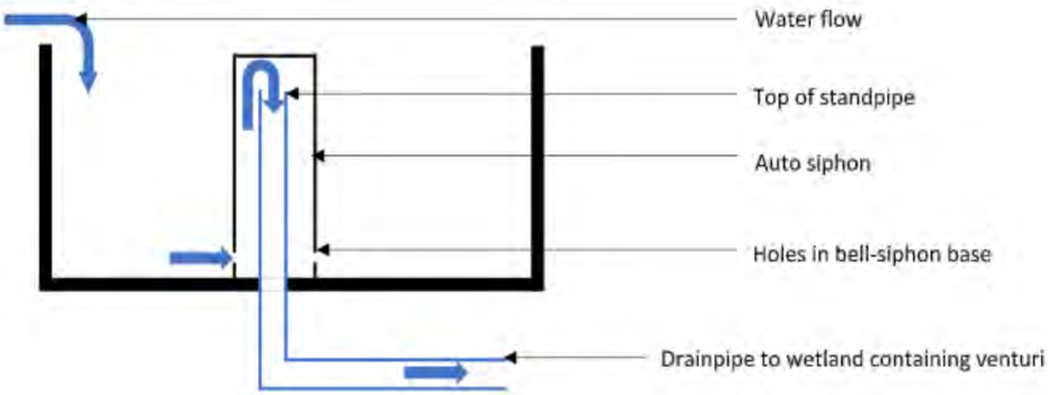

Figure 2.4 Water flow into and out of an ebb and flow filter. Effluent fills the filter and the auto-siphon and begins flowing down the standpipe a vacuum inside the auto siphon is created which causes the effluent to drain out the filter, once the water level falls below the holes in the bell-siphon base the vacuum breaks. The siphon was made from a 110 mm polyvinyl chloride (PVC) pipe and an end cap fitted together and placed over a 55 mm PVC standpipe. The siphon was separated from the gravel by a 200 mm PVC pipe with holes cut into its length 30 mm apart, not pictured here.

Figure 2.5 The top of an ebb and flow filter, with a view of the other filters connected next to it.

2.3.3 Data collection

Dissolved oxygen (DO; mg/L) and temperature (°C) were measured with a DO and temperature meter (HANNA HI98193, Hanna Instruments, USA). Turbidity (nephelometric turbidity units; NTU) was measured using a turbidimeter (2100Q Portable Turbidimeter, product no. 2100Q01, Hach Company, SA). Electrical conductivity (EC; $\mu S/cm^2$) and total dissolved solids (TDS; mg/L) were measured using a conductivity meter (µS/TDS meter Model C8, HJM Electronics, SA). The pH was measured using a pH meter (sensION™ + PH 3, Hach-Lange, DEU). The pH values were converted to H^+ concentrations to calculate the mean using Equation 2.1:

$$H^+ = 10^{-x} \qquad \text{Equation 2.1}$$

where X is the recorded value (Brescia 1966). The mean was then logged for graphical representation. Standard error was calculated from the recorded logged values (Boutilier and Shelton 1980).

Water samples were filtered through 8.0 µm filter paper before analysis in the spectrophotometer (Hach DR 2800 spectrophotometer, product number DR2800-01B1, Hach (Pty) Ltd, USA) to remove turbidity which may influence colourimetric analyses (Hach 2013). The following test kits were used:

- High-range ammonia test (NH_3-N; mg/L; product no. 2606945, Hach (Pty) Ltd, USA);
- Nitrite test (NO_2^-; mg/L; product no. 2107569, Hach (Pty) Ltd, USA);
- Nitrate test (NO_3-N; mg/L; product no. 2106169, Hach (Pty) Ltd, USA);
- Phosphate test (PO_4^{3-}; mg/L; product no. 2244100, Hach (Pty) Ltd, USA);
- Chloride test (Cl^-; mg/L; product no. 2319800, Hach (Pty) Ltd, USA); and
- High-range chemical oxygen demand cell test (COD; mg/L; product no. 2125915, Hach (Pty) Ltd, USA).

The NO_2^- values were all converted to NO_2-N using Equation 2.2:

$$NO_2 - N = {NO_2}^- * 0.3045 \qquad \text{Equation 2.2}$$

The equation comes from the percentage of N in NO_2^-; 30.45 %, using the atomic mass of N and O_2 which are 14.006 g and 15.999 g, respectively. As NO_2-N is a measure of the N in the nitrite molecule the value of the nitrogen is calculated for each sample.

Samples were taken twice a week from the effluent entering the wetland cells (point A; Figure 2.3) and at the outflow of each cell (points B and C; Figure 2.3).

2.3.4 Statistical analysis

The study had limited statistical options as it was not possible to replicate the wetland cells, so the analyses presented here were largely descriptive. Regression analysis was used to investigate changes over time in the parameters. Water quality for each parameter was presented as a change, which was calculated using:

$$Outgoing\ quality - Incoming\ quality \qquad \text{Equation 2.3}$$

The mean, minimum, and maximum values were presented for the two treatments.

2.4 Results

The change in DO varied over time in both treatments; with larger differences found in the nanobubble treatment compared with the ebb and flow treatment. Overall, there was a decrease in DO for both treatments (Figure 2.6 and Table 2.1). There was no relationship between DO and time for either the nanobubble treatment (Multiple linear regression, p = 0.139) or the ebb and flow treatment (Multiple linear regression, p = 0.514).

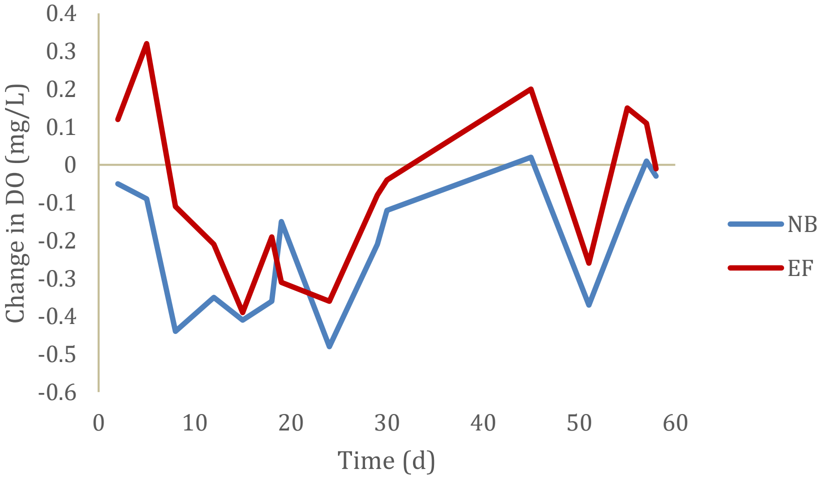

Figure 2.6 The change in dissolved oxygen (DO mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

The nanobubble treatment consistently had increases in NH_3-N whereas the ebb and flow treatment decreased the concentration throughout the trial (Figure 2.7). This is confirmed when looking at the mean incoming and outgoing data where ebb and flow decreased by an average of 31.3 ± 6.5 mg/L and the nanobubble had an average increase of 18.9 ± 5.1 mg/L (Table 2.1). There was no relationship between NH_3-N change and time for the nanobubble treatment (Multiple linear regression, $p = 0.776$), or the ebb and flow treatment (Multiple linear regression, $p = 0.065$).

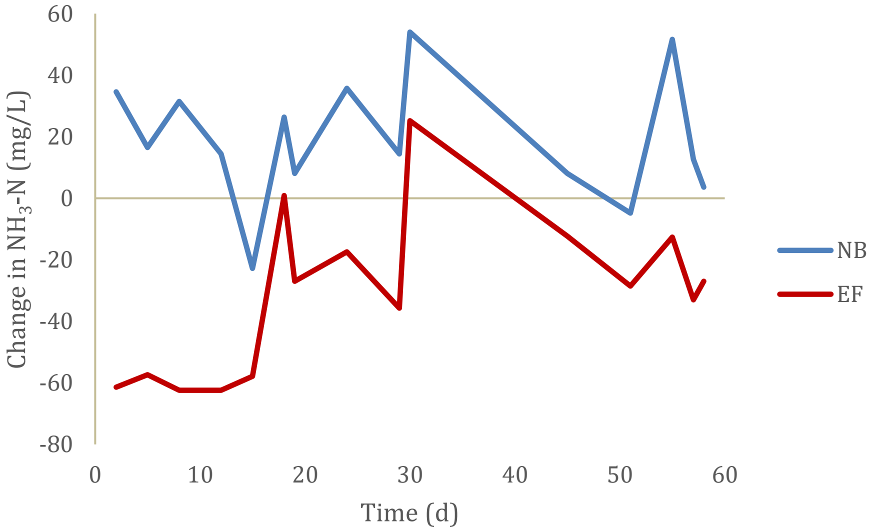

Figure 2.7 The change in ammonia-nitrogen (NH_3-N mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

The NO_2-N for both treatments decreased or showed no difference at the beginning of the trial. This continued in the nanobubble treatment, but the ebb and flow treatment had increases in NO_2-N after day 24 (Figure 2.8). A lower outgoing mean for nanobubble than ebb and flow was shown by the descriptive statistics for this data set (Table 2.1). There was no change from the incoming effluent to the outgoing ebb and flow treatment mean (Table 2.1). There was no relationship between NO_2-N and time for the nanobubble treatment (Multiple linear regression, p = 0.171), or the ebb and flow treatment (Multiple linear regression, p = 0.219).

The change in NO_3-N appeared to be similar in the treatments (Figure 2.9). The means decreased from the incoming quality by 7.2 ± 0.8 mg/L in EF and 6.1 ± 0.9 mg/L in the nanobubble treatment (Table 2.1). There was no relationship between NO_3-N and time for either the nanobubble treatment (Multiple linear regression, p = 0.056) or the ebb and flow treatment (Multiple linear regression, p = 0.296).

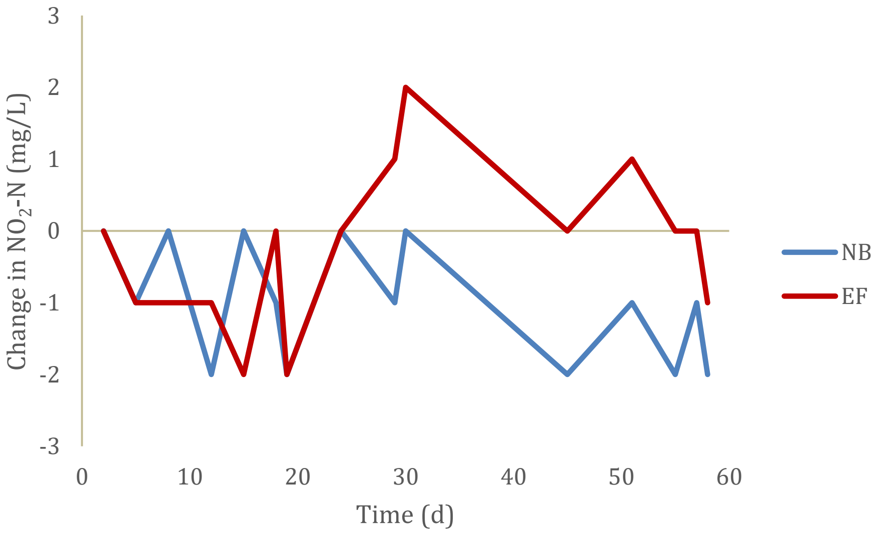

Figure 2.8 The change in nitrite-nitrogen (NO_2-N mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

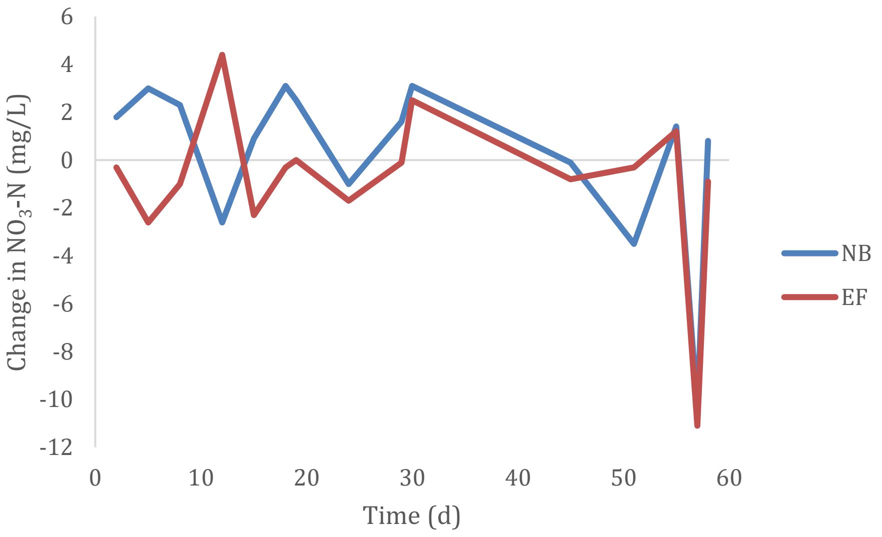

Figure 2.9 The change in nitrate-nitrogen (NO_3-N mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

The nitrogen (NH_3-N + NO_2-N + NO_3-N) exiting the wetland cells showed that the nitrogen concentration decreased in the ebb and flow treatment, whereas it almost always increased in the nanobubble treatment nitrogen (Figure 2.10).

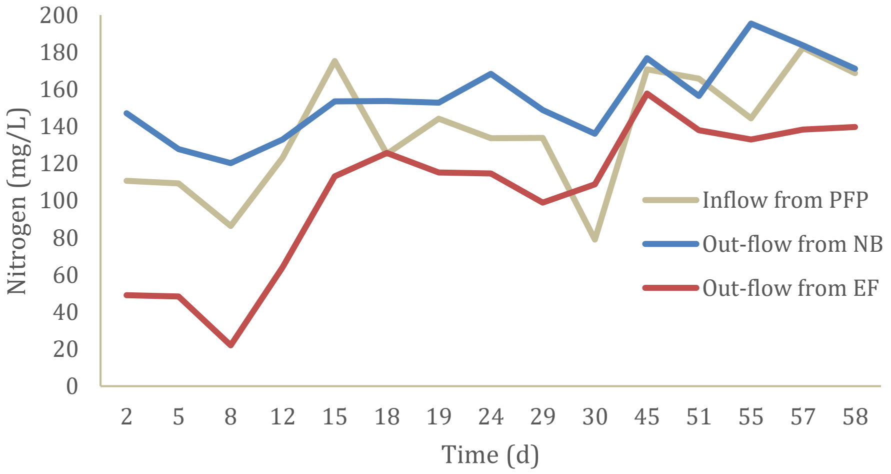

Figure 2.10 The incoming (Incoming from PFP) nitrogen forms (ammonia-nitrogen + nitrite-nitrogen + nitrate-nitrogen) was the effluent source for both the nanobubble (Out-flow from NB) and ebb and flow (Out-flow from EF) effluent treatment systems. The figure also includes the outgoing nitrogen for both the NB and EF systems.

Table 2.1 The mean (± standard error), minimum and maximum values for all water quality parameters for the incoming effluent and the outgoing of each treatment.

	Primary facultative pond			Ebb and flow treatment			Nanobubble treatment		
Parameter	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Dissolved oxygen (mg/L)	0.60 ± 0.05	0.32	0.84	0.52 ± 0.04	0.30	0.87	0.39 ± 0.02	0.30	0.68
Ammonia-nitrogen (mg/L)	131.6 ± 7.5	78.6	172.2	100.3 ± 9.9	19.8	154.2	150.5 ± 5.3	113.7	189.6
Nitrite-nitrogen (mg/L)	2.0 ± 0.0	0.0	4.0	2.0 ± 0.0	-1.0	3.0	1.0 ± 0.0	0.0	2.0
Nitrate-nitrogen (mg/L)	9.70 ± 1.90	0.40	15.70	2.50 ± 0.50	-0.20	7.00	3.60 ± 0.40	0.00	6.50
Chemical oxygen demand (mg/L)	253 ± 36	74	612	110 ± 37	12	634	137 ± 47	35	689
Orthophosphate (mg/L)	16.38 ± 0.87	10.71	24.19	15.34 ± 0.63	12.02	22.25	15.08 ± 0.53	12.44	20.20
Chloride (mg/L)	40.0 ± 6.0	6.0	91.0	33.0 ± 5.0	3.0	66.0	36.0 ± 7.0	6.0	93.0
pH	7.66 ± 0.07	7.18	8.05	7.64 ± 0.05	7.47	8.15	7.91 ± 0.04	7.37	7.84
Total dissolved solids (NTU)	2026 ± 67	218	2590	2040 ± 62	1696	2340	2093 ± 56	1846	2319
Electrical conductivity (µS)	3011 ± 89	2577	3754	3014 ± 79	2459	3455	3108 ± 78	2676	3673
Turbidity (mg/L)	45.17 ± 2.82	26.80	62.40	13.61 ± 2.55	2.20	44.60	19.98 ± 1.90	22.60	27.10
Temperature (^{o}C)	25.39 ± 0.48	23.20	29.00	24.08 ± 0.33	21.40	26.10	24.59 ± 0.26	22.60	27.10

The pH showed a decreasing trend over time in both the nanobubble treatment (Multiple regression; $y = -0.0001x^2 + 0.0176x - 0.377$, $R^2 = 0.61$, $p = 0.001$) and the ebb and flow treatment (Multiple linear regression, $y = 0.0041x + 0.10$, $R^2 = -0.59$, $p = 0.034$). In the nanobubble treatment, the pH decreased at the beginning of the trial and increased towards the end of the trial (Figure 2.11). The ebb and flow treatment showed decreases for the entire trial (Figure 2.11). The decreases in pH were larger further along in the trial.

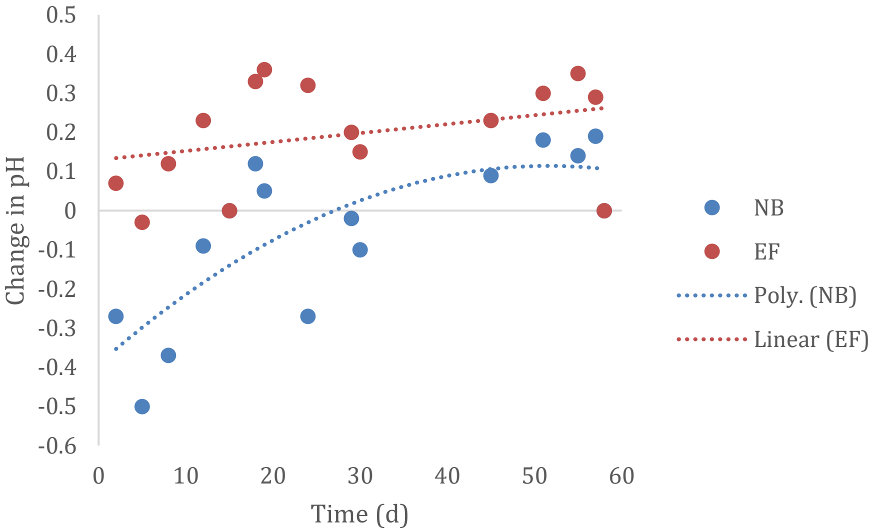

Figure 2.1**1** The change in pH for the ebb and flow treatment (EF) and the nanobubble treatment (NB). There was a decreasing trend over time in both the nanobubble treatment (Multiple regression, $y = -0.0001x^2 + 0.0176x - 0.377$, $R^2 = 0.61$, $p = 0.001$) and the ebb and flow treatment (Multiple linear regression relationship, $y = 0.0041x + 0.10$, $R^2 = -0.59$, $p = 0.034$).

The mean COD of the nanobubble treatment was higher than the ebb and flow treatment (Table 2.1). However, the treatments had similar changes in COD in Figure 2.12. Both treatments decreased the COD.

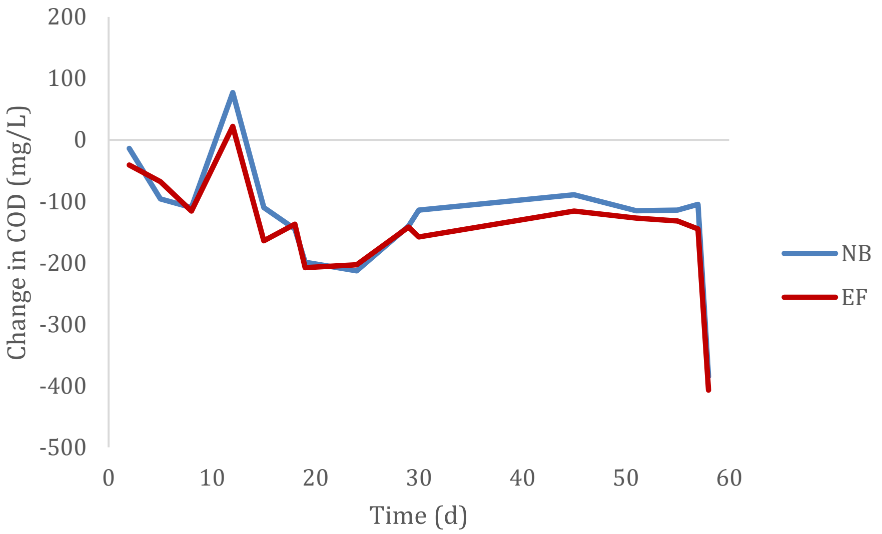

Figure 2.12 The change in chemical oxygen demand (COD mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

Both treatments followed almost identical changes in PO_4^{3-} (Figure 2.13). There was an initial increase in PO_4^{3-} for the first 25 days, then a decrease followed by a sharp increase on day 55, very close to the end of testing (Figure 2.13). The means of the inflow from the PFP, out-flow of EF and out-flow of NB are all similar and indicate no overall change or a minor decrease in PO_4^{3-} (Table 2.1).

The nanobubble and ebb and flow treatments showed similar changes in chloride over time (Figure 2.14). The means of the treatments and the change over time indicated a decrease in Cl^- however, the variation in the data made a clear increase or decrease that was difficult to interpret (Table 2.1). The treatments showed similar trends for TDS and EC except for an increase at day 45 for the EF treatment. Both the change over time and means analysis showed very little change in TDS or EC for either treatment (Figure 2.15, Figure 2.16, and Table 2.1).

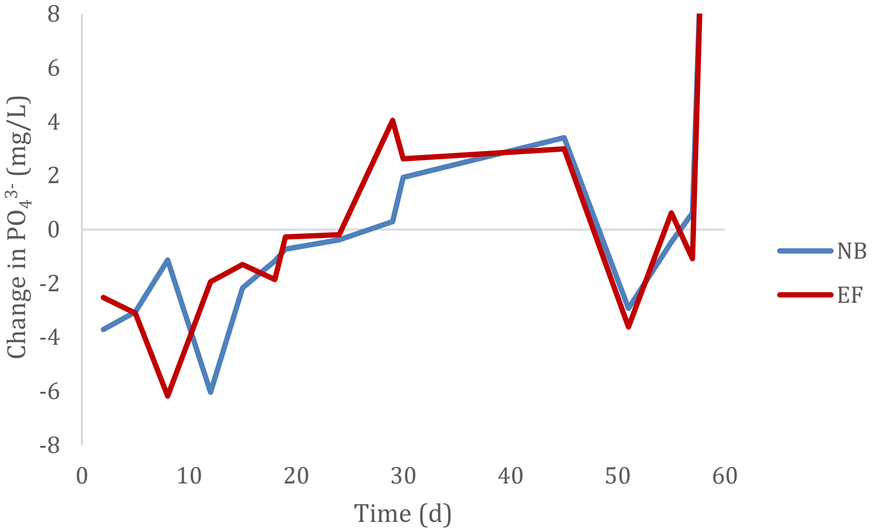

Figure 2.13 The change in orthophosphate (PO_4^{3-}; mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

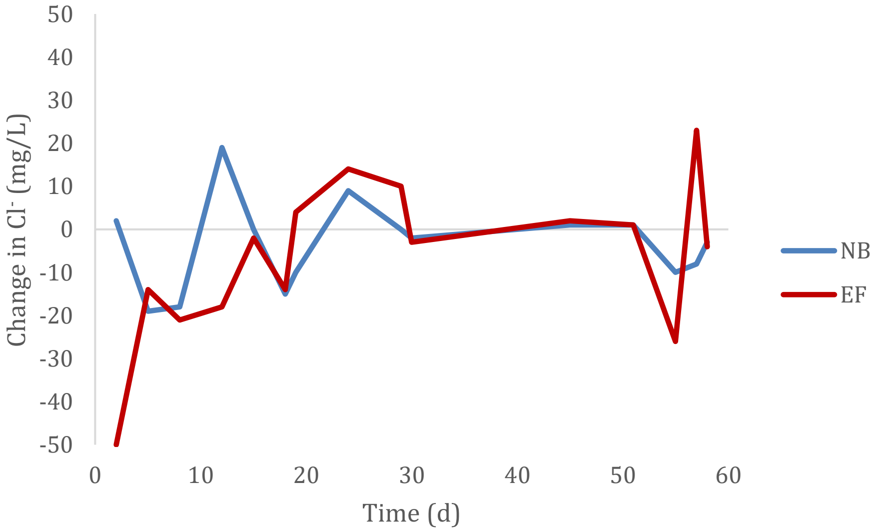

Figure 2.14 The change in chloride (Cl^- mg/L) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

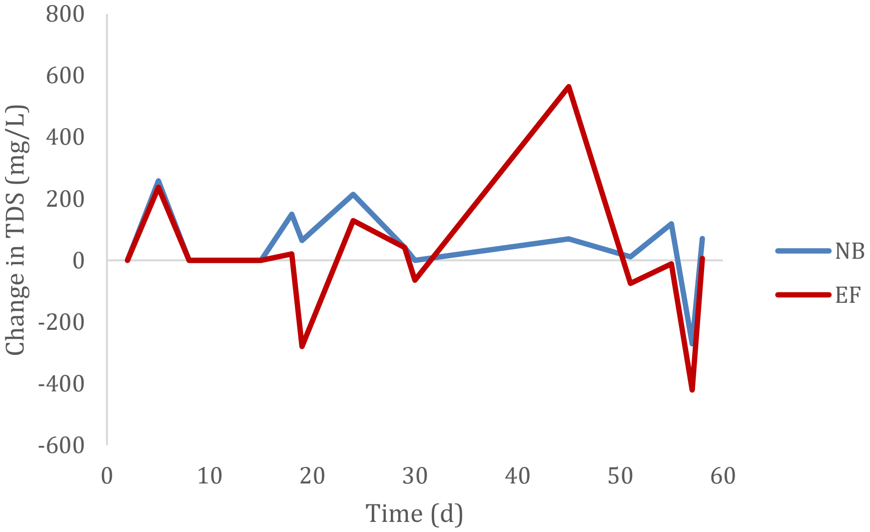

Figure 2.15 The change in total dissolved solids (TDS; mg/L) between the inflow and outflow of the ebb and flow treatment (EF) and the nanobubble treatment (NB).

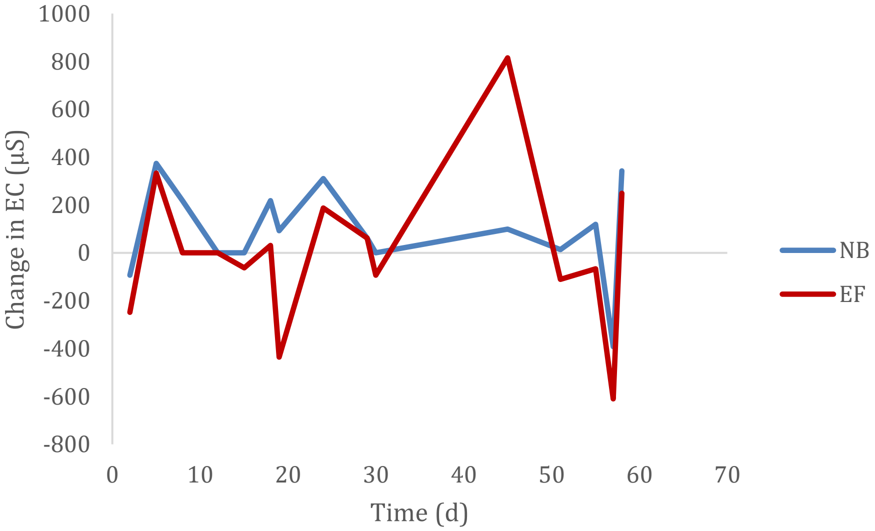

Figure 2.16 The change in electrical conductivity (EC; µS) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

Although the change in turbidity varied over time, both treatments decreased turbidity for the duration of the trial (Figure 2.17). The EB treatment had a mean decrease of 31.56 ± 2.48 (nephelometric turbidity units; NTU), from 45.17 ± 2.82 NTU to 13.61 ± 2.55 NTU. The nanobubble treatment had an average decrease of 25.19 ± 2.71 NTU (Table 2.1).

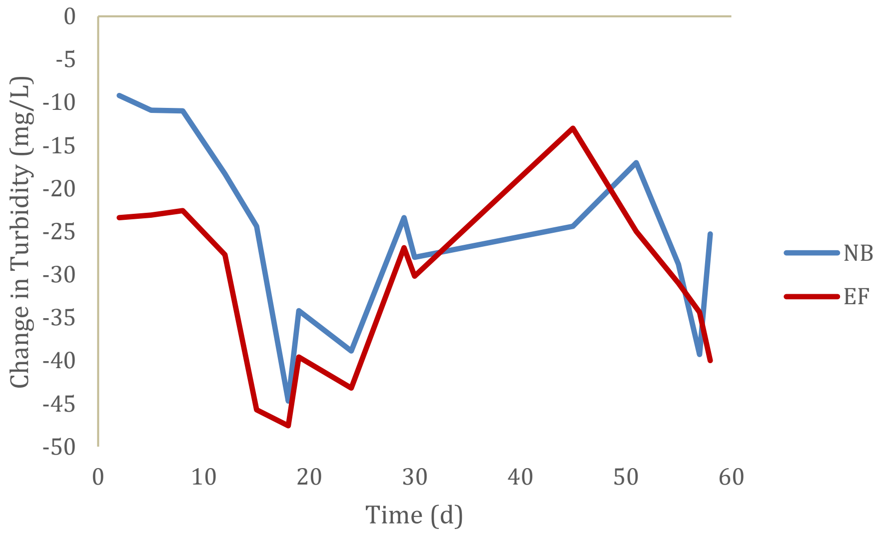

Figure 2.17 The change in turbidity (Nephelometric turbidity units; NTU) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

The temperature decreased in both treatments (Figure 2.18). The mean outgoing for the ebb and flow treatment decreased from the incoming by 1.31 ± 0.47 °C. The nanobubble treatment decreased by 0.80 ± 0.39 °C (Table 2.1).

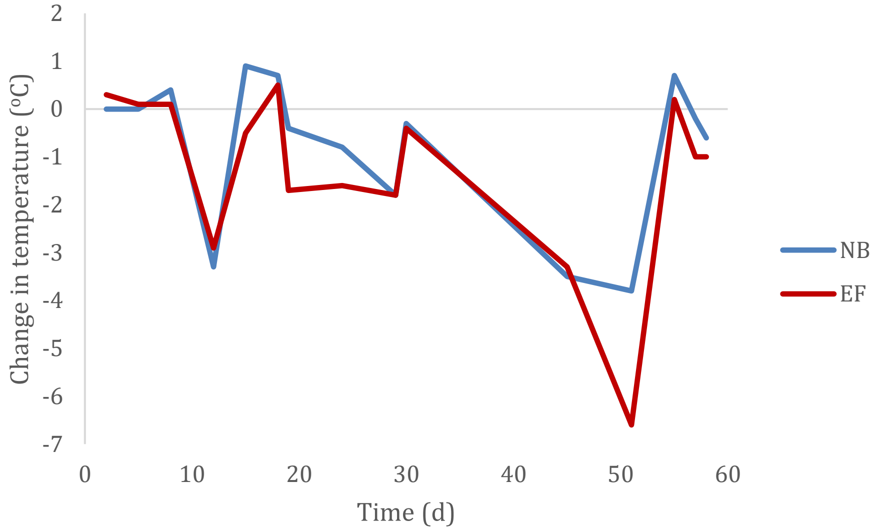

Figure 2.18 The change in temperature (°C) between the inflow and outflow for the ebb and flow treatment (EF) and the nanobubble treatment (NB).

2.5 Discussion

While both units were expected to add DO to the effluent this was not seen in either the EF or NB aeration. Decreases in DO have occurred in full-scale CW studies (Stottmeister *et al.* 2003, Serrano *et al.* 2011, Xiao and Xu 2020). A reduction of DO can be explained by its consumption during nitrification (Kadlec and Knight 1986). For nitrification of NH_3-N and NO_2-N to have occurred in the wetland cells, DO was required (Kadlec and Knight 1986). Nitrification may have consumed any DO that was added by the aerators. Measuring DO at only the inlet and the final effluent cannot fully describe any DO fluctuations during treatment (Vymazal and Kröpfelová 2008). A previous study at Ibhayi Brewery used smaller ebb and flow filters (2 x 0.8 x 0.4 m) to test nutrient reduction (de Jong 2019). The ebb and flow CWs were able to reduce the ammonia in the system from 35.0 mg/L to below 3.0 mg/L in three days (de Jong 2019). The study found an inverse relationship between DO and ammonia concentration (de Jong 2019). The results came from testing the effluent at the exit point of the filter rather than the exit of the cell which may be why significant increases in DO correlating to decreases in ammonia were found by de Jong 2019. Although this study cannot accurately describe the DO within the wetland cells, the results from other studies provide evidence that the ebb and flow filters can provide oxygen to the effluent (de Jong 2019).

Chemical oxygen demand decreased in both systems. Decreases in COD between 29 % and 70 % have been found in hybrid vertical and horizontal flow systems like the ebb and flow treatment in this study (Serrano *et al*. 2011). Nanobubble pumps can decrease the COD by 39 mg/L per day when added to treatment ponds filtering winery wastewater (Kalogerakis *et al*. 2021). Both systems can decrease the oxygen demand of the system.

In the nanobubble treatment, NH_3-N increased which was different from any studies on similar systems which showed decreases in NH_3-N of 55.4 % or pilot-scale systems which had > 62.0 % removal of ammonia (Albuquerque *et al*. 2009, Serrano *et al.* 2011). Which could have been due to the reduction of NO_2-N to NH_3-N (Vymazal 2007). However, the overall nitrogen in the effluent that left the wetland cell was higher than the incoming nitrogen. This indicates a possible outside source of nitrogen. The wetland cell that was used for the nanobubble treatment was located next to the PFP. These ponds were surrounded by plants and were an attraction to wildlife. The wildlife included the Egyptian goose (*Alopochen aegyptiaca*) which could easily access the wetland cell and is known to increase available nitrogen in water and soils through their faecal matter (Cochran *et al.* 2000). The faecal matter of geese can have a nitrogen content of 6.3 % (Cochran *et al.* 2000, Zacheis *et al.* 2002, Ayers 2010). Migratory geese in Michigan were responsible for 27 % of the nitrogen that entered Wintergreen Lake from external sources

(Manny *et al.* 1994). The Canada goose (*Branta canadensis*) increased the N content in wetland systems by 40 % (Kitchell *et al.* 1999). This is a possible source of nitrogen that should be investigated in future work.

In the ebb and flow treatment NH_3-N concentration decreased. There was no NO_2-N change and NO_3-N decreased. Ammonia-N removal from effluent begins with nitrification which is a two-step process beginning with an aerobic conversion of NH_3-N to NO_2-N (Bernhard 2010, Saeed and Sun 2012). Nitrite-N is then oxidised to NO_3-N by nitrite-oxidising bacteria (Daims *et al*. 2016). Nitrate-N undergoes denitrification, an anaerobic conversion to nitrogen gas (Saeed and Sun 2012). Nitrate-nitrogen can also be reduced to NH_3-N but this occurs only in anoxic environments (Vymazal 2007). Nitrification needs aerobic conditions and an increase in DO has been shown to decrease NH_3-N (Vymazal 2007, Nivala *et al.* 2013, Li *et al.* 2014). The nitrification process uses oxygen from effluent (Saeed and Sun 2012). The decrease in NH_3-N in the ebb and flow treatment could be attributed to an increase in DO in the wetland cell that is not seen due to nitrification using any DO that was added by the ebb and flow filters. However, the results in this study cannot appropriately corroborate that.

No difference was found in the NO_2-N concentration in the ebb and flow treatment. However, NO_2-N is formed by the oxidation of NH_3-N in the first step of nitrification (Bernhard 2010). This NO_2-N is not quantified in the incoming effluent as it is a product of a reaction within the system (Dong and Sun 2007). The ebb and flow treatment would contain higher levels of NO_2-N than originally measured due to the oxidation of NH_3-N. An average removal of 11.88 $g/m^3/d$ of NH_3-N coupled with an increase of 0.88 $g/m^3/d$ of NO_2-N was measured by Dong and Sun 2007. Nitrification was found to increase NO_2-N from 0.1 mg/L to 0.5 mg/L by Murphy *et al.* 2016.

Although the levels of NO_2-N measured in this study indicated no change between the incoming and outgoing effluent, the treatment did decrease the overall NO_2-N concentration that was formed during nitrification.

The nanobubble treatment showed a decrease in NO_2-N. There was, however, no oxidation of NH_3-N in this treatment. There was no increase of NO_2-N within the system that was described in the ebb and flow treatment. Although the initial oxidation of NH_3-N was affected the oxidation of NO_2-N was still able to take place. The nitrite-oxidising bacteria responsible for this process can live in pockets of oxygen available in the system (Daims *et al.* 2016). Although horizontal subsurface flow wetlands have poor nitrification, it does still occur (Kadlec and Wallace 2008). In a system with 16 mg/L NH_4-N, Larsen and Greenway 2004 found almost 100% removal of NO_2-N. The nanobubble treatment even with high loads of NH_3-N can still process NO_2-N.

Nitrate-nitrogen was reduced in both treatments. Horizontal subsurface wetlands are known to support anaerobic bacteria even when oxygenated (Chen *et al.* 2022). Aerated and non-aerated horizontal subsurface wetlands have shown decreases in NO_3-N of 1.2 mg/d (Ouellet-Plamondon *et al.* 2006). The wetland cells in this study can reduce the NO_3-N concentration with the incorporation of nanobubble pumps or ebb and flow filters. A change occurred on day 57 in both treatments that may have been influenced by the incoming effluent from the brewery.

Temperature decreased in both treatments. This can affect nitrifying bacteria as they perform better at higher temperatures; approximately 25 – 35 ^{o}C (Kadlec and Wallace 2008, Cho *et al.* 2014). This could indicate a higher capacity for nitrification in both systems with temperature adjustment.

No change was observed in the PO_4^{3-} concentration in either treatment. Phosphorus removal in CWs is poor without the use of media with high adsorption and precipitation capacity (Brix *et al.* 2001, Vymazal 2010). Media such as calcite, crushed marble and oyster shells have high P-binding capacity (Brix *et al.* 2001, Wang *et al.* 2013). Phosphate adsorption to gravel is low which accounts for minimal change in concentration of orthophosphate in this trial (Brix *et al.* 2001, Vymazal 2010, Wang *et al*. 2013).

No differences were found between the incoming and outgoing TDS and EC in either treatment. The TDS in wastewater directly affects the EC levels (Walton 1989). Electrical conductivity and TDS showed little change between incoming and outgoing effluent and between treatments. Total dissolved solids are not reliably lowered by horizontal subsurface flow CWs with removal rates below 4 % (Coleman *et al.* 2001, Baskar *et al.* 2009, Rahi and Faisel 2019). Increases in TDS have been found in planted systems (Coleman *et al.* 2001). A small particle size of 1.2 µm and a ten-day retention time led to 65 % removal in a study by Mora-Orozco *et al.* 2018. When the retention time decreased to five days the TDS removal rate was 15 % (Mora-Orozco *et al.* 2018). In this trial, TDS and EC were not expected to change as two previous studies at Ibhayi Brewery with horizontal subsurface flow CWs saw no change in EC or a 6.5 % decrease attributed to plant uptake (de Jong 2019, Mabasa 2021). As EC and TDS directly impact each other, the lack of change in EC in previous studies on-site and the variable removal rates of TDS in literature explain the results found in this study.

The mean pH was similar between the incoming and outgoing effluent. The pH in both treatments had a negative relationship to time; the pH showed larger decreases as the trial went on. Many factors can influence the pH; carbon dioxide, nitrification, and oxidation of organic matter

(Luklema 1969). Nitrification and denitrification can affect pH levels as the transformations release hydrogen ions (H^+) which decrease pH and hydroxyl ions (OH^-) which increase pH (Kadlec and Knight 1986, Brescia 2012). Nitrification and pH shared an inverse relationship when pH decreased when nitrification took place (Yalcuk and Ugurlu 2009). Effluent also became more acidic during treatment in a CW in a study by Yazdani and Golestani 2019. The pH in this trial stayed within the optimal range (7 – 9) for nitrification and denitrification (Yazdani and Golestani 2019). However, during a longer trial, the pH may drop below the optimal range if the trend in these results continues.

The Cl^- concentration showed no change in either treatment. Chloride is difficult to treat in horizontal subsurface CW and must be specifically targeted with additional removal methods such as reverse osmosis or chemical precipitation (Srivastava *et al*. 2020, Taha *et al.* 2020, Zhao *et al.* 2021, Li *et al.* 2022). The previous studies on site found no change in Cl^- in the horizontal subsurface CW and a decrease in Cl^- in the ebb and flow filters which was attributed to plant uptake (de Jong 2019, Mabasa 2021). Based on these studies the Cl^- concentration was not expected to be affected by either wetland cell.

Turbidity decreased in both the treatments; ebb and flow and nanobubble. The rate that turbidity is lowered is influenced by two factors; substrate type and retention time (Lu *et al*. 2016, Mtavangu *et al*. 2017). The substrate in this trial; gravel, does not usually have enough surface area to affect the turbidity (Lu *et al*. 2016). However, the retention time in the wetland cells is high enough to decrease the turbidity (Lu *et al*. 2016).

Due to the nature of being on a commercial site, this study was constrained by the finances and time requirements of the brewery. This only allowed for one wetland cell to be converted to the new system and for a limited period to collect data.

2.6 Conclusion

There appeared to be differences in dissolved oxygen between the ebb and flow and nanobubble systems; however, this needed to be verified in future work. Furthermore, and possibly linked to the differences in dissolved oxygen, the ebb and flow filters appear to decrease NH_3-N, NO_3-N, COD, and turbidity whereas the same decreases were not always as apparent when the nanobubble system was described. Based on the experimental design and information provided here, it was not possible to conclude that ebb and flow filters were more efficient at nutrient removal than nanobubble pumps. However, this work successfully described changes in the effluent quality of the two systems, and these preliminary findings suggest that there might be differences in effluent treatment efficiency between them. Since this needs to be verified in controlled, replicated studies, it was concluded that further research work was warranted into the use of ebb and flow filters to optimise brewery effluent treatment in horizontal subsurface flow constructed wetlands.

Chapter 3: The effect of plants and worms on the treatment of brewery effluent in ebb and flow filters

3.1 Introduction

The results obtained in Chapter 2 lead to further investigation into the ebb and flow filters as potential components that might contribute to effluent treatment in constructed wetlands (CW). The twenty ebb and flow filters in Chapter 2 increased nutrient removal in the wetland cell but did not reduce the water quality parameters to reuse or discard standards (Table 1.1, DWAF 1996, DWAF 2004). If nutrient removal in each ebb and flow filter could be optimised, and the filters remained linked to the CW, then the CW may be able to filter the water to the required limits.

Many techniques are used to increase nutrient removal in CWs; adding plants and earthworms are two methods that have successfully been employed (Xu *et al*. 2013, Wang *et al*. 2017). Plants in a wetland can increase the level of dissolved oxygen (DO) in the effluent because oxygen diffuses from the plant roots into the outside environment (Rehman *et al*. 2016). They use nutrients themselves and their root structure creates a greater surface area for the bacteria involved in nutrient removal from wastewater such as *Nitrosomonas* (Button *et al*. 2015, Wang *et al*. 2017).

Worms have been used in CWs to increase nitrification and denitrification potentials. They have been linked to increased total nitrogen removal and larger bacterial populations (Singh *et al*. 2021, Xu *et al*. 2013). Earthworms increase the mineralisation of organic-N to its inorganic forms during feeding (Xu *et al.* 2013). These forms of N are vital to plant growth and adding worms to CWs has been shown to increase plant height by 61 % with increased levels of nitrogen found in the stems and leaves of the plants (Xu *et al.* 2013). Worms can influence plant growth through their effects on the nutrients available this in turn increases the amount of nutrients plants can remove from the system and as growth increases due to the available nutrients more nutrition is needed to maintain the plant (Singh *et al*. 2021, Xu *et al*. 2013).

The same ebb and flow filters used in Chapter 2 were utilised here, as a low-cost, moderate land-use way of maximising nutrient removal. Their ability to sustain crops and earthworms was investigated, while still increasing nutrient removal. Swiss chard (*Beta vulgaris)* was chosen for this study since it has been successfully grown in the brewery effluent (Jones *et al*. 2014). It is a crop of economic value and is popular in South Africa which can offset the cost of setting up and running this system and possibly turn a profit or provide food for workers. The plant has a large root structure which allows for greater surface area for bacteria, such as *Nitrosococcus and Nitrosospira* bacteria (Bernhard 2010, Saeed and Sun 2012). These plants need lots of water which the wetland can provide and are affected by very few pests or diseases (Gardner's Path 2023). It can be grown year-round and continually harvested and thrives when given extra nitrogen (Matlala 2017). The worms used were red wigglers or earthworms (*Eisenia fetida*), and are the common worm used in South Africa for vermicomposting and these worms have been used in other similar wetland and filtration systems (Atalla *et al* 2021, Lavrnić *et al*. 2019).

While the effects of plants and worms on wetlands and the effect of wetlands on plants and worms have been studied, this has not been done in South Africa and it has not been done using brewery effluent treatment in an ebb and flow filter system. Furthermore, their effect can be highly variable between wetland type, climate, and type of effluent (Naylor *et al*. 2003, Stottmeister *et al*. 2003, Wu *et al*. 2014, Wang *et al*. 2017, Lavrnić *et al*. 2019).

3.2 Aims and objectives

This study aimed to determine if crops and earthworms influenced the functioning of ebb and flow filters, like those in the previous experiment (Chapter 2), and if the filters could sustain crops and earthworms. This was done by comparing the nutrient load entering each filter and the exiting nutrient load from each filter and assessing the DO levels of the incoming and outgoing effluent.

The objectives were to:

3.1 Compare dissolved oxygen levels between ebb-and-flow filters using different treatments:
  3.1.1 earthworms;
  3.1.2 Swiss chard;
  3.1.3 earthworms and Swiss chard; and
  3.1.4 no additions;
3.2 Determine if any of these systems leads to more efficient water treatment by testing water quality parameters;
3.3 Compare the plant health and biomass production between these treatments; and
3.4 Compare the earthworm biomass between the treatments.

3.3 Materials and methods

3.3.1 Experimental species

One hundred and fifty Swiss chard seedlings were purchased from a commercial seedling supplier (Moorland Seedlings (Pty) Ltd, Humansdorp). One hundred and twenty of these were used in the experiment, the rest were dried, weighed to get an average seedling weight, and then used for chemical analysis.

Six kilograms of worms (including casts) were purchased for this study from a commercial supplier (Wizzard Worms CC, Greytown, KwaZulu Natal). Each worm was removed from the casts and placed into a clean box. Six sets of ten worms were weighed to calculate the average weight of a single worm. The worms were then divided into six separate containers. Each container held 250 g of worm mass (i.e., approximately 500 worms), which were subsequently stocked into six of the bioreactors (Section 3.3.3). This gave a worm stocking density of 250 g/m^3. The use of the worms in this experiment was subject to ethical clearance obtained through Rhodes University's animal ethics policy and procedure (2022-2728-6537).

3.3.2 Experimental systems

Twelve ebb-and-flow filters were built alongside a wetland Cell 2b (Section 2.2.1; Chapter 2). Each filter received effluent from this cell and the effluent from the filters returned to the same cell. Four treatments were investigated:

- Treatment 1 included ebb and flow filters that were planted with 20 Swiss chard seedlings (P);

- Treatment 2 included ebb and flow filters into which 250 g of earthworms were added (W);
- Treatment 3 included ebb and flow filters that were planted with 20 Swiss chard plants and into which 250 g of earthworms were added (PW);
- Treatment 4 included ebb and flow filters that contained only gravel (G).

These treatments were randomly assigned to the 12 bioreactors, and each was represented in triplicate, with one ebb-and-flow bioreactor constituting a single replicate.

3.3.3 Data collection

The same tests and methods for water quality parameters as Experiment 1 were used (Section 2.3.3 Chapter 2). The samples were collected and recorded twice weekly at the inflow and outlet of each filter, for 135 days.

Plant harvest and health parameters

Chemical analyses for nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, copper, zinc, manganese, boron, and aluminium were undertaken on 30 seedlings that were set aside from the initial 150 plants purchased but not planted and on the final harvest of Swiss chard after the experiment. Elements such as N, P, and Na were measured due to their abundance in wastewater, however, a larger range of elements was included to adhere to standards set out by the Agricultural Laboratory Association of Southern Africa (buys *et al*. 1996). They were carried out at a commercial laboratory (Western Cape Department of Agriculture: Institute for Plant Sciences, South Africa), using macro- and trace-elements method 6.1.1 for feed and plants as prescribed by the Agricultural Laboratory Association of Southern Africa (ALASA 1998).

Continual harvesting of the outer leaves of each plant was practised in each bioreactor. These leaves were cut from the plant once per week, after which they were weighed on a scale (0.1 g) and dried in an oven to constant weight at 65 °C (Precision balance WTC, RADWAG Balances and scales, Poland; Series 9000, Thermo Fisher Scientific, USA). After drying the leaves were stored in airtight bags and the dry weight was used to calculate the relative growth rate (RGR; g/g/d) using the formula:

$$RGR = \frac{\overline{lnW_2} - \overline{lnW_1}}{t_2 - t_1} \quad \text{Equation 3.1}$$

where W_2 is the dry weight of the harvest at t_2 and W_1 is the dry weight of the harvest at t_1 (Hoffmann and Poorter 2002).

Earthworm mass

Earthworm mass was estimated at the end of the trial from a representative sample taken from each filter containing worms. After the plants were removed from the bioreactor (if applicable and the treatment included plants), a 40cm x 40 cm x 40cm section of gravel was removed from a standardised position on the south-eastern side of the filters; that is, a sample was taken from the furthest point from the inflow pipe of each bioreactor. The removed gravel, containing earthworms, was placed into a labelled bucket and the lid was placed onto it before the next sample was taken. The gravel was then sorted, one bucket at a time, and all worms and worm eggs were placed into sealed containers corresponding to the bucket and bioreactor they were found in. The worms and eggs were then separated. The eggs from each sample were counted. The mass of worms, excluding the eggs, was weighed to the nearest 0.01 g (Analytical balance

R2, RADWAG Balances and scales, Poland). Worm mass was presented as g/m^3 by dividing worm weight harvested from each sample point by the total volume of the sample, which was 0.064 m^3.

3.3.4 Statistical analysis

The incoming water quality was compared between treatments on each sampling day using a repeated measures analysis of variance (RM-ANOVA) to ensure that the incoming quality was the same for each treatment. If variability was found in the incoming data, only the difference between incoming and outgoing was used to assess that parameter.

The difference between incoming and outgoing water quality for each treatment was tested for variability over time using an RM-ANOVA. If no variability was found a factorial analysis of variance (multifactor ANOVA) was used to compare the percentage change of the parameters, calculated using the formula:

$$\% \, change = \frac{(O-I)}{I} * 100 \qquad \text{Equation 3.2}$$

where O represents the outgoing effluent quality in mg/L and I is the incoming effluent quality in mg/L. When significant differences were detected Tukey's HSD post-hoc test was used to further investigate the interactions between the treatments. Interactions between variables were described by correlation analysis.

All data were checked for equality of variance and normal distribution of the residuals using Levene's test and a Shapiro-Wilk plot of the residuals, respectively, when an ANOVA was used (Levene 1960, Shapiro and Wilk 1965). If the assumptions were not met, the data were log or square-root transformed and rechecked for equal variance and normal distribution of residuals.

If the assumptions were still not met for ANOVA, a non-parametric Kruskal Wallis rank test was used to compare the data between treatments in place of a multifactor ANOVA. If the data did not meet the assumptions for RM-ANOVA, Mauchly's sphericity test was used, and the data were corrected using Greenhouse-Geisser and Huynd-Feldt tests.

Each plant health parameter was assessed between the start and end of the experiment using a Kruskal-Wallis rank test. The total dry weight of the plants was compared between the P and PW treatments with a Student's t-test.

The count and weight of worms found at the end of the trial were compared between treatments using chi-square analysis and Mann-Whitney U test, respectively.

All analyses were performed using STATISTICA 14.0.1. Means were considered different at $p < 0.05$.

3.4 Results

3.4.1 Water quality parameters

Dissolved oxygen

The DO concentration of the incoming effluent showed no difference between treatments, with an overall mean of 3.89 ± 0.29 mg/L (RM-ANOVA, $F_{(3,111)} = 116.50$, $p = 0.470$; Table 3.1). The percentage change in DO was not influenced by the presence or absence of plants or worms, with an overall average increase of 64.37 ± 10.02 % across all treatments (multifactor ANOVA, $F_{(1,8)} = 0.52$, $p = 0.491$, Table 3.2).

Table 3.1 The mean (± standard error) incoming water quality for each treatment; planted (P), with worms (W), planted with worms (PW) and gravel only (G). Values in the same row represented by a different alphabetical letter are significantly different (RM-ANOVA, $p < 0.050$).

Parameter (mg/L)	P	W	PW	G	F	P-value
Dissolved oxygen	3.93 ± 0.29	3.80 ± 0.27	3.85 ± 0.30	3.99 ± 0.31	$F_{(3,111)} = 1.61$	0.065
NH_3-N	14.8 ± 1.7	14.5 ± 1.6	14.5 ± 1.6	14.6 ± 1.6	$F_{(3,111)} = 0.91$	0.717
NO_2-N	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	$F_{(3,111)} = 1.78$	0.310
NO_3-N	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	$F_{(3,111)} = 1.59$	0.275
Chemical oxygen demand	98 ± 44	82 ± 43	122.0 ± 45	80.0 ± 43	$F_{(3,111)} = 1.44$	0.414
Orthophosphate	11.29 ± 3.19	10.29 ± 2.45	11.21 ± 2.90	10.75 ± 2.40	$F_{(3,108)} = 6.22$	<0.001

Table 3.2 The mean (± standard error) percentage change of water quality parameters for each treatment; planted (P), with worms (W), planted with worms (W) and gravel only (G). Values in the same row represented by a different alphabetical letter are significantly different (multifactor ANOVA, $p < 0.050$).

Parameter (%)	P	W	PW	G	F	P-value
Dissolved oxygen	60.77 ± 10.02	75.21 ± 10.02	64.81 ± 10.02	56.69 ± 10.02	$F_{(1,8)} = 0.52$	0.491
NH_3-N	-13.3 ± 2.7	-16.0 ± 2.7	-14.7 ± 12.7	-12.1 ± 2.7	$F_{(1,8)} = 0.20$	0.666
NO_2-N	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	$F_{(1,8)} = 1.78$	0.310
Chemical oxygen demand	6 ± 12	18 ± 12	-26 ± 12	37 ± 12	$F_{(1,8)} = 0.29$	0.606

Ammonia-nitrogen

The incoming NH_3-N concentration showed no difference between treatments (RM-ANOVA, $F_{(3,111)} = 0.91$, $p = 0.717$). The mean incoming NH_3-N for all treatments was 14.56 ± 1.63 mg/L (Table 3.1). The percentage change in NH_3-N was not influenced by the presence or absence of plants or worms (multifactor ANOVA, $F_{(1,8)} = 0.20$, $p = 0.666$, Table 3.2), but there was an average decrease of 14.0 ± 2.7 % across all treatments. Furthermore, NH_3-N and DO had a significant inversely proportional relationship (Multiple regression analysis, $y = -2.96x + 29.20$, $R^2 = 0.32$, $F_{(1,150)} = 69.56$, $p < 0.001$; Figure 3.1).

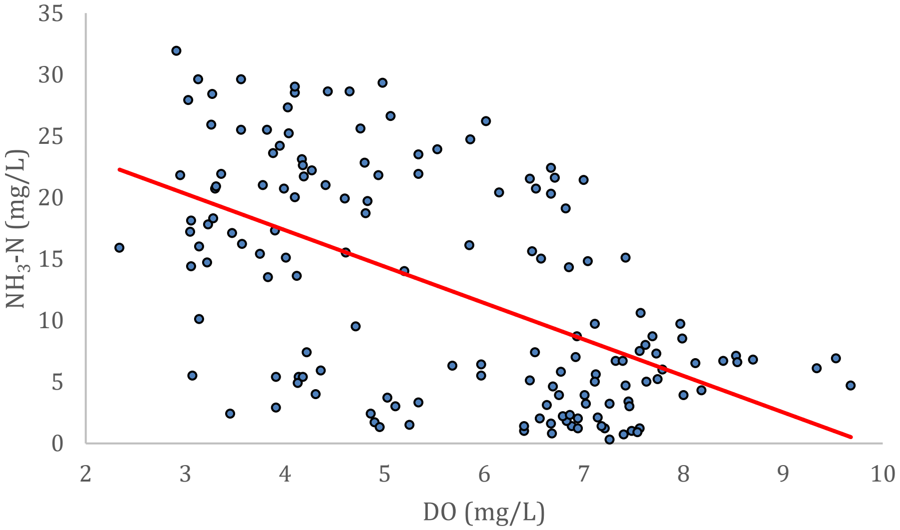

Figure 3.1 Relationship between the effluent ammonia concentration and dissolved oxygen concentration in all treatments (Multiple regression analysis, $y = -2.96x + 29.20$, $R^2 = 0.32$, $F_{(1,150)} = 9.56$, $p < 0.001$).

Nitrite-nitrogen

The incoming NO_2-N did not differ between treatments with a mean of 4.0 ± 0.5 mg/L (RM-ANOVA, $F_{(3, 111)} = 0.76$, $p = 0.545$, Table 3.1). The percentage change between incoming and outgoing NO_2-N was significantly different between treatments (Kruskal-Wallis, $H_{(3, N=12)} = 31.97$, $p < 0.001$, Figure 3.2). The treatment without any additions showed an increase in NO_2-N concentration, whereas the other treatments decreased this parameter (Figure 3.2).

Nitrite-N had no relationship to the DO concentration in any of the treatments; P (Pearson's correlation, $p = 0.066$), PW (Pearson's correlation, $p = 0.543$), W (Pearson's correlation, $p = 0.198$) and G (Pearson's correlation, $p = 0.290$). Nitrite-N showed no correlation to NH_3-N in any of the treatments; P (Pearson's correlation, $p = 0.516$), PW (Pearson's correlation, $p = 0.786$), W (Pearson's correlation, $p = 0.070$) and G (Pearson's correlation, $p = 0.063$).

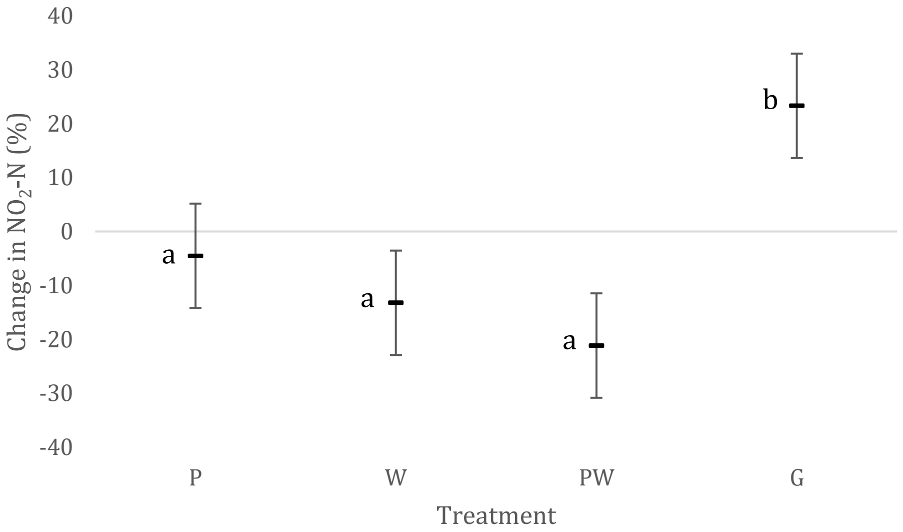

Figure 3.2 The mean (± standard error) percentage change between the incoming and outgoing nitrite-nitrogen (NO_2-N) in the planted (P), worm (W), planted with worm (PW) and gravel only (G) bioreactors. Means that were significantly different are marked by different alphabetical letters (Kruskal-Wallis, H(3, N=12) = 31.97, $p < 0.01$).

Nitrate-nitrogen

The incoming NO_3-N did not differ between treatments with a mean of 1.2 ± 0.1 mg/L (RM-ANOVA, $F_{(3, 111)} = 1.59$, $p = 0.275$, Table 3.1). There was a significant interaction in the change in NO_3-N between the presence and absence of plants and worms (multifactor ANOVA, $F_{(1,8)} = 9.47$, $p = 0.015$, Figure 3.3). The presence of Swiss chard did not influence the change in NO_3-N concentration in the systems with worms (Figure 3.3). Swiss chard did have an effect on this change in the system with worms, decreasing the change (Figure 3.3). Nitrate-N had no significant relationship with NH_3-N for any treatment; P (Pearson's correlation, $p = 0.212$), W (Pearson's correlation, $p = 0.935$), PW (Pearson's correlation, $p = 0.789$) and G (Pearson's correlation, $p = 0.569$). Nitrate-N showed no correlation to NO_2-N in any of the treatments; P (Pearson's correlation, $p = 0.330$), PW (Pearson's correlation, $p = 0.363$), W (Pearson's correlation, $p = 0.087$) and G (Pearson's correlation, $p = 0.729$).

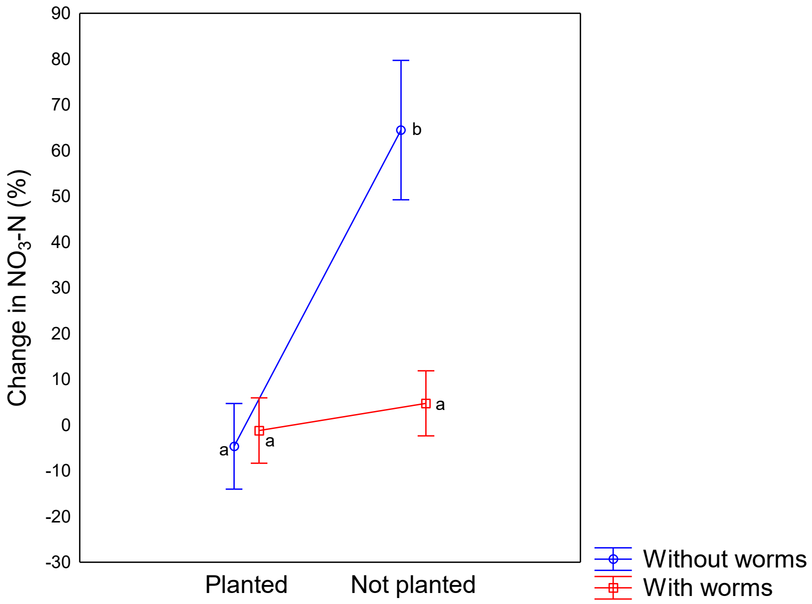

Figure 3.3 The interaction between the presence or absence of plants and worms on the percentage change (± standard error) of nitrate-nitrogen (NO_3-N). Means that were significantly different are marked by different alphabetical letters (multifactor ANOVA, $F_{(1,8)} = 9.47$, $p = 0.015$).

Chemical oxygen demand

The incoming data for COD did not vary between treatments (RM-ANOVA, $F_{(3,111)} = 1.44$, $p = 0.414$, Table 3.1). The percentage change in COD was not influenced by the presence or absence of worms or plants (multifactor ANOVA, $F_{(1,8)} = 0.29$, $p = 0.606$, Table 3.2). No relationship was found between the change in COD and the change in DO (Pearson's correlation, $p = 0.374$).

Orthophosphate

The incoming orthophosphate did not differ between treatments (RM-ANOVA, $F_{(3,108)} = 3.87$, $p = 0.128$, Table 3.1). The percentage change observed between the incoming and outgoing orthophosphate was significantly influenced by the presence or absence of worms and plants (multifactor ANOVA, $F_{(1,8)} = 47.43$, $p < 0.001$, Figure 3.4). The presence of Swiss chard did not influence the change in concentration of PO_4^{3-} in the systems without worms, whereas in the systems with worms, Swiss chard increased the change in this parameter (Figure 3.4).

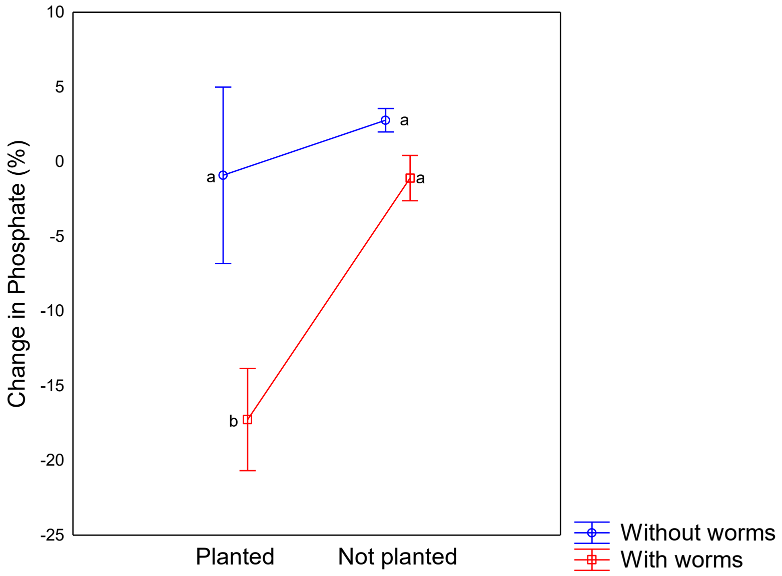

Figure 3.4 The interaction between the presence or absence of plants and worms on the percentage change (± standard error) of orthophosphate (PO_4^{3-}). Means that were significantly different are marked by different alphabetical letters (multifactor ANOVA, $F_{(1,8)} = 47.43$, $p < 0.001$).

3.4.2 Plant health and harvest

No differences were found in the elemental composition of the Swiss chard leaves between treatments (Table 3.3). There was no significant difference found between the start of the experiment and the final Swiss chard plants for any of the tested minerals (Table 3.3).

The mean dry weight of the total leaf harvest was 1702.8 ± 121.8 g in the planted treatment and 1279.3 ± 313.3 g for the planted with worm treatment, this was not significantly different between treatments (T-test, $T_{(1,4)} = 1.03$, $p = 0.362$).

The relative growth rate of 0.04 ± 0.02 g/g/d showed no difference between treatments (RM-ANOVA, $F_{(1,11)} = 0.07$, $p = 0.999$).

Table 3.3 The mean (± standard error) elemental composition of seedlings (at the beginning of the experiment) and Swiss chard plants grown in the planted (P) and plant with worm (PW) treatments after the experiment. Values in the same row represented by a different superscript letter represent significantly different means (Kruskal Wallis, $p < 0.05$).

Element	Seedlings	P	PW	H	P-value
Nitrogen (m/kg)	48.18 ± 3.20	36.03 ± 3.78	43.43 ± 2.06	$H_{(3, N=9)} = 3.82$	0.147
Phosphorus (m/kg)	2.35 ± 0.21	3.17 ± 0.11	3.03 ± 0.14	$H_{(3, N=9)} = 5.15$	0.076
Potassium (m/kg)	9.58 ± 0.33	23.57 ± 1.41	19.97 ± 0.97	$H_{(3, N=9)} = 2.33$	0.127
Calcium (m/kg)	3.92 ± 0.46	4.63 ± 0.39	4.20 ± 0.54	$H_{(3, N=9)} = 0.80$	0.670
Magnesium (mg/kg)	0.64 ± 0.06	0.94 ± 0.04	0.79 ± 0.01	$H_{(3, N=9)} = 4.53$	0.209
Sodium (m/kg)	68.82 ± 3.60	56.62 ± 1.50	52.22 ± 0.57	$H_{(3, N=9)} = 3.49$	0.322
Iron (mg/kg)	155.81 ± 18.70	97.79 ± 3.43	118.10 ± 4.71	$H_{(3, N=9)} = 2.40$	0.301
Copper (mg/kg)	3.10 ± 0.36	5.17 ± 0.18	4.84 ± 0.45	$H_{(3, N=9)} = 7.04$	0.071
Zinc (mg/kg)	42.05 ± 5.12	59.00 ± 2.50	53.90 ± 2.30	$H_{(3, N=9)} = 3.82$	0.148
Manganese (mg/kg)	58.10 ± 14.10	137.23 ± 15.75	128.67 ± 18.19	$H_{(3, N=9)} = 4.62$	0.202
Boron (mg/kg)	33.07 ± 4.23	40.16 ± 2.20	37.05 ± 1.99	$H_{(3, N=9)} = 1.71$	0.635
Aluminium (mg/kg)	122.60 ± 18.69	65.44 ± 3.44	81.75 ± 2.85	$H_{(3, N=9)} = 2.60$	0.458

3.4.3 Earthworms

The count and weight of worms showed no difference between treatments (Chi-square, $X^2_{(2,2)} = 0.65$, $p = 0.723$; Mann-Whitney $U = 2.00$, $p = 0.349$, Table 3.4). The number of eggs found in each treatment did not differ (Chi-square, $X^2_{(2,2)} = 0.35$, $p = 0.838$, Table 3.4). The average biomass of the worms decreased from the original stocking density (250 g/m^3) to 50.26 g/m^3 found in the representative sample (Mann-Whitney $U = 18.00$, $p = 0.001$).

Table 3.4 The mean (± standard error) worm weight, count, and the number of eggs found in the planted with worms (PW) and the worm-only (W) treatments (Chi-square/Mann-Whitney U, $p < 0.050$).

	PW	W	X^2/U	P-value
Weight (g)	3.32 ± 0.41	3.25 ± 0.31	U = 2.00	0.349
Worm count	6.3 ± 0.7	6.3 ± 0.5	$X^2_{(2,2)} = 0.65$	0.723
Egg count	8.7 ± 0.3	7.4 ± 0.5	$X^2_{(2,2)} = 0.35$	0.838

3.5 Discussion

The first objective of this study was to compare the DO concentration between treatments. There were increases in DO across all treatments but no significant difference between them. Worms are used in wastewater treatment to improve nutrient removal, but they are not directly linked to an increase in oxygen (Wu *et al*. 2014, Jin *et al*. 2016). Plants have been linked to DO increases by releasing oxygen into the water through their roots (Wang *et al*. 2017). The release of oxygen from the roots of plants is highly variable and dependent on the root permeability, size, and length as well as the internal oxygen concentration (Sorrell and Armstrong 1994). These factors were not taken into consideration during this study, primarily because there were no significant differences in oxygen between treatments. There may have been oxygen addition through the roots, but one or more of these factors may cause the addition to be too small to make an overall effect on the effluent DO.

Ammonia-nitrogen is a key target for removal from wastewater. Increasing the DO concentration is the best way to increase the ammonia-nitrogen and total nitrogen removal in CWs (Saeed and Sun 2012, Li *et al*. 2014). This can be seen in the inversely proportional relationship found in this study between DO and NH_3-N. The additional oxygen provided by the filters increases the effects of nitrifying bacteria responsible for converting nitrogenous products causing a decrease in NH_3-N in all treatments (Vymazal 2010). As with DO the addition of plants and worms did not influence the NH_3-N decrease any more than the control treatment. Plants and worms, alone or in combination, have been shown to increase nitrification potentials (Xu *et al*. 2013). Earthworms stocked at 32 g/L increased nitrification potentials by 236 % in CWs without plants (Xu *et al*. 2013). Constructed wetlands with earthworms and planted with *Canna indica* increased nitrification potentials by 268 % and denitrification potentials by 15% (Xu *et al*. 2013). This, however, is not always successful; worms stocked at 0.526 kg/m^2 did not influence nitrogen conversions in Attala *et al.* 2021. No difference caused by plants or worms was recorded in a year-long study of four vertical flow wastewater treatment systems containing either worms, plants, worms and plants, or neither (Lavrnić *et al.* 2019). In this study the nitrifying bacterial communities were still limited by the available oxygen and the addition of plants and worms did not affect the DO or NH_3-N.

The oxidation of NO_2-N is known as a rate-limiting step in nitrification because of the slower-growing bacterial communities that oxides NO_2-N to NO_3-N compared to those that oxidise NH_3-N to NO_2-N (Vymazal 2007). The NO_2-N decreased in all the treatments with additions; planted, worms, and planted with worms, whereas an increase was found in the gravel treatment. Nitrite-N oxidation is an aerobic process, therefore dependent on the presence of DO (Saeed and Sun 2012). The addition of dissolved oxygen has led to 100 % nitrite oxidation in low-loaded CWs (Khan *et al*. 2022). The NO_2-N in this study shared no relationship with DO. Although nitrite oxidising bacteria (NOB) such as *Nitrospira* are slower growing, recent studies have shown that (NOB) perform well in varying levels of oxygen (Daims *et al.* 2016). These bacteria which can be outcompeted by faster-growing bacteria such as ammonia oxidising bacteria like *Nitrosomonas* are positively affected by oxygen gradients as multiple species of NOB will occupy different oxygen habitats (Daims *et al.* 2016). Oxygen gradients are found in the areas around plant roots (Berke 2010, Daims *et al*. 2016). Oxygen gradients formed by earthworms have not been studied in wetland-specific environments that do occur in the casts produced by earthworms and they may affect the distribution of oxygen within this system (Taylor *et al.* 2003). The decrease in NO_2-N seen in the treatments with additions may be caused by oxygen gradients formed by those additions. Whereas the treatment with no additions would not have the same oxygen gradients and not be as effective (Daims *et al.* 2016).

Anaerobic conditions are required for denitrification (Vymazal 2007). An increase in NO_3-N was found in all treatments. As nitrogen is processed through the system NH_3-N is converted to NO_2-N which is then converted to NO_3-N (Vymazal 2010). Under aerobic conditions, this leads to a build-up of NO_3-N if there is little or no denitrification (Saeed and Sun 2012). This is seen in multiple studies where nitrogen products build up when there is removal of NH_3 or NH_4-N (Yoo *et al*. 1999, Sun *et al*. 2005, Saeed and Sun 2012).

While all the treatments increase in nitrates the increase is less in the treatments with plants. This could indicate that some of the NO_3-N formed in the bioreactor or entering the bioreactor is being removed. This can be attributed to NO_3-N uptake by plants as it is used for growth (Kant 2018). Plants are known to remove NO_3-N from effluent and have been shown to increase NO_3-N removal by 49.9 % compared to unplanted wetlands (Lin *et al*. 2002). Plants can remove NO_3-N from the system which is demonstrated in the treatment differences found in this study.

The increases in NO_3-N were expected as denitrification requires anaerobic conditions which are not found in the ebb and flow bioreactors (Vymazal 2007). The horizontal subsurface flow wetland cell that follows the ebb and flow filters in an anaerobic environment would be the main mechanism of NO_3-N removal.

Traditional gravel CWs require extra steps or adjustments to be able to remove phosphate from wastewater (Sun *et al.* 2021). Three treatments; the planted bioreactor, worm bioreactor, and gravel bioreactor, did not affect the PO_4^{3-} concentration in the brewery effluent. Phosphate removal is highly dependent on the media used in biofilters (Sun *et al*. 2021, Wang *et al*. 2013). Phosphate does not readily adsorb to gravel (Mann and Bavor 1993, Mann 1997). The lack of PO_4^{3-} removal in the above treatments can be explained by the use of gravel in the bioreactors.

Contrasting this finding is the planted treatment with worms, which resulted in a decrease in PO_4^{3-} of 15.84 ± 2.27 %. Utilising earthworms to improve the uptake of phosphate by plants is effective and well-studied in soil (Huang *et al*. 2013, Singh *et al*. 2021). However, this is caused by the worms processing organic forms of phosphate and converting them to orthophosphate (Huang *et al.* 2013). In such instances, they are creating more PO_4^{3-} for plants to take up and are not directly increasing the uptake by plants (Tuffen *et al*. 2002). The plant health and growth parameters did not differ between the planted treatment and the combination treatment indicating that worms did not increase the PO_4^{3-} uptake by the Swiss chard. The combination of plants and worms affected the PO_4^{3-} concentration in the bioreactors but it is an irregular finding that has not been definitively described in the literature.

The gravel treatment had no consistent change in the oxygen demand. The change in COD varied day to day, sometimes increasing, and at others decreasing. The measure of COD indicates how much oxygen would be needed to oxidise the organic matter in the effluent (Jain and Singh 2003). An increase or decrease in organic matter would influence the amount of oxygen needed (Jain and Singh 2003). Organic matter was not quantified in this research and may have affected the COD measured.

The planted treatment and the treatment with worms had no overall effect on the COD, with similar values for incoming and outgoing effluent. Both plants and worms had varying effects on COD. In certain cases, worms have reduced the demand for oxygen by 11 % (Xu *et al*. 2013) but did not affect others (Attala *et al*. 2021). Some planted systems have had higher COD than the unplanted variations, some with only a slight effect showing a 2 % decrease in COD (Kaseva 2004, Akratos and Tsihrintzis 2007). Others had no difference between planted and unplanted systems (Ong *et al*. 2010). However, all these systems have lowered the COD, unlike the systems in this study. The only treatment with a decrease in COD was the combination treatment with plants and worms. The combination of plants and worms has decreased COD in other studies (Xu *et al.* 2013, Attala *et al.* 2021). However, this is caused by the effect of earthworms on the plants in a soil environment (Xu *et al*. 2013). In sand and gravel CWs these effects are not seen (Nuengjamnong *et al*. 2011, Attala *et al*. 2021). The decrease of COD in the combination treatment and the lack of effect in the P and W treatments could not be explained by the literature available and requires further investigation.

The plant chemical analysis and harvest showed no difference between treatments indicating that the worms did not affect their growth and health likewise, the plants did not affect the abundance of worms. There are many instances of worms increasing nutrient uptake and growth of plants, but these studies take place mainly in the soil where dynamics are different to gravel (Nuengjamnong *et al*. 2011, Attala *et al*. 2021). The main improvements in plant growth are seen from earthworms' effect on soil structure which is not the case in gravel and the excretion of inorganic forms of nitrogen, which was readily available in these systems and any produced by the worms may have been in excess rather than helpful to the plants (van Groenigen *et al*. 2014). Worms can increase Ca and decrease K, Mg and Mn, however there was no difference in the concentration of these substances in plants growing with worms or without, this may be because of the abundance of nutrients already available in the system (Zaltauskaite *et al.* 2022) Subsurface flow systems do not always result in improved plant production when integrated with worms (Nuengjamnong *et al*. 2011, Attala *et al*. 2021). A study by Nuengjamnong *et al.* (2011) showed that plants could perform better (44 % more biomass) without the presence of worms compared to Xu *et al*. (2013) where plant height increased by 15 – 61 % with the presence of worms. Plants generally aid worms by providing decaying matter for the worms to feed on, however in a high-nutrient environment, like the bioreactors in this study, worms would not be impacted by the plants (Brown and Doube 2004). The interactions with worms and plants are varied in subsurface flow wetlands so seeing no difference between treatments is not unexpected.

The worms decreased in biomass by the end of the study. Reasons for the lowered biomass could have been the death of the worms however worms are known to survive in tidal systems (Lavrnić *et al*. 2019). No dead worms were found in the gravel samples taken. The filters were not enclosed and escape by the worms into the environment was possible. The sampling technique used to collect the earthworms may have affected the results as digging samples can exclude worms that escape by burrowing further downwards (Singh *et al.* 2016). Other methods include chemical irritants which were excluded due to the harmful effect they would have on the bacterial population in the filters (Singh *et al.* 2016).

The absence of dead worms and the presence of eggs in the gravel samples indicated that the earthworms could survive in this effluent.

3.6 Conclusion

Crops and earthworms influenced the functioning of ebb and flow filters. Their inclusion in the ebb and flow filters improved the rate of nutrient removal, particularly the combination of the two increased the NO_2-N removal compared to the gravel treatment. The two treatments with plants also showed lower NO_3-N levels than the other treatments. The combination treatment, incorporating both plants and worms, performed the best, reducing the PO_4^{3-} concentration and COD found in the effluent.

While the combination treatment was the most effective at reducing the nutrient level, the increased performance at decreasing COD and PO_4^{3-} could not be fully explained.

The addition of plants and worms did not affect the DO concentration in the filters. All filters resulted in increased DO.

The filters were able to sustain crop production and led to plant growth which did not differ between treatments. Worm production could not be appropriately quantified, and for that reason, it was decided not to include the worms in the final trial, however, their inclusion in future studies with suitable containment and possibly different media types to further explore this results should be considered by researchers.

Chapter 4: The effect of ebb and flow filters and trickle filters on the treatment of brewery effluent

4.1 Introduction

In Chapter 3 the ebb and flow bioreactors were able to increase dissolved oxygen (DO) and decrease ammonia-nitrogen, but the system could not impact many of the other water quality parameters significantly. Another system that is common in wastewater treatment that requires a minimal economic cost to run is the trickle filter (Vayenas 2011). Trickle filters might, therefore, be a useful addition to the constructed wetland (CW) treatment system at Ibhayi Brewery and an alternative loop in the system that currently run as ebb and flow filters. These filters are passively aerated treatment filters that use a biofilm growing on media to treat wastewater; which classifies trickle filters as biological filters or bioreactors (Rezai and Allahkarami 2021). The biofilm has access to oxygen as air enters the spaces in the media via the temperature gradient between the outside air and the air contained between the media (Vayenas 2011). Trickling bioreactors are often circular, and water is distributed by sprinklers attached to a rotating arm; however, square and rectangular trickle bioreactors use fixed distribution systems (Butler and Boltz 2014). In both types of trickle bioreactors, wastewater is constantly supplied to the open surface of the bioreactor, the water trickles down through the media putting it in contact with the biofilm where filtration occurs (Butler and Boltz 2014). The distribution design is important to allow the entire filter to be utilised, even with good surface distribution wastewater channels; creating paths through the filter media and creating unused spaces within the filter where a biofilm will not be supported (Wik 2004).

Trickle filters have been found to significantly decrease total nitrogen, phosphorus, biological oxygen demand (BOD_5) and chemical oxygen demand (COD) in industrial brewery wastewater (Lemji and Eckstädt 2014). An experimental treatment plant in 1938 used trickling bioreactors to lower the BOD_5 in brewery wastewater (Bushee 1939). On their recommendation, a treatment plant for the brewery was built consisting of settling tanks, two trickling filters connected in series and ending with sludge processing (Bushee 1939). Trickling bioreactors have been used; alone, in more complex treatment systems and in connection with horizontal subsurface flow wetlands, to treat wastewater successfully (Bushee 1939, Vucinic *et al*. 2012, Lemji and Eckstädt 2014). With well-documented success at treating brewery wastewater and the ease of adapting the current design at Ibhayi Brewery, including trickle filters in the system could be beneficial to the treatment process.

While plants did not have the expected improvement for most parameters tested in Chapter 3, the project took place on a commercial site and the brewery requested the inclusion of celery (*Apium graveolens*) due to the economic potential. Growing celery requires large amounts of water and fertiliser, which the CW can provide (Glines 1922, Tanwar *et al.* 2013). Celery has been grown in CWs treating sewage, the system managed to decrease the total nitrogen by <70 % (Hussain *et al.* 2018). Celery has also been grown in the Ibhayi Brewery wetlands successfully (Dr R Taylor personal communication 2021). Celery is a good candidate to be grown in wetlands because of its need for water and nutrients and the success it has already shown in similar systems.

4.2 Aims and objectives

This study aimed to determine if trickle filters would perform the same as the ebb and flow filters from the previous experiments. It also assessed the viability of crop production in both systems. This was done by comparing the nutrient load entering and exiting the filters and assessing the DO levels of the effluent.

The objectives were to:

4.1 Compare dissolved oxygen content between ebb and flow filters and trickle filters using:
  4.1.1 planted; and
  4.1.2 unplanted treatments;
4.2 Determine if any of these systems leads to more efficient water treatment by testing water quality parameters; and
4.3 Compare the plant health and biomass between these treatments.

4.3 Materials and methods

4.3.1 Experimental species

One hundred and twenty celery (*Apium graveolens*) seedlings were purchased from a commercial seedling supplier (Moorland Seedlings (Pty) Ltd, Humansdorp). Ninety of these were used in the experiment and the rest were dried and weighed to get an average seedling weight at the start of the trial.

4.3.2 Experimental systems

This experiment utilised the twenty original bioreactors alongside constructed wetland cell 1b cell that was used to treat brewery effluent at Ibhayi Brewery in Port Elizabeth (Figure 2.2; Section 2.3.2 Chapter 2). All 20 bioreactors were originally ebb and flow systems receiving and returning effluent from and to wetland cell 1b. Ten bioreactors remained as the ebb and flow systems, and the other 10 were randomly selected and converted to trickle systems. The 10 converted systems had the bell siphons removed, which allowed effluent to drain through the filter without flooding. A grid made of piping with holes in it was fitted to the inflow of each filter and spanned the surface of the bioreactor (Figure 4.1 and Figure 4.2). The grid of the trickle filter was designed using 30 mm PVC piping, and an outer rectangle of 1000 mm x 900 mm was constructed with a 45 mm adapter attached perpendicularly to the inflow of the tank. Three pieces of piping were added lengthwise in the middle of the outer rectangle 225 mm apart (Figure 4.2). Holes were drilled into the piping approximately 70 mm apart. This allowed effluent to slowly enter the bioreactor through multiple streams and trickle through the gravel until it reached the bottom where it could freely exit into the wetland cell. Four treatments were investigated in this experiment:

- Treatment 1 included trickling systems planted with nine celery plants per bioreactor (PT);
- Treatment 2 included ebb and flow systems planted with nine celery plants (PE);
- Treatment 3 was trickling systems with no plants (T);
- Treatment 4 was unplanted ebb and flow systems (E).

These treatments were randomly assigned to the 20 bioreactors, so each treatment had five replicates, with a bioreactor constituting a replicate.

Figure 4.1 The trickle filters were made with 30 mm piping with holes drilled every 7 cm to release steady streams of effluent over the surface of the gravel. The gravel guards (blue piping pictured in the left-hand corner) remained in place from the ebb and flow filters but the bell-siphon and standpipe inside were removed to allow the effluent to exit the filter continuously.

Figure 4.2 On the left the trickle filter grid attaches to the original inflow of the ebb and flow filter. On the right, the celery was planted against the hole in the trickle grid ensuring the effluent reached the roots, next to the celery plant a stream of effluent can be seen exiting the trickle filter grid.

4.3.3 Data collection

The same collection methods and water quality tests that were presented earlier were used here (Section 2.3.3; Chapter 2), with the exceptions of electrical conductivity and total dissolved solids which were not recorded during this experiment due to equipment availability/maintenance. The samples were collected twice weekly at the inflow and outflow of each bioreactor for a period of 192 d.

Plant harvest and health parameters

Biomass was recorded using the dry weight of the seedlings planted subtracted from the dry mass of the harvested plants at the end of the experiment. The relative growth rate (RGR; g/g/d) was calculated using the equation:

$$RGR = (\frac{1}{W})(\frac{dW}{dt}) \qquad \text{Equation 4.1}$$

where W is the plant dry weight at the start of the trial, dW is the dry weight at the end of the trial and dt is the time (days) from the end to the start of the trial (Briggs 1920). This equation was used instead of Equation 3.1 (Section 3.3.3; Chapter 3) as only one harvest occurred at the end of this experiment.

Plants were removed from all bioreactors, they were then weighed with a scale to 0.1 g (Precision balance WTC, RADWAG Balances and scales, Poland). After weighing the wet plant material, the celery was spread flat in an oven and dried to constant weight at 65°C (Series 9000, Thermo Fisher Scientific, USA). The plants were then weighted (0.1 g; Precision balance WTC, RADWAG Balances and scales, Poland) and stored in airtight bags.

Dry plant tissue samples were sent for chemical analysis of nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, copper, zinc, manganese, boron, and aluminium at a commercial laboratory (Western Cape Department of Agriculture, Institute for Plant Sciences, South Africa). The analysis was performed using the same methods described in Chapter 3; Section 3.3.3.

4.3.4 Statistical analysis

All data were checked for equality of variance and normal distribution of the residuals using Levene's test and a Shapiro-Wilk plot of the residuals, respectively (Levene 1960, Shapiro and Wilk 1965). 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.

The incoming water quality was compared using repeated measures analysis of variance (RM-ANOVA) to check that the incoming quality was the same for each tank on each day. If variability was found in the incoming data, only the difference between incoming and outgoing was used to assess that parameter.

The difference between incoming and outgoing water quality for each treatment was tested for variability over time using an RM-ANOVA. If no variability was found a multifactor analysis of variance (multifactor ANOVA) was used to compare the outgoing treatment means and the percentage change of the treatments. When significant differences were detected Tukey's HSD post-hoc test was used to further investigate the interactions.

If the assumptions for ANOVA were not met, a non-parametric Kruskal Wallis test was used to compare the data between treatments in place of it. If the data did not meet the assumptions for repeated measures ANOVA, Mauchly's sphericity test was included in the analysis, and were corrected using Greenhouse-Geisser and Huynd-Feldt tests.

Relationships between variables were investigated using multiple regression analysis and Pearson's correlation.

All plant health parameters were assessed between treatments using a Mann-Whitney U test. The total dry weight of the plants was compared between the planted ebb and flow treatment and the planted trickle treatment with a Mann-Whitney U test.

All analyses were performed using STATISTICA 14.0.1. Means were considered different at $p < 0.050$.

4.4 Results

4.4.1 Water quality parameters

Dissolved oxygen

The DO concentration of the incoming effluent showed no difference between treatments (RM-ANOVA, $F_{(3,102)} = 1.00$, $p = 0.209$). The mean incoming DO for all treatments was 6.41 ± 0.13 mg/L (Table 4.1). The percentage difference was significantly higher in both systems when they were planted with celery, compared with the unplanted bioreactors, and, in all instances the change in DO concentrations of the ebb and flow filters were greater than those of the trickle filters (multifactor ANOVA, $F_{(1,16)} = 1758.80$, $p < 0.001$, Figure 4.3).

Table 4.1 The mean (± standard error) incoming water quality for each treatment; planted trickle systems (PT), planted ebb and flow systems (PT) or trickle systems (T) and ebb and flow systems that were not planted (E). Values in the same row represented by a different alphabetical letter are significantly different (RM-ANOVA, $p < 0.050$).

Parameter (mg/L)	PT	PE	T	E	F	P-value
Dissolved oxygen	6.41 ± 0.13	6.41 ± 0.13	6.40 ± 0.13	6.41 ± 0.13	$F_{(3,102)} = 1.00$	0.209
NH_3-N	16.6 ± 1.6	16.5 ± 1.6	16.5 ± 1.6	16.5 ± 1.6	$F_{(3,102)} = 1.64$	0.506
NO_2-N	2.0 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	2.0 ± 0.3	$F_{(3,102)} = 1.10$	0.261
Orthophosphate	31.42 ± 0.27	31.37 ± 0.29	31.44 ± 0.29	31.47 ± 0.27	$F_{(3,102)} = 1.00$	0.363
Chemical oxygen demand	61.0 ± 6.2	61.0 ± 6.3	61.0 ± 6.3	61.0 ± 6.3	$F_{(3,102)} = 1.00$	0.551

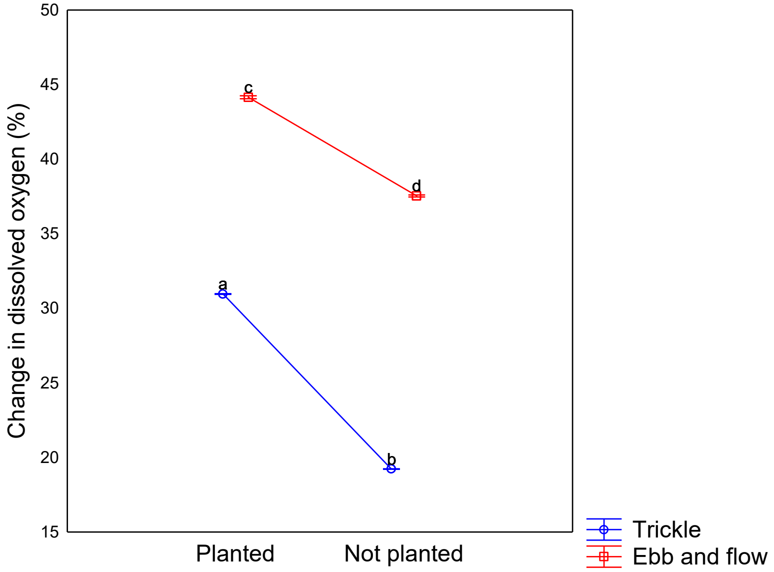

Figure 4.3 The influence of the interaction between filter type and the presence of plants or not on the percentage change (± standard error) of dissolved oxygen. Means that were significantly different are indicated by a different alphabetical letter (multifactor ANOVA, $F_{(1,16)} = 1758.80$, $p < 0.001$).

Ammonia-nitrogen

The incoming NH_3-N did not differ between treatments with a mean of 18.4 ± 1.6 mg/L (RM-ANOVA, $F_{(3, 102)} = 1.64$, $p = 0.506$, Table 4.1). Differences were found in the change in NH_3-N between incoming and outgoing concentration in all treatments (RM-ANOVA, $F_{(3,102)} = 5.21$, $p < 0.001$). All treatments differed on the same days; 70, 84, 98 and 108, on these days the incoming NH_3-N was below 2.5 mg/L. The days were left out of the subsequent analysis. Once removed, the change between incoming and outgoing showed no difference within each treatment (RM-ANOVA, $F_{(3,90)} = 1.20$, $p = 0.116$). The percentage change in NH_3-N was influenced by an interaction between the filter type and whether it was planted or not (multifactor ANOVA, $F_{(1,16)} = 684.06$, $p < 0.001$, Figure 4.4). The percentage increase in NH_3-N was significantly higher in the ebb and flow filters compared with the trickle filters (Figure 4.4). The addition of plants to the trickle filter increased the percentage change of NH_3-N, whereas plants in the ebb and flow filter saw a decrease in this parameter (Figure 4.4). The outgoing DO mg/L and NH_3-N mg/L showed no relationship for any treatment over time (Multiple regression analysis, $p = 0.894$).

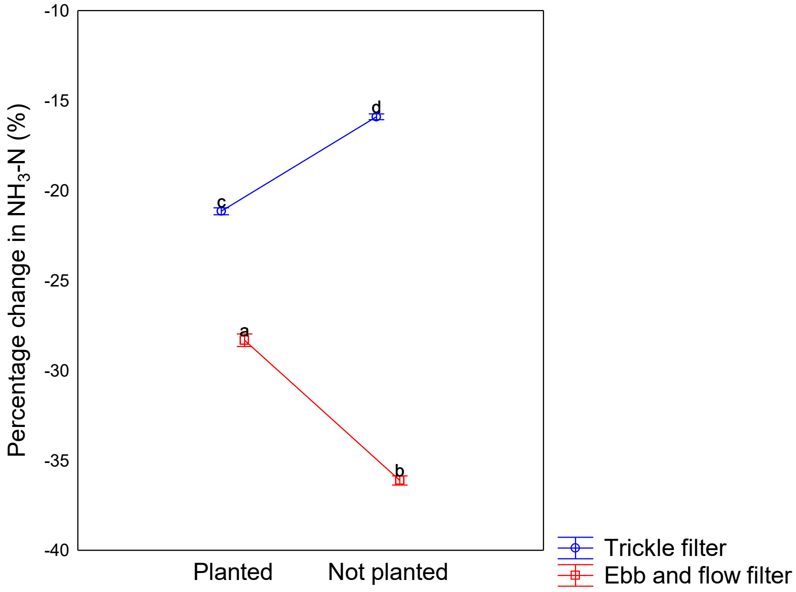

Figure 4.4 The interaction between filter type and the presence of plants or not on the percentage change (± standard error) in ammonia-nitrogen (NH_3-N). Means that were significantly different are indicated by a different alphabetical letter (multifactor ANOVA, $F_{(1,16)} = 306.58$, $p < 0.001$).

Nitrite-nitrogen

No differences were found in the incoming effluent between treatments (RM-ANOVA, $F_{(3,120)} = 1.10$, $p = 0.261$). There was a significant interaction in the change in NO_2-N between filter type and the presence and absence of plants (multifactor ANOVA, $F_{(1,16)} = 5.05$, $p = 0.039$; Figure 4.5). The presence of plants had no significant effect on the change in NO_2-N in both filters; however, this change was significantly greater in the trickle filter compared to the ebb and flow filter, but only when the filters were planted (Figure 4.5). The outgoing DO mg/L and NO_2-N mg/L showed no relationship with any of the treatments over time (Multiple regression analysis, $p = 0.482$). A positive correlation was found between NH_3-N and NO_2-N (Pearson's correlation, $r = 0.38$, $p = 0.033$, Figure 4.6).

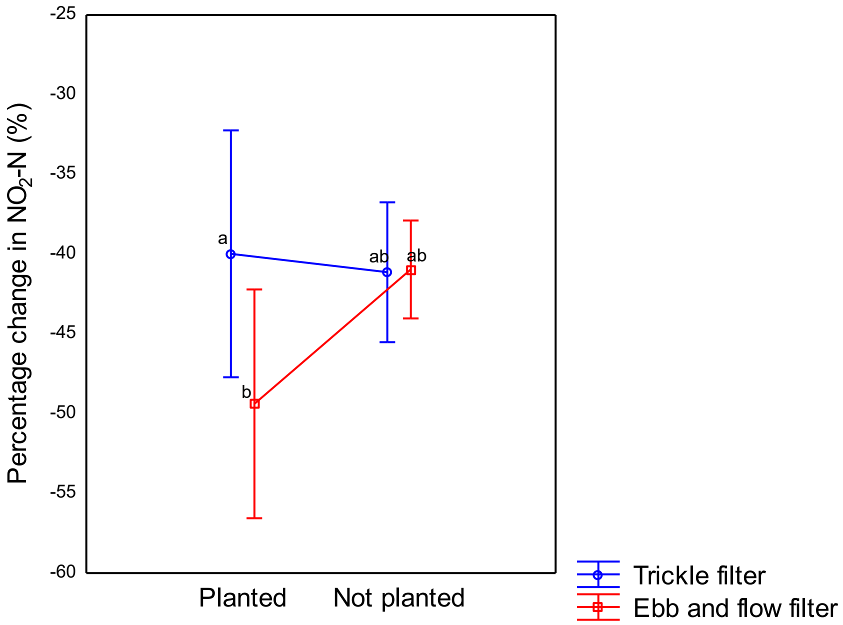

Figure 4.5 The interaction between filter type and the presence of plants or not on the percentage change (± standard error) of nitrite-nitrogen (NO_2-N). Means that were significantly different are indicated by a different alphabetical letter (multifactor ANOVA, $F_{(1,16)} = 5.05$, p = 0.039).

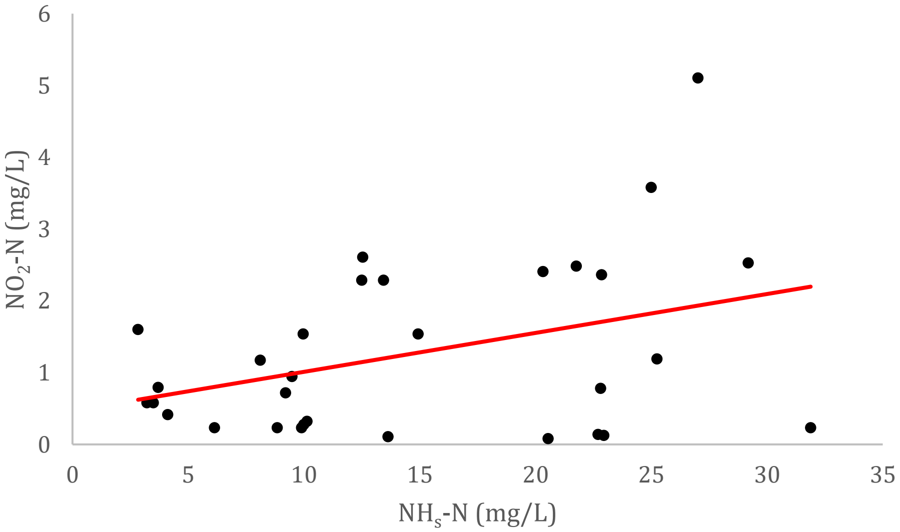

Figure 4.6 A comparison of ammonia-nitrogen (NH_3-N mg/L) and nitrite-nitrogen (NO_2-N mg/L) in the outgoing effluent (Pearson's correlation, *r* = 0.38, p = 0.033).

Nitrate-nitrogen

The incoming NO_3-N showed a difference between treatments (RM-ANOVA, $F_{(3,102)} = 8.03$, $p < 0.001$). There was a significant interaction in the change in NO_3-N between filter type and the presence and absence of plants (multifactor ANOVA, $F_{(1,16)} = 77.37$, $p < 0.001$; Figure 4.7). The change was significantly greater in the ebb and flow filters than in the trickle filters (Figure 4.7). The addition of plants to the trickle filter increased the percentage change of NO_3-N, whereas plants in the ebb and flow filter saw a decrease in this parameter (Figure 4.7).

The change in DO mg/L and the change in NO_3-N mg/L showed no relationship for the PT treatment (Pearson's correlation, $r = 0.64$, $p = 0.247$), the PE treatment (Pearson's correlation, $r = 0.04$, $p = 0.948$), the T treatment (Pearson's correlation, $r = -0.67$, $p = 0.220$) or the E treatment (Pearson's correlation, $r = -0.68$, $p = 0.207$). No correlation was found between the outgoing NH_3-N and the outgoing NO_3-N for the PT treatment (Pearson's correlation, $r = 0.18$, $p = 0.336$), the PE treatment (Pearson's correlation, $r = 0.17$, $p = 0.363$), the T treatment (Pearson's correlation, $r = 0.23$, $p = 0.204$) or the E treatment (Pearson's correlation, $r = 0.08$, $p = 0.686$). Nitrite-nitrogen and NO_3-N were not correlated for the PT treatment (Pearson's correlation, $r = 0.02$, $p = 0.915$), the PE treatment (Pearson's correlation, $r < 0.01$, $p = 0.998$), the T treatment (Pearson's correlation, $r = 0.05$, $p = 0.778$) or the E treatment (Pearson's correlation, $r = 0.09$, $p = 0.617$).

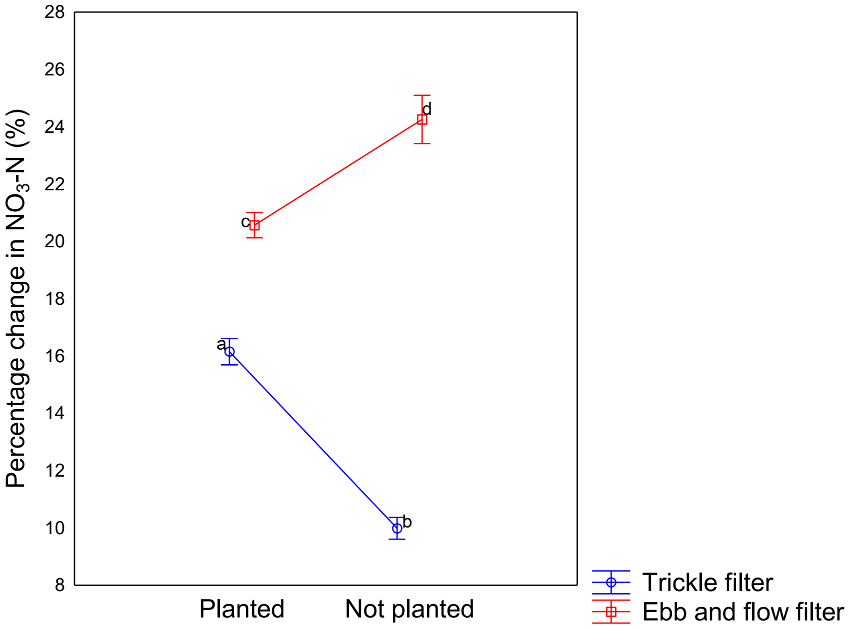

Figure 4.7 The interaction between filter type and the presence of plants or not on the percentage change (± standard error) of nitrate-nitrogen (NO_3-N). Means that were significantly different are indicated by a different alphabetical letter (multifactor ANOVA, $F_{(1,16)} = 77.37$, $p < 0.001$).

Chemical oxygen demand

There was no difference in the incoming COD between treatments, the mean COD was 61.0 ± 6.2 mg/L (RM-ANOVA, $F_{(3,120)} = 1.00$, $p = 0.551$, Table 4.1). The type of filter and whether it was planted affected the percentage change in COD (multifactor ANOVA, $F_{(1,16)} = 11.26$, $p = 0.004$, Figure 4.10). The presence of plants had no significant effect on the change in COD in the ebb and flow filters, whereas this change was significantly greater in the trickle filters that were planted compared to those that were unplanted (Figure 4.8).

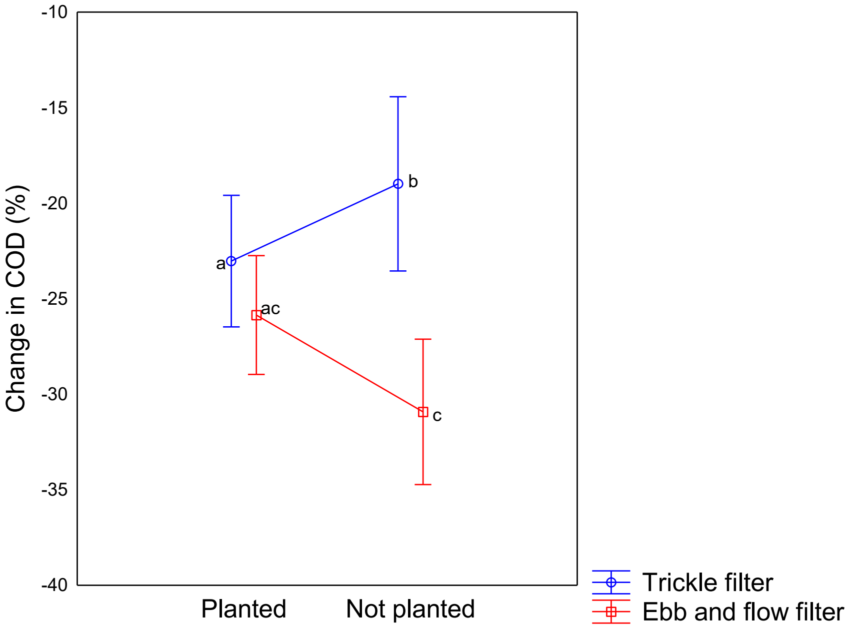

Figure 4.8 The interaction between filter type and the presence of plants or not on the percentage change (± standard error) of chemical oxygen demand (COD). Means that were significantly different are indicated by a different alphabetical letter (multifactor ANOVA, $F_{(1,16)} = 11.26$, $p = 0.004$).

Orthophosphate

The incoming PO_4^{3-} had no difference between treatments (RM-ANOVA, $F_{(3,120)} = 1.00$, $p = 0.363$). The mean incoming PO_4^{3-} was 31.43 ± 0.28 mg/L (Table 4.1). There was no difference between percentage change (multifactor ANOVA, $F_{(1,16)} = 0.83$, $p = 0.375$). With an average change of -0.04 % the difference between the outgoing PO_4^{3-} and the incoming PO_4^{3-} was not significant (ANOVA, $F_{(1,38)} = 0.15$, $p = 0.693$).

4.4.2 Plant health and harvest

Of the chemicals analysed potassium, sodium, boron, and aluminium showed no difference between treatments (Table 4.2). Nitrogen was higher in the ebb and flow planted treatment (Mann-Whitney, U = 1.00, p = 0.022, Table 4.2) with an average of 51.94 ± 1.29 g/kg compared to the trickle planted system with had 46.34 ± 0.75 g/kg. Phosphorus showed a difference between treatments (Mann-Whitney, U = 0.00, p = 0.012, Table 4.2). There was higher phosphorus content in the planted trickle treatment (6.56 ± 0.15 g/kg) compared to the ebb and flow planted treatment (4.30 ± 0.23 g/kg). Calcium was different between treatments (Mann-Whitney, U = 0.00, p = 0.012, Table 4.2), with the ebb and flow treatment having a higher calcium content (14.30 ± 0.62 g/kg) than the trickle treatment (10.14 ± 0.49 g/kg). Magnesium (Mann-Whitney, U = 0.00, p = 0.012), zinc (Mann-Whitney, U = 0.00, p = 0.012), iron (Mann-Whitney, U = 2.00, p = 0.037), copper (Mann-Whitney, U = 0.00, p = 0.012), and manganese (Mann-Whitney, U = 0.00, p = 0.012) were all higher in the ebb and flow plants compared to the trickle plants as seen in Table 4.2. The ebb and flow plants had a magnesium content of 4.10 ± 0.03 g/kg, zinc of 33.91 ± 0.74 mg/kg, copper of 2.75 ± 0.13 mg/kg, and manganese of 91.18 ± 0.57 mg/kg. Compared to 2.82 ± 0.03 g/kg of Mg, 21.77 ± 0.46 mg/kg of Zn, 1.47 ± 0.03 mg/kg of Cu, and 59.77 ± 1.49 mg/kg Mn in the trickle plants.

The final mass of the harvested celery showed no significant difference between treatments (Mann-Whitney, U = 6.00, p = 0.210). The mean final mass was 75.5 ± 10.2 g/m^2. The relative growth rate of 0.2 ± 0.1 g/g/d showed no difference between treatments (Mann-Whitney, U = 6.00, p = 0.210).

Table 4.2 The mean (± standard error) mineral composition of the celery plants at the end of the trial in the planted trickle treatment and the planted ebb and flow treatment. Values in the same row represented by a different superscript letter represent significantly different means (Mann-Whitney U, $p < 0.050$). The data from this trial is presented alongside the mean mineral content in celery stalks found by Singh *et al.* 2022.

Element	Trickle	Ebb and flow	U	P-value	Singh *et al.* 2022
N (g/kg)	46.34 ± 0.75^{a}	51.94 ± 1.29^{b}	1.00	0.022	21.0
P (g/kg)	6.56 ± 0.15^{a}	4.30 ± 0.23^{b}	0.00	0.012	0.94
K (g/kg)	21.68 ± 0.63	22.72 ± 0.52	7.00	0.296	15.79
Ca (g/kg)	10.14 ± 0.49^{a}	14.30 ± 0.62^{b}	0.00	0.012	46.3
Mg (g/kg)	2.82 ± 0.03^{a}	4.10 ± 0.03^{b}	0.00	0.012	N/A
Na (g/kg)	21.62 ± 1.32	15.88 ± 2.13	5.00	0.144	6.51
Fe (mg/kg)	147.14 ± 18.74^{a}	197.48 ± 6.69^{b}	2.00	0.037	0.03
Cu (mg/kg)	1.47 ± 0.13^{a}	2.75 ± 0.03^{b}	0.00	0.012	0.007
Zn (mg/kg)	21.77 ± 0.75^{a}	33.90 ± 0.46^{b}	0.00	0.012	0.11
Mn (mg/kg)	59.77 ± 0.57^{a}	91.18 ± 1.49^{b}	0.00	0.012	0.021
B (mg/kg)	41.59 ± 0.88	41.30 ± 0.76	11.00	0.835	N/A
Al (mg/kg)	88.50 ± 9.44	99.42 ± 7.17	8.00	0.403	N/A

4.5 Discussion

All treatments increased DO levels. However, the ebb and flow treatments had a larger increase than the trickle bioreactors. Both systems are passively oxygenated by ambient air (Stottmeister *et al*. 2003, Vayenas 2011, Butler and Boltz 2014). Ebb and flow bioreactors use a pressure gradient from the draining of the bioreactor while trickling bioreactors are oxygenated by a temperature gradient between the air outside the bioreactor and inside (Stottmeister *et al*. 2003, Vayenas 2011, Butler and Boltz 2014). Most trickling filters have more than one way for air to enter the system, an underdrain underneath the filter media is a common part of trickle bioreactor design (Butler and Boltz 2014). In the brewery treatment study by Lemji and Eckstädt (2014) where gravel trickling bioreactors were used, aeration holes were added to the side of the bioreactor for additional air exchange. In this experiment, the trickle bioreactors used were not equipped with an underdrain as the bioreactors were adapted from the existing ebb and flow bioreactors. This left the only site of air exchange on the surface of the bioreactors, whereas the ebb and flow bioreactors could draw oxygen into the entire void space during the draining phase. This allowed the ebb and flow bioreactors to draw in more oxygen. If this study were to be repeated, the trickle filters could be redesigned to allow better movement of air into the filter; this may result in better performance of the trickle filters.

Both planted treatments had higher increases in DO than their unplanted counterparts. When plants are added to the effluent treatment, they transport oxygen from the external environment into the system through their roots (Zhai *et al*. 2013, Wang *et al*. 2017). The celery in the experiment significantly increased the DO content in the effluent. However, both ebb and flow treatments had a higher increase in DO than the trickle treatment that included celery.

Ammonia-nitrogen had a varied outgoing concentration from the beginning to the end of the study, largely influenced by the fluctuating incoming NH_3-N levels from the brewery. All treatments still consistently decreased the NH_3-N concentration. The ebb and flow treatments decreased the NH_3-N concentration more than the trickle filters. The NH_3-N decrease in the ebb and flow treatment could be attributed to the additional oxygen available in the systems. Increased DO in bioreactors has been linked to increased removal of nitrogen. Nitrogen removal has been increased by over 200 % in constructed wetlands when receiving air at 2.0 L/min versus receiving no outside aeration (Ouellet-Plamondon *et al.* 2008). However, regression analysis did not find a significant relationship between DO and NH_3-N. The trickle bioreactors could have had less of an impact due to the full bioreactor not being used correctly, in this case, possible channelling with the trickle systems. If the effluent was not being distributed evenly throughout the bioreactor it would not be performing to its full capacity (Wik 2004). The inclusion of plants had different effects on the two filter types. In the trickle filters the presence of plants aided the removal of NH_3-N. The ebb and flow filters without plants performed better than the planted ebb and flow filters. Although plants have been found to contribute to 9.0 % of total Kjeldahl nitrogen removal in some systems, they do not always lead to increased removal of nitrogen compounds (Gottschall *et al.* 2006). A total nitrogen decrease of 80 % was found in an unplanted horizontal subsurface flow CW, this decreased to 69 % in an aerated system with the inclusion of *Phragmites australis* (Ong *et al.* 2010).

Nitrite-nitrogen decreased in all treatments. The planted ebb and flow improved NO_2-N removal compared to the planted trickle filters. The NO_2-N in the measured incoming effluent does not include the NH_3-N oxidised to NO_2-N. Nitrite-nitrogen is formed by the oxidation of NH_3-N in the first step of nitrification (Bernhard 2010). This NO_2-N is not quantified in the incoming effluent as it is a product of a reaction within the system (Dong and Sun 2007). The treatments with higher NH_3-N removal would have additional NO_2-N produced during the oxidation process. This is substantiated by the positive correlation between NH_3-N and NO_2-N in all treatments described in the results.

The NO_3-N increased in all treatments. The unplanted ebb and flow bioreactors showed the largest increase in the treatments. The unplanted trickle showed the lowest increase. Dissolved oxygen concentration and nitrification can both affect denitrification in a system (Sun *et al.* 2005, Murphy *et al.* 2016). An increase in oxygen can have adverse effects on denitrifying bacteria populations, decreasing the denitrification occurring in the system (Dong and Sun 2007, Murphy *et al.* 2016). Nitrate-nitrogen is a product of nitrification (Vymazal 2010). If sufficient denitrification does not take place a build-up of NO_3-N can occur (Saeed and Sun 2012). The treatment with the lowest increase of NO_3-N, which was the unplanted trickle filter, had the lowest addition of DO and the lowest decrease in NH_3-N. The unplanted ebb and flow, which had the highest increase in NO_3-N, had the best addition of DO and the second-highest decrease in NH_3-N. However, regression analysis did not find a significant relationship between NO_3-N and DO or NH_3-N.

In the planted filters, ebb and flow and trickle filters, the NO_2-N removal showed large variability compared to the NO_3-N in the same treatments. The ebb and flow showed the largest decrease in NO_2-N and that was followed by an increase in NO_3-N which could be attributed to the NO_3-N formed during the oxidation of NO_2-N. Whereas the trickle treatment had the least change in NO_2-N and a lower increase in NO_3-N. This shows that less NO_2-N was oxidised to NO_3-N. Although the NO_2-N change was the highest in the planted ebb and flow treatment, the unplanted ebb and flow had a higher NO_3-N change. This can be explained by the NO_3-N uptake by the celery. The unplanted trickle treatment, however, had lower NO_3-N than the planted treatment which may indicate that the celery was not taking up enough nitrogen to be sustainable.

All treatments were able to lower COD. The unplanted trickle filter was the least effective at decreasing COD. Lower COD is seen in aerated horizontal subsurface CWs (Ouellet-Plamondon *et al.* 2006, Li *et al.* 2014). The unplanted trickle filter saw the least addition of DO compared to the other treatments, this could lead to the higher COD than in other treatments.

The treatments did not affect the PO_4^{3-}. While there has been some success using biofilters to decrease PO_4^{3-} these results are in line with the findings of the previous trial where a PO_4^{3-}

decrease only occurred in gravel biofilters with both plants and worms (Section 3.4.1, Chapter 3; Ong *et al*. 2010, Sun *et al*. 2021).

The mineral composition of the celery varied between treatments. Nitrogen, Fe and Mn were higher in the ebb and flow treatment than in the trickle treatment. Both treatments had higher concentrations than those found by Singh *et al.* 2022 when comparing the mineral content of stalks, leaves and seeds in 20 celery genotypes. Excess N can reduce flower and fruit production (Rao 2009). This would not be relevant to celery production at the brewery as celery would ideally be harvested before flower or fruit production if the plants were being harvested for sale (AgMRC 2021). Iron in large quantities can inhibit growth however, the Fe content is highly regulated in plants, and it is rare to see Fe in excess (Kim and Guerinot 2007, Adamski 2011). Iron concentration can be up to 250 mg/kg in many plants (Rao 2009). Although the Fe content in this study is higher than Singh *et al.* 2022, this does not indicate an excess of Fe as plants have various control mechanisms to increase or decrease their Fe content (Kim and Guerinot 2007, Adamski 2011). Manganese is a micronutrient but in large quantities it can have a variety of adverse effects on plants such as inhibition of growth and delayed seed germination (Rao 2009). These effects were not seen in the growth between treatments, as both treatments had similar weight and growth rates even though the ebb and flow treatment had higher Mn. This indicates that the Mn concentration in the study was not excessive.

Other minerals that were higher in this study compared to Singh *et al.* 2022 were P, K and Na. Phosphorus can inhibit enzymatic reactions and reduce Zn uptake (Yoneyama 1988, Imran *et al.* 2016). Potassium in high quantities is not dangerous to the health of a plant but the uptake of K can be inhibited by excessive Na (Rao 2009). Although Na appears in high quantities in the plants of this study it has not adversely affect the uptake of K.

Calcium, Cu and Zn are essential nutrients for plant growth (Rao 2009). These minerals were lower in the trickle treatment than in the ebb and flow. Both treatments had concentrations lower than Singh *et al.* 2022. A Ca deficiency can prevent new growth, low Cu can cause dieback of shoot tips and Zn deficiency results in mottled leaves (Rao 2009). Although there were differences in mineral content between treatments, they did not impact the growth of the plants as no differences were found in final weight or relative growth rate between treatments.

4.6 Conclusion

All treatments decreased the levels of nitrogen in the effluent, however, the ebb and flow filters showed higher DO than the trickle treatments as well as increased NH_3-N removal. The celery in the systems did affect nutrient removal, increasing dissolved oxygen, denitrification and the removal of NH_3-N in the trickle filters. Plants also affected the ebb and flow filters, leading to increased DO, however the unplanted filters performed better at removing NH_3-N and denitrification.

The celery showed higher levels of N, Fe and Mn in the ebb and flow treatment but no difference in mass or growth. The production of a celery is viable in both systems.

For all parameters the ebb and flow bioreactors had the same or better performance than the trickle bioreactors; they led to more efficient water treatment.

Chapter 5: Discussion

Ebb and flow filters as additions to constructed wetland (CW) cells have the potential to increase nutrient removal from brewery wastewater coupled with the ability to support economic crop production. Nutrient removal by ebb and flow filters was demonstrated in Chapters 3 and 4 where individual filters were able to reduce the ammonia load by 2.2 - 4.6 mg/L in one cycle. Adding these filters to the wetland cells allows for multiple cycles through many of the filters, with the potential of increasing the wetland cells' capacity for removing nutrients from brewery effluent.

In Chapters 3 and 4 Swiss chard and celery were successfully grown with the effluent providing nutrients to the plants, however more testing should be done on the health of the plants to ensure they can survive. These crops had varying effects on the nutrient removal capacity of the filters, and it was possible to produce them using brewery effluent as water and nutrient supply.

5.1 Oxygenation in constructed wetlands

Oxygen addition to CWs is well studied and a key method for improving nutrient removal in effluent treatment systems (Sun *et al.* 2005, Vymazal 2007, Nivala *et al.* 2013). Artificial aeration of horizontal subsurface CWs resulted in lower chemical oxygen demand (COD), NH_4-N and NO_3-N (Ouellet-Plamondon *et al.* 2006). Total nitrogen (TN) and COD were decreased by 70.3% and 24.6% respectively in a horizontal subsurface flow CW (Li *et al.* 2014). When the CW was aerated, the removal increased to 51.3 % for TN and 90.1 % for COD (Li *et al.* 2014). The method of oxygen addition can affect the success of nutrient removal (Ilyas and Masih 2017). Wetlands can be aerated in various ways; artificial aeration by blowers and nanobubbles, tidal action, and drop aeration (Nivala *et al.* 2013, Ilyas and Masih 2017, Xiao and Xu 2020). The placement and timing of the aeration can also affect the success of these methods (Wu *et al.* 2016, Ilyas and Masih 2017). Intermittent aeration removed between 85.83 and 87.88 % of NH_4-N compared to tidal flow systems which removed 96.19 and 98.30 % in a study on vertical flow subsurface CWs by Li *et al.* 2019.

When dissolved oxygen (DO) was measured between the inflow and the outflow from a wetland cell with nanobubble aeration and another with ebb and flow filtration a decrease in DO was found. When further investigation was done measuring DO from the ebb and flow filters rather than the cell all filters increased DO. This indicates that ebb and flow filters do oxygenate the effluent however that DO is used up by further nutrient cycling in the wetland cell.

The tidal cycle of the ebb and flow filters increased oxygen more than the trickle system tested in Chapter 4.

Celery improved the DO concentration in the ebb and flow filters whereas Swiss chard did not affect the DO in the filters. Different plant species are known to have different rates of oxygenation loss into surrounding water (Lai *et al.* 2012). The amount of oxygen that diffuses from a plant's roots is dependent on the root permeability, size, and length as well as the internal oxygen concentration (Sorrell and Armstrong 1994). These variables can change under different growing conditions (Santamaria *et al.* 1999). The root system of celery is shorter and thicker than that of Swiss chard when exposed to high levels of ammonium and NO_3-N (Santamaria *et al.* 1999).

The two studies were run on different flow rates which could impact the differences a plant makes to these filtration systems. The dry weight, root length and leaf surface area of Swiss chard were studied under different flow rates by Baiyin *et al.* 2021. It was found that these parameters increased when the flow rate was increased from 2 to 4 L/min (Baiyin *et al.* 2021). However, these parameters decreased when the rate was further increased to 8 L/min (Baiyin *et al.* 2021).

The flow rates and different plant species used account for the differences in oxygen addition. This could be researched in the future testing different plant species in the system under the same conditions rather than in separate studies.

Ebb and flow filters are the best options for adding DO to the constructed wetland (CW) at Ibhayi Brewery. Dissolved oxygen and nutrient removal were correlated in only one instance where NH_3-N was inversely correlated with DO. A similar result was found in a previous study carried out at Ibhayi Brewery on a smaller scale which found an inverse relationship between DO and ammonia concentration (de Jong 2019).

5.2 Additions to ebb and flow filters

Plants are commonly added to CWs to improve nutrient removal (Stottmeister *et al.* 2003, Vymazal 2011). Wetland-specific plants are often used (Brix 2003). However, economic crops such as cabbage (*Brassica oleracea*), tomatoes (*Lycopersicum escolentum*) and Swiss chard have been successfully grown with the effluent from Ibhayi Brewery (Power and Jones 2016, Taylor *et al.* 2018, de Jong 2019, Mabasa 2021).

The combination of Swiss chard and earthworms in the ebb and flow filters decreased both the chemical oxygen demand (COD) and the PO_4^{3-}. No other treatment plan led to a decrease in PO_4^{3-} which is expected as phosphate does not readily adsorb to gravel (Mann and Bavor 1993, Mann 1997). Better results have been found with steel slag media with phosphate accumulating organisms, with an 80 % phosphate removal (Sun *et al.* 2021). Chemical oxygen demand was lowered by both horizontal subsurface flow wetland cells; one with nanobubble aeration and the other with ebb and flow filtration. Additions of worms or plants separately did not influence the COD. The effects caused by the combination of plants and worms were not found in other studies. This was not further investigated in this study due to the issues in containing and quantifying the worms encountered in Chapter 3.

Swiss chard alone did not affect nutrient removal more than treatments without plants. This may be because not enough plants were grown compared to the effluent quantity and quality. Celery was able to affect nutrient removal, running smaller trials with a variety of plants to discover the most effective crop may be valuable research for Ibhayi Brewery.

5.3 Future recommendations and research

Substrate type can also play an important role in nutrient removal. The ebb and flow filters without both Swiss chard and earthworms did not affect the PO_4^{3-}. The substrate used in the ebb and flow filters could aid in a reduction of PO_4^{3-} (Sun *et al*. 2021, Wang *et al*. 2013). Broken oyster shells showed the highest capacity for phosphorus when compared to broken bricks, volcanics and zeolite (Wang *et al.* 2013). All the mentioned substrates were able to adsorb phosphorus and were suitable for plant growth (Want *et al.* 2013). Oyster shells and broken bricks are by-products increasing sustainability and keeping costs low (Wang *et al.* 2013). The reduction of total Kjeldahl nitrogen and total phosphorus were 2.64 g/m^2 and 0.2 g/m^2, respectively when using clay bricks (Marcelino *et al.* 2020). Recycled concrete aggregates have shown phosphorus removal of 89.5 % (Li *et al.* 2021). Combining polystyrene with traditional CW fillers such as gravel was found to reduce COD, ammonia and phosphate (Khalifa *et al.* 2020). A soil substrate could also be utilised to further investigate the impacts on worms in these systems which showed promise but many of the benefits of worms are seen in soil not in the gravel substrate used here (Nuengjamnong *et* *al*. 2011, Attala *et al*. 2021). Further investigation into different types of substrate in the Ibhayi Brewery system could be beneficial to the treatment of the effluent, especially when using by-product media such as construction waste and plastics.

Studying the whole wetland system at multiple points could also give better insight into how and where oxygen is being utilised. The ebb and flow filters could also be more thoroughly examined through multiple cycles as this project focused on the efficiency of only one cycle.

Using the nanobubble pumps in conjunction with the ebb and flow filters may be of economic value to Ibhayi Brewery. These filters were already purchased and installed. While they did not provide enough DO to adequately filter the effluent, they could be used to pump aerated effluent to the ebb and flow filters without incurring more cost.

5.4 Conclusion

The addition of ebb and flow filters to the CW at Ibhayi Brewery has the potential to improve nutrient removal, increase DO and allow effluent to be reused. Ebb and flow filters are better at aerating brewery effluent than nanobubble pumps and trickle filters. The ebb and flow filters had the highest nutrient removal capacity of the systems tested and many of these filters can be added to a single wetland cell. The removal of nutrients from the effluent allows it to be reused, decreasing the water costs of the brewery.

Both ebb and flow and trickle filters provided the necessary nutrients to the plants. Although there was limited evidence of plants positively impacting the nutrient removal in these systems. Economic crops can contribute to the system by providing an additional revenue source, creating jobs, increasing local food security, and improving the aesthetic of the CW.

The presence of worm eggs indicates the ability of worms to be sustained in the wetland system. The inability to appropriately quantify the abundance of worms in the system did not allow this to be studied further.

Implementing the ebb and flow filtration system can benefit Ibhayi Brewery by treating effluent and providing a crop with economic potential.

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