THE EFFECT OF EBB-AND-FLOW TECHNOLOGY, SUBSTRATE TYPES AND SALT-TOLERANT CROP (*Apium graveolens* L.) ON NUTRIENT REMOVAL FROM A BREWERY EFFLUENT

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DECLARATION

Declaration by the candidate

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DEDICATION

I dedicate this work to Almighty God, for His grace has been sufficient. I also dedicate this thesis to my late father, ZB Benjamin Obado, a philomath, to my beloved Mum, Mary Obado, to my siblings, Philip, Emma, Anne, Job, and Nicholas, to my son, Curtis, my nephew and nieces, Carson, Debra and Meghan.

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ABSTRACT

Brewing industries face economic and environmental challenges of water use, energy consumption, and effluent disposal. The limited water availability and strict effluent discharge regulations in the South African Brewery justify wastewater treatment and recycling. Constructed wetland (CW) design and operation have been optimised for sustainable wastewater treatment. An ebb-and-flow technology is a CW design that creates aerobic and anaerobic conditions essential for wastewater treatment. South African Ibhayi Brewery effluent is treated onsite using a commercial-scale anaerobic digester (AD) and activated sludge units. However, the anaerobically digested effluent has high treatment costs and does not meet the environmental discharge standards. Therefore, the post-AD effluent is further treated using a low-cost CW technology. This study investigated the effect of ebb-and-flow CW operation, substrate types and celery plant growth on nutrient removal from a post-AD brewery effluent. Two experiments were conducted; Experiment 1 examined the effect of retention times (10, 20 and 40 minutes) and gravel sizes (7, 13, and 19 mm) on nutrient removal. The retention times (RT) and gravel sizes were allocated as treatment combinations and replicated thrice in a completely randomised design. In the second experiment, an ebb-and-flow CW was used to investigate the effect of media types planted with a celery crop on nutrient removal. Eight media (clay pebbles, clay bricks, sand, bioballs, recycled plastic, and gravel sizes), either alone or mixed, were tested. A 2:1:1 ratio of each media, pine bark and granular activated carbon, respectively, was used for mixed treatments. A uniform celery planting density of eight seedlings per m^2 and a 12-minute effluent retention time were used. Results for experiment 1 indicated significant differences at $p \leq 0.05$ between RT and gravel size treatment combinations on nutrient removal. The 10-minute RT at 19-mm gravel achieved the highest reduction in chemical oxygen demand (COD) of 8.2 %, 7.7 % ammonia-N and 38 % total inorganic nitrogen removal. The 40-minute RT at 7-mm gravel had the highest nitrate-N removal of 18.6 %. Orthophosphate removal was below 5 % in all treatments. The peak removal for ammonia and total inorganic nitrogen was after 8 and 10 weeks, respectively, suggesting that the efficiency of the ebb-and-flow design on nutrient removal is time-dependent. The aerobic and anaerobic conditions of ebb-and-flow operation, gravel surface area and effluent retention time influence nutrient transformation and removal. Results for Experiment 2 indicated significant differences between unmixed and mixed media on nutrient removal ($p \leq 0.05$). The unmixed media of clay pebbles had the highest mean reduction chemical oxygen demand of 7.5% and 8.1% ammonia-N removal. Mixing resulted in better overall mean removal efficiency of nitrite-N (7.6%), nitrate-N (15.3%), total inorganic nitrogen (29.9%), orthophosphate (12.7%) and plant biomass (11158.5 gm^{-2}) than unmixed media. Media porosity, surface area, chemical composition and celery plant growth enhanced nutrient removal through synergistic interactions. Experiment 1 recommends a short effluent retention time of 10 minutes on 19-mm gravel for improved nutrient removal in the ebb-and-flow system design. Experiment 2 recommends unmixed clay pebbles, bioballs, and 19-mm gravel for improved ammonia-N removal and mixed media for multiple pollutant removal and celery productivity in the ebb-and-flow constructed wetland.

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LIST OF ABBREVIATIONS AND ACRONYMS

AD	Anaerobic digester
AOB	Ammonia-oxidising bacteria
ANOVA	Analysis of variance
CCI	Chlorophyll concentration index
CEC	Cation exchange capacity
COD	Chemical oxygen demand
CW	Constructed wetland
CWs	Constructed wetlands
DO	Dissolved oxygen
DIFS	Department of ichthyology and fisheries science
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organisation
GAC	Granular activated carbon
HF	Horizontal flow
HRT	Hydraulic retention time
HRL	Hydraulic loading rate
HN-AD	Heterotrophic nitrification anaerobic denitrification
Mv	Millivolts
NWA	National Water Act
NOB	Nitrite oxidizing Bacteria
Pty	Proprietary Limited
PVC	Polyvinyl chloride
PCA	Principal component analysis
PC	Principal component
RT	Retention time
RTs	Retention times
SAB Ltd	South African Brewery Limited
SSF	Subsurface flow
TIN	Total inorganic nitrogen
USEPA	United states Environmental protection Agency
VF	Vertical flow
VFA	Volatile Fatty Acids
WEI	Water exploitation index

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Water is a worldwide scarce resource and its availability affects economic, social and environmental sustainability (Naik, 2017; Manungufala, 2021). Physical water scarcity occurs when climate change causes temperature rises and changes in rainfall patterns. This can be mitigated through effective water management practices (Naik, 2017). Economic scarcity refers to the limited human capacity to utilize and invest in water resources (Perveen & James, 2011; Manungufala, 2021). Nearly half of the world's population is experiencing water scarcity and the global water demand will rise to 55 % by the year 2050 (Almuktar *et al.*, 2018). In European Union, 11 % of the European Union population is already under a water crisis (European Environmental Agency, 2010; Cui et al., 2018). The Water Exploitation Index (WEI) states that 20 % of the Mediterranean population experiences constant water scarcity, affecting 50 % of the population during summer (European Environment Agency, 2007; Cui et al., 2018; Jelonek *et al.*, 2020). About 250 million people in Africa will be experiencing water shortages by the year 2030 (Food and Agriculture Organisation, 2013).

The global growth in human population, industries, urbanisation, and agricultural activities increases the demand for freshwater while reducing its availability (Almuktar *et al.*, 2018; Biswas *et al.*, 2021). Food and beverage processing industries are among the main sources of pollution due to high water consumption and the release of large volumes of wastewater to the environment (Karambiri, 2018; Schneider *et al.*, 2021). Industrial effluent contains high concentrations of pollutants that are detrimental to aquatic life when directly discharged into natural ecosystems (Simate *et al.*, 2011; Jaiyeola & Bwapwa, 2016).

Circular management approaches for water, energy, and nutrients have been proposed in modern technologies to minimise environmental degradation (Schneider *et al.*, 2021). Green infrastructure is an approach that promotes the recycling and recovery of resources to derive ecological, economic, social and complementary benefits (Mell, 2008; Stefanakis, 2019). Urban agriculture is bridging the food demand gap by recovering nutrients from wastewater to produce crops (Badejo *et al*., 2014; Mohareb *et al*., 2017). The urban farming methods that recycle and recover nutrients comprise aquaponics, hydroponics, recirculating systems and permaculture (Palm *et al.*, 2018; Lennard & Ward, 2019; Schneider *et al.*, 2021).

South Africa is a water-scarce country with competing water uses for economic development (Cilliers, 2012). Wastewater management is a practical problem faced by food and beverage processing industries. South African Breweries (SAB) (Pty, Ltd) produces more than 1.8 billion hectoliters of wastewater per year (Fillaudeau *et al.*, 2006). The average usage by the SAB Miller group is about 4.6 L of water per litre of beer produced. Large volumes of wastewater originate from the brewing process, cooling, cleaning and sanitation (Simate *et al.*, 2011). About 70 % of the water used in the brewing process goes to waste (Jones *et al.*, 2014).

South African Breweries' Miller group reported that about 7 billion liters of water can be recovered from the brewery plant (Jaiyeola & Bwapwa, 2016). Water use was reduced from 4.6 L to 3.6 L for every liters of beer produced by applying a sustainable strategy of ‘make more beer using less water’ (Jaiyeola & Bwapwa, 2016). The high concentration of nutrients in the brewery effluent requires appropriate treatment before discharge into the waterbodies (Badejo *et al.*, 2014). The South Africa National Water Act established wastewater discharge standards on water parameters such as ammonia, phosphorus, organic matter, pH, temperature, suspended solids and heavy metals to protect natural waterbodies (Ramukhwatho *et al.*, 2016).

Conventional wastewater treatment methods include anaerobic digesters (AD), activated sludge and membrane filtration. These methods require high capital, energy, operation and maintenance costs (Tauseef *et al.*, 2013; Badejo *et al.*, 2014; Jaiyeola & Bwapwa, 2016). The limited water availability, high treatment costs and strict wastewater discharge regulations justify onsite treatment and reuse of brewery effluent (Götz *et al.*, 2014; Jaiyeola & Bwapwa, 2016).

South African Ibhayi Brewery effluent is treated on the brewery site using a commercial-scale anaerobic digester and activated sludge units before being channeled to the municipal sewerage treatment plant. A portion of the post-anaerobic digester (AD) effluent is further treated and reused in a research facility at the brewery using low-cost alternative technologies of primary facultative ponds, high-rate algal ponds and constructed wetlands (Jones *et al.*, 2014; Power & Jones, 2016).

A constructed wetland (CW) is an engineered wastewater treatment technology that utilises natural processes through the interconnected and concerted actions of a substrate, microorganisms, and plants (Vymazal, 2007; Faulwetter *et al.*, 2009; Saeed & Sun, 2012). This technology employs physicochemical (filtration, adsorption, precipitation, evapotranspiration, photodegradation) and microbial (degradation, nitrification, anaerobic ammonia oxidation, denitrification) processes to remove pollutants (Cui *et al.*, 2013; De

La Varga *et al*., 2016). Wetlands are designed and operated to mimic the unique ecological processes of the natural ecosystem (Vymazal, 2011; Sandoval-Herazo *et al.*, 2018). Constructed wetlands have a high efficiency for pollutant removal at a low energy consumption and maintenance costs (Eck *et al.*, 2019; Rahman et al., 2020). Garfí *et al.* (2017), in a life cycle analysis comparative study for wastewater treatment, reported activated sludge conventional system had an environmental impact that was 2 to 5 times higher than that of the constructed wetlands, which had a smaller footprint in terms of capital, operation and maintenance costs. CWs have been used for treating domestic, industrial, agricultural and urban runoffs wastewater (García *et al.*, 2010; Vymazal, 2014; Almuktar *et al.*, 2015). Constructed wetlands work similarly to natural wetlands processes in a more controlled environment, which can be optimised to achieve high pollutant removal (Dordio & Carvalho, 2013). The efficiency of CWs in pollutant removal relies on wetland design, operation, and environmental factors (Saeed & Sun, 2012; Wu *et al.*, 2015; Kumar & Dutta, 2019).

Substrate or media is an important wetland design component where biological, chemical and physical reactions take place to remove pollutants (Vymazal, 2011). Substrates are classified by their physical, chemical, and biological properties (Wu *et al.*, 2015). The physical properties are particle size, specific surface area, shape, porosity, hydraulic and electrical conductivity, and mechanical strength. Chemical properties are their ion exchange capacity and acid-base properties, while biological aspects include electron donors and acceptors properties (Ge *et al.*, 2015; Yang *et al.*, 2018). The choice of CW substrate affects effluent treatment efficiency, investment cost and sustainability (Ji *et al.*, 2022).

Microorganisms are the key drivers for the biological removal of pollutants under a wide range of environmental conditions in CWs (Faulwetter *et al.*, 2009; Meng *et al.*, 2014). The microbial degradation processes of respiration and fermentation are performed by bacteria, fungi, protozoa and archaea under aerobic and anaerobic conditions (Faulwetter *et al*., 2009; Meng *et al.,* 2014; Rose, 2021). Aerobic heterotrophs use oxygen in breaking down organic matter to produce energy, carbon dioxide and water while, anaerobic heterotrophs degrade organics through fermentation and methanogenesis (Faulwetter *et al*., 2009; Meng *et al*., 2014). The metabolism of microorganisms is mainly influenced by environmental factors such as oxygen, pH, temperature, salinity, and organic carbon availability (Truu *et al*., 2009; Meng *et al.,* 2014). Studies in microbial ecology increase the understanding of their functioning and are crucial for optimising wetland design and operation.

Vegetation is an important wetland component with various functions, including providing the surface for microorganisms to attach and nutrient adsorption sites (Vymazal, 2011). A beneficial relationship exists between plant roots and the supporting media in breaking down organic nutrients into simple forms for uptake (Vymazal, 2011). The length of time for contact between plant roots and the effluent influences the uptake of nutrients (Stottmeister et al., 2003). Wetland plants are very productive, and the amount of nutrients uptake by plants can be quantified as g per m^{-2} biomass gain (Vymazal, 2011; Jesus *et al.*, 2018), contributing to wetland design for wastewater treatment. Nutrients in pretreated brewery effluents have been used in producing hydroponic vegetables (Power & Jones, 2016; Taylor *et al.,* 2018). Optimising wetland design components can create an economic impact on wastewater treatment.

This study contributes to the scientific knowledge for addressing the innovative approaches of low-cost wastewater treatment techniques, water reuse and recovery of nutrients from brewery effluent for crop production. The study optimised wetland design and operation parameters by examining the combined effect of hydraulic retention time and substrate surface area on nutrient removal using ebb-and-flow constructed wetlands. The potential of various alternative substrates planted with celery (*Apium graveolens L*.) on nutrient removal from a post-anaerobic digester effluent was investigated in the ebb-and-flow CWs.

1.2 Statement of the problem

Water, energy consumption and effluent disposal are economic and environmental challenges for brewing industries (Masi *et al.,* 2015; Jaiyeola & Bwapwa, 2016; Junge *et al.*, 2017). Large volumes of water used in brewing increase the demand for freshwater (Simate *et al*., 2011; Jaiyeola & Bwapwa, 2016). Strict environmental effluent discharge standards and high treatment costs in municipal sewers have forced industries to treat effluents before discharge into natural water bodies. The onsite effluent treatment using an anaerobic digester and activated sludge at the South African Brewery has high energy consumption. Additionally, post-anaerobic digester effluent does not meet the discharge standards since it has high concentrations of organic matter, nitrogenous compounds and inorganic pollutants requiring further treatment (Cilliers, 2012; Taylor *et al*., 2018).

High organic matter and nitrogenous compounds in the effluent stem from the raw materials and by-products of the brewing process, including mash, hops, and spent grains (Cilliers, 2012; Jaiyeola & Bwapwa, 2016). Cleaning agents such as sodium hydroxide, caustic soda, chlorine, calcium sulphate, and diatomaceous earth used in the brewery elevate the pH and electrical conductivity of the effluent (Fillaudeau *et al.,* 2006;

Senthilraja *et al*., 2013). The high salinity, indicated by electrical conductivity, has been reported to exceed 3000 µS/cm in the brewery effluent (Cilliers, 2012; Jones *et al.*, 2014).

Studies show that the efficiency of CWs in pollutant removal relies on wetland hydraulic design, substrates, plants and environmental variables (Saeed & Sun, 2012; Wu *et al*., 2015; Almuktar *et al.*, 2018). The hydraulic loading rate/ retention time is a principal parameter determining the space required for wetland operation. High loading rates reduce contact time between the effluent and substrate, thus compromising contaminant removal (Ghosh & Gopal, 2010). Low hydraulic loading effectively removes pollutants but requires a large area of land (Dong *et al.*, 2012). Due to the limited availability of land, CW hydraulic parameters can be optimized to achieve a high treatment efficiency and reduce land and space requirements.

Wetland substrate accounts for large operation and investment costs; consequently, an appropriate choice of substrate surface area can reduce the wetland area and at high removal efficiency (Dong *et al*., 2012; Zhang *et al*., 2021). The natural, industrial, and man-made products and by-products have shown potential as media for nutrient removal in CWs (Cheng *et al*., 2018; Wang *et al.*, 2020). For example, activated carbon is a stable substrate with a large surface area and a high adsorption capacity for pollutants. However, it has a high processing cost and is thus not viable for sustainable wastewater treatment (Fu *et al.*, 2020; Marlena & Nowicki, 2022). Recent studies have examined the potential of emerging substrates on a laboratory-scale treatment of synthetic effluents (Liu *et al.*, 2014; Kizito *et al*., 2017; Li *et al.*, 2019; Zhang *et al*., 2021). However, limited studies have utilized actual wastewater. Moreover, the use of alternative media alone or a combination has not been tested for the treatment of brewery effluent.

Brewery effluent is a potential water and nutrient source for plant irrigation (Jones *et al*., 2016; Taylor *et al*., 2018). However, high pH and salinity in the effluent are major constraints limiting the bioavailability of nutrients for plant growth (Power & Jones, 2016; Zhao et al., 2021). High salinity in the brewery effluent increases plant osmotic pressure and induces ionic stress (i.e. sodium ions) in the plant tissues (Su *et al*., 2020; Zhao *et al.*, 2020). The tolerance of plants to wastewater conditions is an important factor to consider when choosing plant types for use in a constructed wetland. The uptake of nutrients by plants in CW differs depending on plant type, species, effluent retention time and system design (Vymazal, 2011, Saeed & Sun, 2012). Therefore, a salt-tolerant celery crop can be suitable for growth in the high salinity brewery effluent.

Celery (*Apium graveolens* L.) is a salt-tolerant perennial food crop of the family Umbelliferae (Kooti & Daraei, 2017; Ashmawi, 2019). It has bioactive compounds comprising flavonoids, alkaloids, phenolic acids, terpenoids, and tannins, all with medicinal properties (Stephen *et al*., 2020). Despite its intrinsic properties and salt tolerance, celery has not been grown in the brewery effluent and thus, a need to test the potential growth and how it copes with the high salinity of the brewery effluent. The current study optimised a constructed wetland design by varying the hydraulic retention time, wetland substrate and incorporated celery plant crop to evaluate the potential of an ebb-and-flow system on nutrient removal.

1.3 Justification of the study

Wastewater treatment and recycling are the potential alternatives for addressing water scarcity and the high demand for industrial use (Almuktar *et al.*, 2015; Riera-Vila *et al.*, 2019). The high cost of wastewater treatment in municipal sewers necessitates alternative technologies for treating various effluents. Constructed wetlands have been used as a low-cost treatment method to enable reuse for secondary industrial activities while minimizing environmental pollution. Wetland components comprising substrate, microorganism, plants, and their interactive functions purify wastewater through chemical, physical, and biological processes.

Optimisation refers to making the best use of a resource. A constructed wetland system can be devised from a previously developed design to improve pollutant removal efficiency. This involves varying the design, operation parameters and wetland components to regulate the functioning of the CW. The design parameters can be examined/ over time to determine effective performance in wastewater treatment. Manipulating wetland operation variables can achieve a high treatment efficiency of South African Ibhayi brewery effluent on a small land area.

Ebb-and-flow/tidal wetland is an innovative technology that fills and drains wastewater, creating aerobic and anaerobic conditions essential for nutrient removal. This technology simultaneously transforms and removes multiple pollutants in a single compartment, thus reducing land area, space requirements and aeration costs. This system operation mimics natural ecosystems by promoting the establishment of diverse microbial consortia for enhanced removal of pollutants (Austin, 2006; Gregory, 2012). The operation efficiency of a tidal design is based on the number of fill-and-drain cycles, which is used to determine the hydraulic loading rate/ retention time (Austin 2006; Zhang *et al.,* 2021).

The hydraulic loading rate (HLR) of a CW is the average water flow rate determined in a wetland area. The hydraulic retention time (HRT) is the average time an effluent remains in the wetland bed, and is influenced by the wetland surface area, substrate porosity and flow depth (Wallace, 2009; Dong *et al.*, 2012). The laboratory and full-scale effluent treatment studies on wetland hydraulics have prompted further optimisation for improved efficiency of wastewater treatment (Dong *et al.*, 2012; Li *et al*., 2015; Al-wahaibi *et al*., 2021; Zhang *et al*., 2021).

A substrate is a bioreactor that interconnects with plants and microorganisms in a CW for improved wastewater treatment (Wang *et al.*, 2020). Substrates' physical and chemical properties are essential for removing specific or multiple pollutants. Substrates with large surface area and high cation exchange capacity can achieve high pollutant removal with small space requirements (Vohla *et al.*, 2011; Kizito *et al.*, 2017; Zhang *et al.*, 2021). Combining locally available materials and industrial by-products substrates in CWs has several advantages in wastewater treatment; the physicochemical characteristics complement each other in achieving desired properties for nutrient removal, investment and treatment costs are reduced, thereby adding economic value to waste materials (Enaime *et al.*, 2020).

Research is advancing on emerging wetland substrates based on availability, cost-effectiveness and competitive efficiency in pollutant removal, to outperform traditional sand and gravel substrates. Examples of emerged substrates are construction wastes, tyre chips, clay bricks, lightweight clay aggregates, polyethene terephthalate (plastics) and biochar (Liu *et al.*, 2014; Lima *et al.*, 2018; Sandoval 2019; Wang *et al*., 2020; Obeng *et al*., 2023). Organic wood mulch and pine bark are potential sources of organic carbon to support biological nutrient removal processes, compared to traditional substrates that lack carbon (Saeed and Sun, 2011, 2012; Yang *et al.*, 2018).

Wetland plants are essential in the recycling of wastewater and the recovery of nutrients. Brewery effluent is a unique effluent with a high nutrient load, high pH and salinity. The adjustments of a brewery effluent pH improved nutrient availability and led to the successful production of tomatoes, lettuce and cabbage (Power & Jones, 2016; Taylor *et al.*, 2018). Halophytes are plants adapted to high salinity environments and can benefit from irrigating saline brewery effluent for growth, survival and development. Salt-tolerant plants such as Swiss chard, maize, sunflower, sesame, lucerne, and saltbush have been grown using the brewery effluent (Senthilraja *et al.*, 2013; DeJong, 2019; Mabasa *et al.*, 2021). Cultivating salt-tolerant crops such as celery (*Apium graveolens* L.) in a CW is an economical method that can increase celery food crop production while treating high-salinity brewery effluent.

1.4 Aim and Objectives of the Study

This study aims to determine the optimal effluent retention time, suitable wetland substrate, and appropriate plant species to enhance the design and operational efficiency of an ebb-and-flow constructed wetland system for treating post-anaerobic digestion brewery effluent. This was achieved by addressing the following objectives;

1.4.1 General Objective

To optimize ebb-and-flow constructed wetland design by varying the hydraulic retention time, wetland substrate and celery plant crop on nutrient removal from a pretreated brewery effluent.

1.4.2 Specific Objectives

1. To determine optimal retention time and gravel particle sizes on nutrient removal in the ebb-and-flow constructed wetland

2. To determine the period of maximum nutrient removal for the retention time and gravel particle sizes in the ebb-and-flow constructed wetland
3. To determine a suitable media type for optimal nutrient removal in the ebb-and-flow constructed wetland
4. To determine the maximum period of nutrient removal for media types in the ebb-and-flow constructed wetland
5. To investigate celery growth, survival and chemical composition between media in the ebb-and-flow constructed wetland.

1.5 Hypotheses of the Study

H_{01}: Effluent retention time and gravel sizes do not affect the efficiency of nutrient removal in the ebb-and-flow constructed wetlands

H_{02}: The period of maximum nutrient removal does not differ between retention time and gravel sizes in the ebb-and-flow systems

H_{03}: Media types do not differ in their efficiency on nutrient removal in the ebb-and-flow wetland

H_{04}: The maximum period of nutrient removal does not differ between media types in the ebb-and-flow constructed wetlands

H_{05}: Celery growth, survival and chemical composition do not differ between media types in the ebb-and-flow constructed wetlands

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

It has been extensively discussed in the literature that the efficiency of CWs in pollutant removal relies on wetland design, operation, and environmental factors (Saeed & Sun, 2012; Dordio & Carvalho, 2013; Meng *et al.*, 2014; Wu *et al.*, 2015; De La Varga *et al.*, 2016; Almuktar *et al.*, 2018; Kumar & Dutta, 2019; Liu *et al.*, 2022). This literature review focuses on the evolution of a constructed wetland, nutrient removal processes in various wetland designs, and the roles of alternative wetland substrates and plants in nutrient removal. In addition, it discusses research progress on biological removal of nitrogen from wastewater and suggests optimised strategies with potential application in constructed wetlands. This review will help identify gaps for optimising wetland components to improve wastewater treatment efficiency using low-cost treatment technologies and potential applications for various effluents in future research.

2.2 Factors influencing nutrient removal in constructed wetland

2.2.1. The nature and strength of wastewater

The agricultural, domestic and industrial effluents have a varied concentration of organic and inorganic nutrient load (Vymazal, 2014). The effluent strength estimate concentration ranges from low chemical oxygen demand (COD) of about 700 mgL^{-1}, also known as ‘grey water’, to a stronger COD of around 35,000 mgL^{-1} called ‘black water’ with variations in daily composition (Tauseef *et al*., 2013; Sheela & Beebi, 2014). Wastewater from petroleum refineries contains organics up to 4000 mgL^{-1}, suspended solids, ammonia, oil and grease, heavy metals and phenolics (Wallace, 2009; Vymazal, 2014). Tannery wastewater has a high inorganic and organic loading of 3800 kg COD $ha^{-1}d^{-1}$ comprising suspended and dissolved solids, and high ammonia, nitrogen and chromium (Calheiros *et al.*, 2014). Acid mine drainage landfill leachates contain heavy metals such as iron (Fe), copper (cu), zinc (Zn), manganese (Mn), chromium (Cr), nickel (Ni), and lead (Pb). The concentrations of heavy metals in wastewater are lethal to aquatic ecosystems' health (Machemer & Wildeman, 1992; Saeed *et al.,* 2021).

Brewery wastewater contains high concentrations of ammonia, phosphorus and organic matter in the form of sugars, soluble starch, ethanol and volatile fatty acids, emanating from raw materials used in brewing (Taylor *et al*., 2018). The effluent has high salinity and pH, depending on the amount and type of chemicals used for brewing and cleaning (Simate *et al.*, 2011; Cilliers, 2012; Jaiyeola & Bwapwa, 2016) (Table 1).

Table 1: Characteristics of brewery wastewater (Rao et al., 2007; Simate *et al.*, 2011)

Parameter	**Value**
pH	3 - 12
Temperature (°C)	18 - 40
Chemical oxygen demand (COD) (mg L^{-1})	2000 - 6000
Biological oxygen Demand (BOD) (mg L^{-1})	1200 - 3600
COD: BOD ratio	1.667
Volatile fatty acid (VFA) (mg L^{-1})	1000 - 2500
Phosphates as PO_4 (mg L^{-1})	10 - 50
Total Kjeldahl Nitrogen (TKN)(mg L^{-1})	25 - 80
Total solids (TS) (mg L^{-1})	5100 - 8750
Total suspended solids (TSS) (mg L^{-1})	2901 - 3000
Total dissolved solids (TDS) (mg L^{-1})	2020 - 5940

2.2.2 Constructed wetland design for wastewater treatment

Constructed wetlands are classified based on hydrology, flow direction, vegetation types and hybrid systems (Vymazal, 2007; Wallace, 2009). There are two types of wetlands based on free water surface and subsurface water flow wetlands. Free water surface (FWS) flow wetlands consist of a shallow bottom with a water surface above the substrate and emergent vegetation (Truu *et al.,* 2009; Vymazal, 2010). Pollutants are removed through sedimentation, filtration, aggregation, denitrification and ammonia volatilisation and uptake by emergent plants (Vymazal, 2007, 2010). Sizing of FWS flow wetland is based on either volume or area; Volume-based methods use hydraulic retention time while area-based methods use the overall wetland area to determine pollutant removal (Wallace, 2009). Free water surface systems have high dissolved oxygen concentrations due to the rapid diffusion of atmospheric oxygen on the water surface (Vymazal, 2007). During the day, high ammonia removal occurs at a pH above 8 through volatilisation (Vymazal, 2011, 2014). The emergent plant litter upon decay, provides organic carbon to support denitrification (Vymazal, 2007, 2013). Phosphorus removal is low in FWS systems and only limited amounts can be utilised by plants (Vymazal, 2010).

Subsurface flow constructed wetland (SSFCW) is a wetland in which the bottom substrate is planted with different plant species (Truu *et al.,* 2009). Subsurface wetlands are classified as vertical and horizontal systems depending on the flow direction (Vymazal, 2010). These wetlands effectively remove suspended solids, organic pollutants and heavy metals (Vymazal, 2011). Removal of nitrogenous compounds depends on the availability of dissolved oxygen for nitrification and organic carbon to facilitate denitrification under anaerobic conditions (Babatunde *et al*., 2010). Pollutant removal in SSCWs is determined per m^2 surface area compared to free water surface wetlands that use volume-based methods (Saeed & Sun, 2012).

2.2.2.1 Horizontal flow (HF) subsurface constructed wetlands

Horizontal flow (HF) subsurface CWs are cells filled with gravel/rocks or soil and planted with vegetation (Vymazal, 2007). Water levels in the HF system are kept below the gravel to prevent algal growth, reduce fouling and vector breeding (De La Varga *et al.*, 2016). These systems have been used to treat wastewater from domestic sources, agricultural, industrial, landfill leachates and urban runoffs (Saeed & Sun, 2012; Calheiros *et al.*, 2014; Wu *et al.*, 2015). The effluent is fed at the inlet following a horizontal flow path in the substrate and is collected in the outlet drainage. A constant waterlogged condition in HF systems depletes dissolved oxygen creating anaerobic and anoxic conditions favourable for denitrification (Wallace, 2009; Vymazal, 2014; Zhang *et al.*, 2014). Organic pollutants are removed through filtration, settling, flocculation and biomass adsorption (Vymazal, 2014). HF wetlands are recommended for treating effluents with high nitrate-nitrogen concentrations through denitrification (Almuktar *et al.*, 2018).

Phosphorus removal in horizontal flow subsurface constructed wetlands (HSSF CWs) is low and relies on the physical and chemical properties of the wetland substrate (Gupta *et al.*, 2009; García *et al.*, 2010; Wang *et al*., 2020). Plants grown in HF have well-structured roots for microbes attachment and root aeration, and large above-ground biomass to insulate the substrate bed in the tropics (Wallace, 2009; Vymazal, 2011; Jesus *et al.*, 2018) (Figure 1). Land area requirement is a limitation for designing HFSS wetlands, therefore, VSSF is preferred due to its smaller footprints (Vymazal, 2011; Foladori *et al.*, 2013).

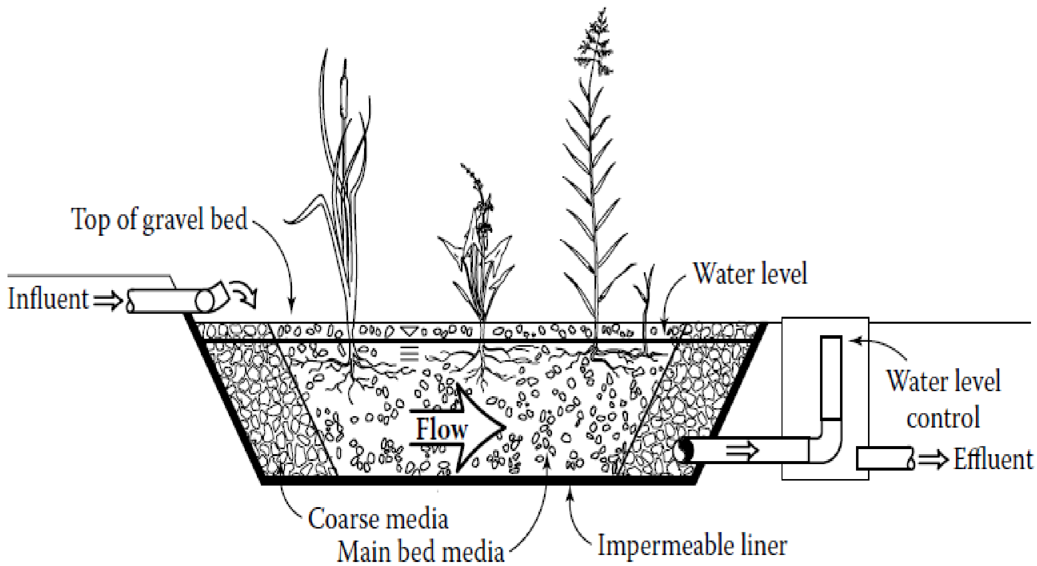


Figure 1: Horizontal subsurface flow constructed wetland (Wallace, 2009)

2.2.2.2 Vertical flow (VF) Constructed Wetland

Vertical flow (VF) subsurface wetlands have been used to treat various effluents due to their small footprint, ease of construction and low maintenance costs (Stefanakis *et al*., 2008; Jia *et al*., 2010). Vertical flow constructed wetlands (VFCWs) gained prominence after identifying low oxygen and water-logged conditions in HF wetlands (Stefanakis & Tsihrintzis, 2014). Water flow in VF is downwards by gravity through a porous substrate up to the bottom drainage pipe, and a new batch of wastewater is fed immediately after the substrate bed is drained off (Vymazal, 2007; Wallace, 2009). Periodical filling and draining improve oxygen transfer, within the substrate bed to facilitate oxidation of ammonia and organic matter (De La Varga *et al*., 2016). Efficient operation of VFCWs is achieved when wastewater is first screened to remove solid wastes (Stefanakis & Tsihrintzis, 2012).

Vertical flow CWs are categorised into three types depending on the hydraulic regime (Figure 2); up-flow, down-flow and tidal flow. Down-flow trickles wastewater down through the substrate and water can be recirculated (Wallace, 2009; Weinheimer, 2015). Up-flow effluent feeding is from the bottom and flows upwards until it floods the wetland surface. Wastewater feeding is stopped and the effluent is held for some time for interaction with microbes attached on the biofilm of the substrate, then draining occurs by gravity. During draining, atmospheric oxygen diffuses into the voids left in the porous substrate (Cui *et al.*, 2012; Li *et al.*, 2015). Liu *et al*. (2022) in a study on the effects of flow mode in tidal CWs on nutrient removal reported down-flow mode to be beneficial for the reduction of chemical oxygen demand (COD) (98.28–99.62%), while the up-flow mode favored the removal of total inorganic nitrogen (81.46–88.26%). High salinity limited the growth of nitrifying bacteria causing accumulation of ammonia and nitrite, while denitrifying bacteria were tolerant to high salinity levels. Therefore, water flow mode and salinity influence microorganisms' growth and metabolic activities. Figure 2 illustrates a down-flow VF system where effluent flows from the surface through different-sized gravel layers (Wallace, 2009, Almuktar *et al*., 2018).

The organic pollutants removal in VSSF relies on operation parameters i.e. hydraulic loading rate, effluent retention time, and influent feeding mode (Saeed & Sun, 2012; Li *et al*., 2015). Vertically fed systems have high oxygen transfer which facilitates nitrification, but with limited denitrification. Biological nitrogen removal in VSSF is often restricted by limited organic carbon to support denitrification (Fan *et al.*, 2013; Zhou *et al.*, 2017). Determining the organic carbon to nitrogen (C: N) ratio in an effluent is important in improving denitrification in VF constructed wetland systems (Fan *et al.*, 2013; Winkler & Straka, 2019).

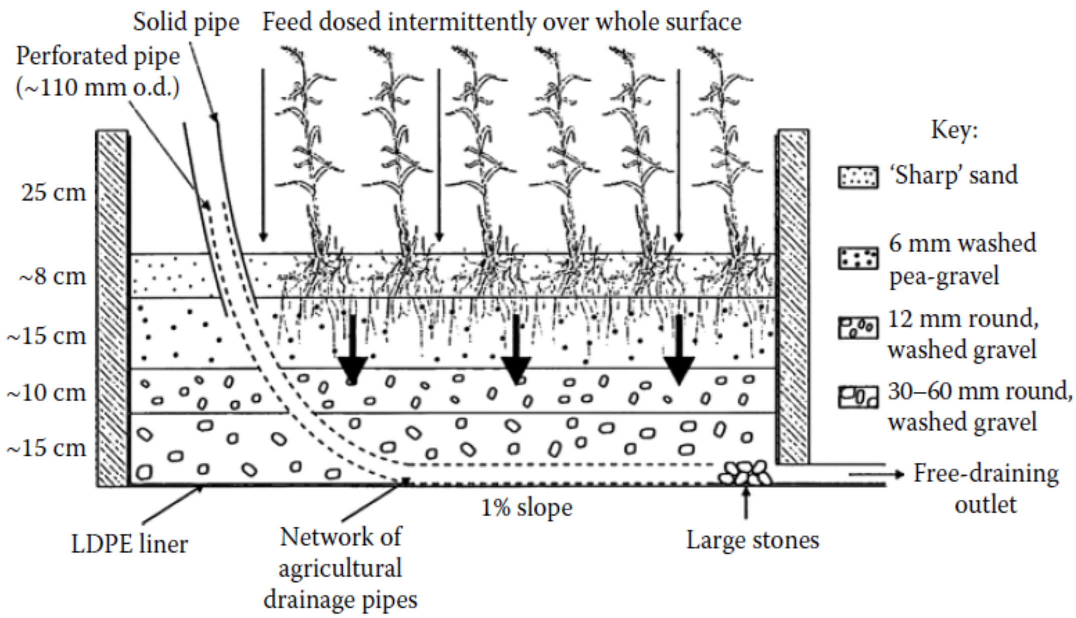


Figure 2: Vertical flow subsurface constructed wetland (Wallace, 2009; Almuktar et al., 2018).

Phosphorus removal in VFSS is low unless substrates with high adsorption capacity are used (Babatunde *et al.*, 2010). Clay pebbles, alum sludge, coal, and fly ash contain elements such as iron, aluminium, calcium, and magnesium for improved P removal efficiency (Babatunde *et al.*, 2009; Vohla *et al.*, 2011; Wang *et al.*, 2020). Phosphorus removal in CW is through adsorption, precipitation and ligand exchange reactions, whereby phosphate ions displace water or hydroxyls from the surface of either aluminium or aluminium hydroxide (Vymazal, 2007; Gupta, *et al.*, 2009).

2.2.2.3 Intermittent Aeration in Vertically Constructed Wetlands

Recent research on the operation of VFSS advanced the use of vertical systems with intermittent aeration for improved efficiency of removal of nitrogen and organic matter (Foladori *et al.*, 2013; Zhou *et al.*, 2017; Nivala *et al.*, 2019). The feeding mode of influent (continuous, batch and intermittent) influences the amount of oxygen transferred in the wetland bed. Jia *et al.* (2010) compared the intermittently aerated and continuously flooded vertical flow systems planted with *Phragmites australis*. The study reported 90% ammonia removal in the intermittent system, attributed to more oxidised conditions.

Caselles-Osorio & García (2007a) investigated the continuous and intermittent wastewater feeding and plant biomass in the horizontal flow system treating urban wastewater. The author reports above 80% ammonia removal, 0.58 to 0.67 g N/m^2/day in an intermittently fed system. The continuously fed system had low dissolved oxygen conditions, achieving 76% sulphate removal. Saeed & Sun (2011) also reported high chemical oxygen demand removal above 90% in intermittent operation with different flooding regimes. Denitrification is effectively achieved under anaerobic conditions with an adequate organic carbon supply (Hu *et al.,* 2012). Saeed & Sun, (2011) reported 99.6% of ammonia and 60.3% total phosphorus removal when using organic media (wood mulch) in VFSS. The high removal efficiencies were primarily due to higher dissolved oxygen conditions favouring nitrification, while organic mulch supplied organic carbon for total nitrogen and phosphorus removal. Studies recommend a 10:1 COD/N ratio and intermittent aeration for improved nitrogen removal in subsurface flow CW (Zhao *et al.,* 2010; Fan *et al.*, 2013; Chen *et al.,* 2018). Therefore, intermittent aeration and carbon supply improve the total nitrogen removal.

2.2.2.4 Tidal Flow Constructed Wetlands

The transformation steps involved in nitrogen removal have led to a combination of vertical flow (VF) and horizontal flow (HF) designs to provide aerobic and anaerobic conditions for nitrification and denitrification (Vymazal, 2007). This system design was devised to exploit the advantages of different wetland designs. Combining VF and HF has improved the efficiency of wetland operation through the simultaneous removal of multiple pollutants in a single compartment (Austin, 2006; Dong *et al.*, 2012; Weinheimer, 2015; Kizito *et al.*, 2017).

A tidal wetland is an innovative design that bridges the gap between highly engineered systems with less energy, space and operation costs (Austin & Nivala, 2008; Zhi *et al.*, 2015). The term “tidal flow”, also known as reciprocating flow, refers to an operation that allows the substrate to be filled with wastewater and completely drained repeatedly (Austin, 2006; Weinheimer, 2015). This system design increases oxygen transfer into the substrate for improved treatment performance (Li *et al.*, 2015). Flood and drain systems are also known as tidal flow wetlands with several flood and drain cycles (Behrends, 2000).

The tidal system design consists of adjacent wetland cells filled with gravel to create a habitat for microorganisms. The pair of wetland cells is serially connected with pumps and pipes and operates in phases of alternate flooding and draining (Borkar & Mahatme, 2015). During filling, oxygen is used up, while in the draining phase, atmospheric oxygen diffuses and occupies the spaces between the gravel substrate (Cui *et al.*, 2012).

A tidal flow CW acts like a pump in expelling water and drawing air into the CWs, improving nutrient removal through nitrification and denitrification processes (Austin &

Nivala, 2008; Zhang *et al.*, 2021). The aerobic and anaerobic phases created through subsequent filling and draining promote the establishment of diverse microorganisms for enhanced nutrient removal (Behrends, 1999; Austin, 2006; Weinheimer, 2015). The improved oxygen transfer in tidal systems has enhanced the oxidation of ammonia and organic matter (Austin, 2006). During flooding ammonium ions (NH_4^+) are adsorbed onto the biofilm attached to substrate surfaces (Austin & Nivala, 2008). When the wetland cell drains, atmospheric oxygen diffuses in the biofilm of the substrate to oxidise ammonium ions (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) through nitrification. In the next flood phase, nitrate (NO_3^-) and nitrite (NO_2^-) anions are released into the bulk water, which are terminal electron acceptors for microorganism respiration (Austin, 2006).

The efficiency of microbial activities in these systems depends on temperature, salinity, pH and the presence of organic carbon in the effluent (Truu *et al*., 2009). Lack of organic carbon limits denitrification depending on the nature of wastewater, substrate and operating conditions (Wang *et al.*, 2015; Xu *et al.*, 2019). The inflowing organic carbon from the effluent is an energy source for heterotrophic denitrification (Austin & Nivala, 2008; Borkar & Mahatme, 2015). Plants can be grown in the tidal wetland cells to utilise transformed nutrients from wastewater (Weinheimer, 2015).

2.2.2.5 Hybrid Constructed Wetland

The hybrid CW was developed in 1960 and gained popularity in 1990 -2000 due to the strict effluent discharge limits imposed by the environment regulating bodies (Vymazal, 2013). A hybrid wetland design consists of two stages of parallel vertical flow beds followed by two or three horizontal flow beds, all in a series. Vertical beds promote aerobic conditions to facilitate nitrification while horizontal bed with constant flooding create an anaerobic environment that favours nitrogen removal through denitrification. The hybrid design creates a redox environment for microbes to simultaneously remove contaminants such as ammonia, total nitrogen and phosphorus (Meng *et al*., 2014). The system treats sewage, industrial and municipal effluents (Vymazal, 2013, 2014).

The application of hybrid systems meets the effluent discharge standards; however, it is expensive and unaffordable in rural settings (Chen et al., 2014; Wu et al., 2015). Figure 3 illustrates pollutant removal in a combined vertical flow and horizontal bed for the hybrid wetland. Figure 4 is a summary diagram for the classification of various constructed wetlands due to the evolution and advanced research on nutrient removal.

2.2.3 Hydraulic Operation Variables

The term "hydraulic" refers to water movement through wetlands as determined by flow volumes, forces, velocities, rates, flow patterns and other characteristics (USEPA, 2000). Cilliers (2012) defined hydraulic retention time (HRT) as the average time an effluent remains in the wetland bed. The hydraulic loading rate (HLR) is determined by the average water flow rate (m^3d^{-1}) in a given wetland area (m^2), expressed in md^{-1} (Wallace, 2009). The HRT is determined by the wetland surface area, substrate porosity and flow depth (Dong *et al.*, 2012). These hydraulic variables influence the settling of solids and microbial metabolic processes in the wetland (Stefanakis, 2018).

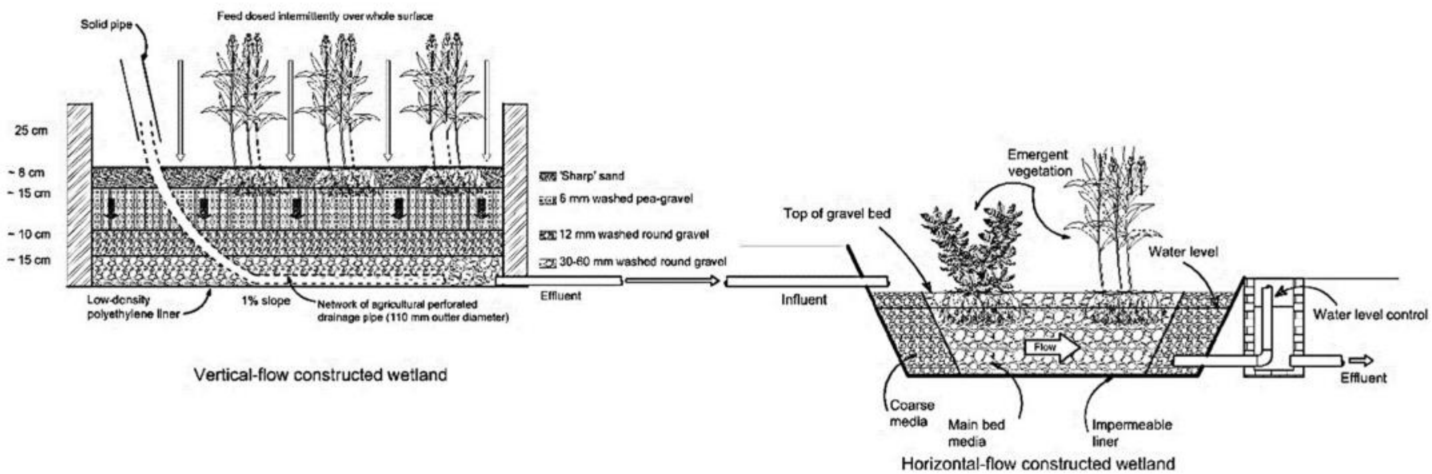


Figure 3: Design of a Hybrid constructed wetland (Almuktar *et al.*, 2018)

Past studies reveal that low hydraulic loading rates of effluent have high treatment efficiency (Stottmeister *et al.*, 2003; Akratos & Tsihrintzis, 2007; Kadlec *et al.*, 2010). However, the high cost of land limits wetland operation; thus, a high hydraulic loading points to a smaller land area requirement. Ghosh and Gopal (2010) evaluated four HRTs (1–4 days) with varied inflow rates of 300, 150,100 and 75 L d^{-1} in vertical wetlands planted with *Typha angustata* for secondary wastewater treatment. The 100% ammonia removal, above 90% for nitrate-nitrogen and Kjeldahl nitrogen, achieved at 4 days HRT was attributed to a combination of nitrification, denitrification, anaerobic ammonia oxidation and plant uptake processes.

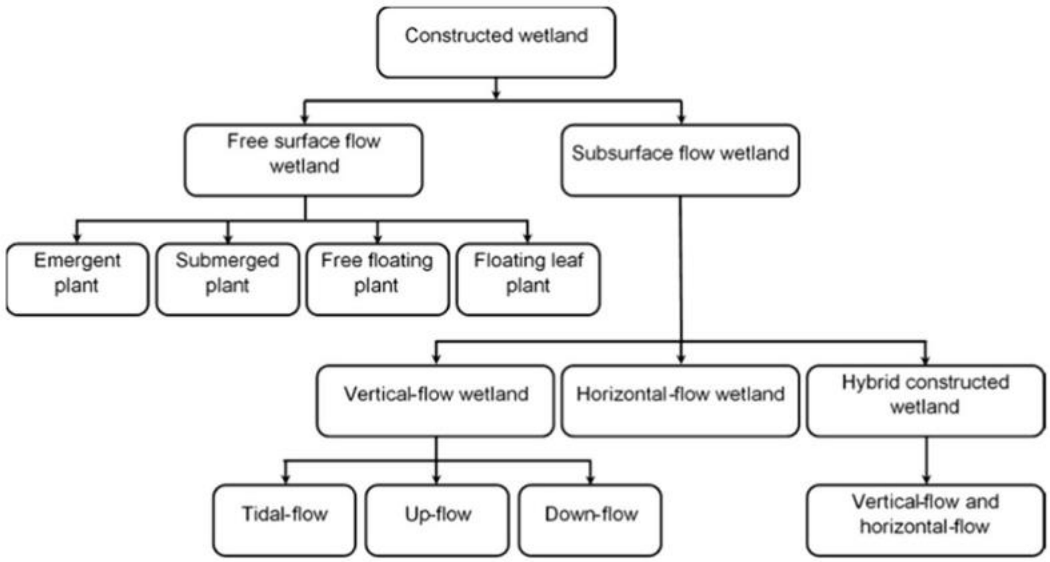


Figure 4: Classification of constructed wetlands for wastewater treatment (Vymazal, 2007).

The study recommended a shorter HRT to have higher treatment efficiency, which had a comparatively high treatment performance. Dong *et al*. (2012) examined nutrient removal efficiency in unaerated, continuous and intermittently aerated CWs, operated at hydraulic loading rates (HLR) of 19, 38 and 76 cm day^{-1}. High removal efficiencies above 80 % for ammonia and chemical oxygen demand (COD) were achieved at an HLR of 19 cm day^{-1} in a continuously aerated system. The intermittently aerated system at HLR of 38 cm day^{-1} had the highest total nitrogen removal of 57 % at reduced operation costs. Fan et al. (2013) further reported that an intermittent aerated system with an influent carbon supply ratio of 10:1 COD/N ratio achieved multiple pollutant removals of 99 %, 96 %, and 90 % for ammonia-N, COD and TN, respectively (Appendix I).

Studies have demonstrated that high hydraulic loading rates improve oxygen transfer with high treatment efficiency at low operation costs. Li *et al*. (2015) investigated nitrogen removal in a tidal CW under varying effluent filling times of 12 to 48 h. The system achieved a 77–94 % reduction of COD, 55–82 % ammonia and 60–84 % total nitrogen removal, thus eliminating the need for costly aeration. The fill and drain cycles of tidal wetland systems have been used to determine the hydraulic loading rate and amount of oxygen transferred. DeJong (2019) examined the effect of filling and draining time (FDT) (15, 30, and 60 minutes) on dissolved oxygen profiles and nutrient removal in the effluent of an anaerobic brewery digester using a tidal CW filled with 19-mm gravel substrate. The highest dissolved oxygen concentration was at 15 minutes FDT (6.52 ± 0.09 mg L^{-1}) followed by 30 and 60 minutes FDTs with (5.74 ± 0.09 mg L^{-1}) and (5.40 ± 0.09 mg L^{-1}), respectively. High dissolved oxygen concentration in 15- and 30-minute FDTs removed ammonia faster than in the 60-minute cycles of tidal CWs. Therefore, dissolved oxygen levels are a key factor in ammonia-nitrogen removal.

High porosity and cation exchange capacity substrates have shown higher treatment efficiencies, with the feasibility of short effluent retention time in tidal wetlands (Liu *et al.*, 2014; Kizito *et al*., 2017; Li *et al.*, 2019; Zhang *et al*., 2021). A rapid nitrification in the draining phase facilitates regeneration of the adsorption sites. The organic matter biodegradation complements the process for a higher treatment efficiency and prolonged period (Liu *et al*., 2014; Kizito *et al*., 2017). Therefore, a short effluent retention time in a tidal system design has complementary benefits of multiple pollutant removal, and low operation costs with potential application in large-scale systems (Appendix I).

2.2.4 Redox Condition/Potential in Wetlands

Wastewater treatment technologies have advanced the operation and design of CWs for removing specific or multiple pollutants (Faulwetter *et al.*, 2009). Microbial activities are the cornerstone for water purification in advanced wastewater treatment technologies (Ojeda *et al.*, 2008; García *et al.*, 2010; Corbella *et al.*, 2014). Dissolved oxygen content in a wetland bed is an important aspect determining the conditions associated with wastewater purification (Szogi *et al.*, 2013). Oxidation-reduction is the chemical and microbial processes influencing wastewater treatment within a wetland substrate bed (Duˇseka *et al.*, 2008). Oxidation-reduction processes are characterised by oxidation (loss) and reduction (gain) of electrons under aerobic and anaerobic conditions.

During respiration, microorganisms induce electron transfer from organic carbon (high energy) to low-energy-state electron compound acceptors like oxygen (O_2), nitrite (NO_2^-), and nitrate (NO_3^-), and sulfate (SO_4^{2-}) to produce energy for growth and metabolic activities (Scholz & Lee, 2005; Faulwetter *et al*., 2009; Szogi *et al.*, 2013). These conditions can be determined by measuring the oxidation-reduction potential of wastewater (Scholz & Lee, 2005; Szogi *et al*., 2013). Subsurface flow CWs have a basic characteristic of redox conditions, enabling the occurrence of biological and physicochemical processes simultaneously (Ojeda *et al*., 2008; García *et al.*, 2010). Biochemical processes have different redox potentials with an oxidative state at +250 to +700 mV. The reductive state (anaerobic) is at +250 to −400 mV, with temperature and pH as the influencing factors (Bezbaruah & Zhang, 2004; Scholz & Lee, 2005; García *et al*., 2010).

High redox conditions are associated with an aerobic environment that promotes nitrification, while low redox conditions are related to anaerobic processes such as denitrification, methanogenesis and sulfate reduction (Faulwetter *et al*., 2009). Oxygen is used as an electron acceptor for aerobic respiration, and when depleted, anaerobic respiration takes place sequentially using nitrate, manganese oxide, iron oxide, sulfate and carbon dioxide (Patrick and Reddy 1985; Szogi et al., 2013). Under anaerobic conditions, about +250 mV nitrate (NO_3^-) is reduced to nitrite (NO_2^-) and then to nitrous oxide (N_2O) and nitrogen gas (N_2). The different redox potentials influence the distribution of specific microbes in CWs (Scholz & Lee, 2005; Paredes *et al.*, 2007; Truu *et al*., 2009).

Redox condition in SSF CWs is influenced by organic loading, mode of operation (tidal/ intermittent, batch, continuous feeding), presence of plant roots (rhizosphere) and water depth (Wallace, 2009; Faulwetter *et al*., 2009; García *et al.*, 2010). Both batch and intermittent effluent feeding create a temporary redox environment within the substrate that favours the survival of various species of microorganisms (Caselles-Osorio & García, 2007; Meng *et al*., 2014). Manipulating the redox condition in tidal systems improve the activities of microbes in removing pollutants (Wu *et al*., 2014; Wu *et al*., 2015). Adjusting the hydraulics and providing a suitable substrate and plant species improved the redox state (Caselles-Osorio & García, 2007a; Hu *et al.*, 2012; Meng *et al*., 2014). Therefore, redox conditions can be manipulated in a wetland to facilitate the growth of specific microbial groups in removing selected pollutants.

2.2.5 Wastewater Recirculation

Recirculation of wastewater is a potential technology for treating large volumes of effluents (Cui *et al*., 2012; Kumar & Dutta, 2019; Al-wahaibi *et al.*, 2021). It involves transferring a portion of treated effluent back into the influent of a wetland system (Wu *et al*., 2015). Effluent recirculation redistributes oxygen between treated and untreated water to increase interactions between microorganisms and nutrients (Lian-sheng *et al.,* 2006; Wu *et al*., 2015). Recirculation of high nutrient load wastewater can increase the volume of treated effluent on a small land area (Cui *et al*., 2012; Saeed *et al.*, 2021). Saeed *et al*. (2021) reported that increased recirculation, coco peat and construction bricks substrates planted with *Phragmites australis* in a hybrid CW improved the removal of heavy metals, zinc, chromium, nickel and lead, achieving 75 – 98%, 29 – 41%, 14 – 48%, 23 – 26%, respectively. Therefore, an increased effluent recirculation, incorporation of plants and various substrates system design can increase nitrogen and heavy metal removal. The oxygen and no oxygen conditions and inorganic carbon between the treated and untreated effluent improved nitrification and denitrification (Al-wahaibi *et al.*, 2021). Arias *et al* (2005) recycled a nitrified wastewater in a vertical system with pre-treated wastewater and reported nitrogen removal rates of 52%, 66% and 68% for 1, 2, and 3-times recycling, respectively. The author recommended an optimal recycling of 1 and 2 times of a pretreated effluent. The intermittent wastewater feeding, recirculation and addition of organic carbon have been reported to enhance total nitrogen removal (Cui *et al.*, 2012; Al-wahaibi *et al.*, 2021, Appendix II). Cui *et al*. (2012) investigated recirculation times and (0.5, 1 and 1.5 day^{-1}) HLRs on removing total phosphorus, ammonia-nitrogen, and total nitrogen in a hybrid tidal flow CW. Total phosphorus removal decreased with an increase in HLR, from 74.08% to 46.09%. Total nitrogen removed was 72.71%, at an HLR of 1.0 m day^{-1}, suggesting that recirculation increases the volume of treated effluent. Saeed *et al.* (2021), reported that increased recirculation and the presence of *Phragmites australis* in mixed substrate improved the removal of heavy metals: zinc, chromium, nickel and lead, achieving 75 - 98 %, 29 – 41 %, 14 – 48 %, and 23 – 26 % respectively.

2.3 Processes involved in nitrogen removal in a constructed wetland

The removal of nitrogen in constructed wetlands is low, and the efficiency can be improved by a combination of wetland types or by improving the design and operation (Almuktar *et al.*, 2018). Nitrogen removal in CWs occurs through a series of transformation steps of nitrification, denitrification, and anaerobic ammonia oxidation, performed under aerobic and anaerobic conditions (Seifi & Fazaelipoor, 2012; Meng *et al.*, 2014). These removal processes are majorly influenced by the nature of wastewater, hydraulic operation conditions, substrate type and environmental factors (Truu *et al*., 2009; Wu *et al*., 2015).

Nitrogen in wastewater occurs in various transformed forms with seven valency states ranging from -3 (in NH_4^+) to +5 (in NO_3^-) in the biochemical cycle (Vymazal, 2007). Organic nitrogen exists as amino acids, urea, uric acid, purine, and pyrimidines (Wallace, 2009). The inorganic forms are ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), and dissolved nitrogen gas (N_2). Gaseous nitrogen includes nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO), and free ammonia (NH_3) (Vymazal, 2007; Saeed & Sun, 2012). Dissolved inorganic nitrogen occurs as ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) (Taylor *et al.*, 2005). Organic nitrogen is further divided into particulate and dissolved organic nitrogen, as shown in Figure 5.

Plants and autotrophic bacteria prefer the uptake of ammonium since it can be easily oxidised (Vymazal, 2007). Un-ionised ammonia is toxic even at low concentrations of 0.02 mg L^{-1}(Wallace, 2009). Nitrogen removal in CWs is influenced by the transformation processes of the biochemical reactions (Senzia *et al.*, 2003; Cui *et al.*, 2013). These processes are nitrification, denitrification, assimilation by microorganisms and plants, mineralisation (ammonification), ammonia volatilisation, nitrogen fixation, anaerobic ammonia oxidation (anammox), adsorption and ion exchange (Vymazal, 2007; Saeed & Sun, 2012). A community of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), heterotrophic denitrifiers, and anaerobic ammonia-oxidizing bacteria is effective in these processes (Meng *et al*., 2014).

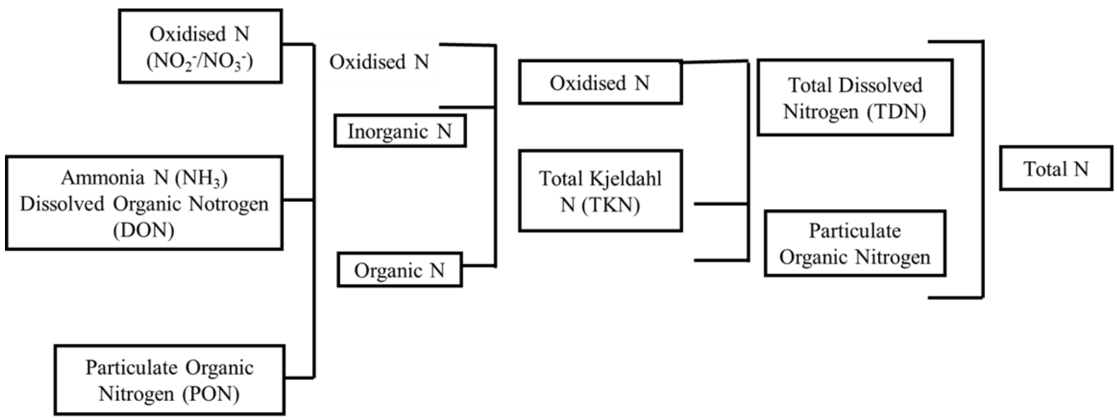


Figure 5: Constituents of nitrogen element and their named combinations (Taylor *et al.*, 2005).

2.3.1 Ammonification

Ammonification is the initial transformation process of organic nitrogen to ammonium ions (Vymazal, 2007; Lee & Fletcher, 2009). Biochemical oxidation of organic nitrogen occurs when amino acids are subjected to oxidative and reductive deamination processes to produce energy and ammonia gas (De Datta, 1995), as illustrated in Equations 1 and 2.

Amino acids → Imino acids → Keto acids → NH_3 (Eq. 1)

Reductive deamination:

Amino acids → saturated acids → NH_3 (Eq. 2)

Ammonification depends on temperature, pH, C/N ratio, available nutrients, and the nature of the substrate (Patrick 1985; USEPA, 2000). An optimal temperature range of ammonification is reported to be 40–60 °C with an optimal pH range from 6.5 to 8.5 (Vymazal, 1995). Ammonia can be further reduced by adsorption, volatilisation and plant uptake (Vymazal, 2007).

2.3.2 Volatilisation in Wastewater Treatment

Volatilisation refers to a physicochemical process of ammonia removal under high wastewater pH (García *et al*., 2010). Volatilisation rates depend on temperature, pH, concentration of ammonia, and cation exchange capacity of the substrate (Freney *et al.*, 1990). Poach *et al.* (2001) reported 7–16 % free ammonia volatilisation in a steady state enclosure with a loading rate of 2.7–3.9 kg N ha^{-1} day^{-1} at a pH of 7.1-7.3 in treating swine wastewater treatment. Luo *et al.*, (2016) reported a 58% reduction of ammonia through volatilisation in a plant polyculture microcosm, simulating CWs, implying that plant species richness and composition reduce ammonia volatilisation. Therefore, ammonia volatilisation occurs at low quantities in CW depending on the effluent characteristics and operating conditions.

2.3.3 Nitrification in Wastewater Treatment

Nitrification is a two-step biological oxidation process of ammonia to nitrate with nitrite as an intermediate product in the reaction (Vymazal, 2007). In the first step, ammonia-oxidizing bacteria (*Nitrosomonas, Nitrosococcus and Nitrosospira)* oxidise ammonia to nitrite under alkaline conditions (Vymazal, 2007; Jaramillo *et al*., 2018), (Eq. 3). Autotrophic bacteria use ammonia as an energy source, oxygen as an electron acceptor and carbon dioxide to produce new cells (Lee & Fletcher, 2009).

$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \rightarrow C_5\ H_7O_2N + 54\ NO_2^- + 57\ H_2O + 104\ H_2\ CO_3$ (Eq. 3)

In the second step, facultative nitrite-oxidizing bacteria (*Nitrobacter* and *Nitrosomonas*) oxidise nitrite to nitrate (Saeed & Sun, 2012) with nitrite as an electron acceptor and organic compounds as a carbon source for cell metabolism (Vymazal, 2007), Equation 4;

$$400\ NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195\ O_2 \rightarrow C_5\ H_7\ N + 3H_2O + 400\ NO_3^- \quad \text{(Eq. 4)}$$

Stoichiometric equation of nitrification (Vymazal, 2007);

$$NH_4 + + 1.5\ O_2 \rightarrow NO_2 + H_2O + 2H^+ \qquad \text{(Eq. 5)}$$

$$NO_2 - + 0.5\ O_2 \rightarrow NO_3^- \qquad \text{(Eq. 6)}$$

The conversion of ammonia to nitrite produces hydrogen ions that reduce water pH (Anthonisen et al., 1976). At pH below 8.3, the hydrogen ions are neutralised by bicarbonate ions in the water (Eq. 7), (Amatya et al., 1970).

$$H^+ + HCO_3- \rightarrow CO_2 + H_2O \qquad (7)$$

Saeed & Sun (2012) stated that about 7.14 mgL^{-1} of alkalinity (as CaCO3) is consumed for every one mg L^{-1} of ammonia, and 1.98 mol of H^+ is released for every one mole of ammonia-nitrogen consumed. The nitrification process is limited by oxygen supply for AOB and sufficient carbon for the NOB. At low temperatures (<5°C) and oxygen conditions (≤1.5 mg L^{-1}), nitrous oxide (N2O) is normally released in wastewater treatment plants (Massara et al., 2017)

2.4 Factors affecting the nitrification in constructed wetland

The rate of nitrification is affected by temperature, dissolved oxygen, pH, alkalinity (carbonate and bicarbonate concentration), inorganic carbon source, moisture, microbial biomass, ammonia- nitrogen concentration (Lee & Fletcher, 2009; Kim *et al.*, 2011).

2.4.1 Dissolved Oxygen (DO)

Dissolved oxygen (DO) is a critical factor influencing complete nitrification since it is an oxygen-demanding process (Ruiz *et al*., 2003). Rodríguez-Gómez *et al*. (2021) reported 4.57g of oxygen to oxidise 1 gram of ammonia. About 4.3 g of oxygen is consumed for 1 mg of ammonia-N oxidised. Oxygen concentration below 0.5 mg L^{-1} inhibits the activity of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) hence a build-up of ammonia and nitrite in the effluent (Rodríguez-Gómez *et al*., 2021). There is a competition for oxygen between nitrifying bacteria and microbes that decompose organic matter, thus the need for adequate supply (Carrera *et al.*, 2003). Wetland aeration technology achieves effective nutrient removal with increased energy demand (Boog *et al*., 2017). Boog *et al*. (2014) reported a 70 % nitrate reduction in an intermittently aerated vertical flow (VF) wetland, saving 33 % of energy cost for aeration. A tidal design aerates the system through subsequent fill and drain cycles thus reducing the energy used in artificial aeration (Weinheimer, 2015).

2.4.2 pH Potential

The nitrification process is sensitive to changes in pH, and the optimal range is 7.5 to 9. The maximum activity of ammonia-oxidizing bacteria is at a pH of 8.0 (Amatya *et al*., 1970). Nitrification declines as pH drops below 7. Low pH favours the growth of nitrite-oxidizing bacteria over ammonia-oxidizing bacteria. Methane-forming bacteria are effective at a pH range of 6.5–7.5 for anaerobic decomposition of organic matter (Paredes *et al*., 2007). Oxidation of ammonium to nitrite uses 2 moles of bicarbonate to oxidise 1 mole of ammonium, and low alkalinity inhibits complete nitrification (Paredes *et al*., 2007). During nitrification, 7.2 g of alkalinity is consumed in converting 1 g of ammonia to nitrate, hence a decrease in alkalinity (Saeed & Sun, 2012). At pH above 8.5, ammonium ions exist as ammonia (NH_3) gas, increasing their toxicity (Mahne & Megus, 1998).

2.4.3 Temperature Level

Temperature affects the metabolism and growth of ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB) (Hülsen *et al*., 2016; Chen *et al.*, 2018; Yuan *et al*., 2018). At a temperature range of 10-15 °C, NOB grows faster than AOB, and above 15 °C, AOB outcompete NOB (Peng & Zhu, 2006). Below 10 °C, the activity of NOB is decreased and can cause the build-up of nitrate in the effluent (Faulwetter *et al*., 2009; Truu *et al.*, 2009; Young *et al*., 2017). The optimal temperature for nitrification ranges from 25–36 °C. Bio-augmentation into activated sludge has been reported to improve nitrification at temperatures below 15°C (Yuan *et al*., 2018). Sawdust and plastic film insulating layers retained heat in CW and enhanced wastewater treatment during cold seasons (Yan & Xu, 2014). Nivala *et al*. (2007) reported 93%–98% ammonia removal in treating landfill leachate using HSSCWs with an insulated layer of sawdust, indicating the possibility of nitrification in cold seasons.

2.4.4 The Denitrification Process

Denitrification is a metabolic process occurring under anaerobic conditions in a reduction sequence of nitrate (NO_3^-) to nitrite (NO_2^-) to nitrogen gas (N_2) (Ni *et al.*, 2017), (Eq. 8).

$$NO_3^- + 1.08\ CH_3OH + 0.24\ H_2CO_3 \rightarrow 0.056\ C_5H_7NO_2 + 0.47\ N_2 + 1.68\ H_2O + HCO_3^- \quad \text{(Eq. 8)}$$

Denitrifying microorganisms are classified into two groups: heterotrophic and autotrophic denitrifiers. Heterotrophs use organic matter from the effluent as energy sources for growth and development. Autotrophs obtain energy from inorganic substances and utilise carbon dioxide as a carbon source for metabolism (Lee & Fletcher, 2009). Several species of denitrifiers are chemoheterotrophs, using organic carbon and carbon dioxide as energy sources during respiration (Vymazal, 2007). Examples are the genera *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Enterobacter*, *Micrococcus*, *Spirillum*, *Proteus*, and *Aerobacter* (Meng *et al*., 2014; Ni *et al.*, 2017).

The denitrification process increases the alkalinity of the effluent. About 3 g bicarbonate (as $CaCO_3$) is produced for every gram of nitrate-nitrogen reduced (Saeed & Sun, 2012). Denitrification occurs in the flooding phase of a tidal CW. Low oxygen conditions favour the activities of denitrifiers in reducing nitrate and nitrite to nitrogen gas released into the atmosphere (Austin, 2006; Gregory *et al*., 2012).

The rate of denitrification is influenced by nitrate concentration, microbial flora, quality of carbon source, low oxygen conditions, redox potential, temperature and pH (Bastviken *et* *al*., 2005; Sirivedhin & Grey, 2006; Vymazal, 2007; Meng *et al*., 2014; Ni *et al*., 2017). Organic carbon used as an electron donor and energy source for nitrogen removal is usually limited in various wastewater (Saeed & Sun, 2012). The three main carbon sources are organic matter in the influent, microorganism death and decomposition and external sources (Ni *et al*., 2017).

External synthetic sources are methanol, glucose, acetate, ethanol, lactate starch, cellulose and plant material (Khin & Annachhatre, 2004; Meng *et al.*, 2014). About 2.47 g of methanol is required to reduce 1 gram of nitrate-N for complete denitrification (Khin & Annachhatre, 2004). High ammonia concentration in the effluent normally reflects a low carbon-to-nitrogen ratio (Winkler & Straka, 2019). Fan *et al.* (2013) reported a simultaneous reduction of COD, ammonia-N, and total nitrogen by 96%, 99% and 90%, respectively, at a COD: N ratio of 10:1. The COD: N ratio varies depending on the organic carbon available in the effluent and system design (Michaud *et al*., 2006). Denitrification is effective at a temperature range of 20 –25 ^{0}C, pH range of 7.0 – 7.5 and decreases at pH< 6.0 (USEPA, 1975). Studies recommend a 10:1 COD/N ratio and intermittent aeration for improved inorganic nitrogen removal in subsurface flow CW (Zhao *et al*., 2010; Fan *et al*., 2013; Chen *et al*., 2018).

Phosphorus is an essential element for the growth and metabolism of denitrifying bacteria (Hua *et al*., 2016). The end product of denitrification is the release of nitrogen gas and energy stored in the form of ATP (adenosine triphosphate) and utilised by heterotrophic bacteria for respiration (Vymazal, 2007). Nitrogen gas released into the atmosphere can be fixed to organic nitrogen by cyanobacteria (blue-green algae) in a nitrogen fixation process at the plant roots or water surface (Scholz & Lee, 2005; Rose, 2021) (Figure 6).

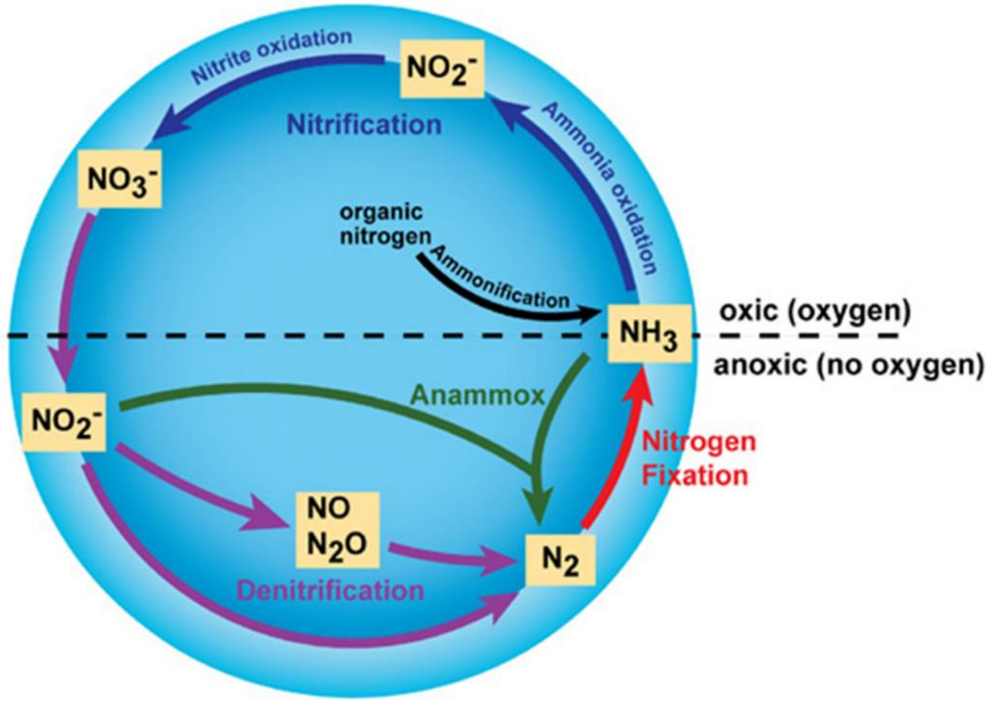


Figure 6. Schematic diagram of processes involved in the nitrogen cycle in a wastewater treatment plant (Rose, 2021).

2.4.5 Assimilation by microorganisms

Heterotrophic bacteria and autotrophic microalgae readily assimilate ammonium ions in the intracellular spaces and convert it to amino acids that build up cell biomass (Vymazal, 2007; Li *et al*., 2017). Li *et al.* (2017) reported that the marine bacterium *Vibrio* species assimilated 8.0 % of nitrate (100 mg l^{-1}) and 80.4 % of total nitrogen (TN) from municipal wastewater. Simultaneous removal of pollutants by specific microorganisms has been reported through heterotrophic nitrification and aerobic denitrification (Yang *et al*., 2019; Liu *et al*., 2020; Shukla *et al*., 2020; Hao *et al*., 2022). Yang *et al*. (2019) reported that enzymatic activities of nitrite, nitrate reductase and hydroxylamine oxidase in

Pseudomonas putida strain accounted for the simultaneous assimilation of ammonium ions and phosphorus at 30 °C temperature and a C: N ratio of 10.

Microalgae are competitive in recovering nutrients and biomass production in wastewater treatment (Han & Zhou, 2022). Algae and heterotrophic bacteria symbiotically exist in wastewater treatment plants (Cilliers, 2012). During respiration, microorganisms release carbon dioxide and inorganic substances for the growth and development of microalgae (Johnson 2010; Han & Zhou, 2022). Microalgae have the potential to use energy from light and organic carbon to assimilate nutrients under aerobic, anaerobic, and anoxic conditions (Munasinghe-Arachchige *et al*., 2020). Therefore, the availability of nutrients in wastewater can influence microalgal activities and treatment efficiency (Johnson 2010; Han & Zhou, 2022). Further studies are necessary to evaluate the potential of diverse strains of microorganisms for nutrient assimilation and its application in tidal wetland system design.

2.4.6 Adsorption and ion exchange on wetland substrates

Adsorption refers to the physical or chemical accumulation of substances on a solid surface (Gupta *et al.*, 2009). Physical adsorption is a reversible attraction between the adsorbed molecules through weak van der Waals forces. Chemisorption is an irreversible attraction of molecules to a solid surface through chemical bonding (Gupta *et al*., 2009). Examples are substrates with oxides and hydroxides of aluminium, iron, and calcium in limestone, zeolite, vermiculite, ceramsite, alum and slag (Gupta, *et al*., 2009; Wang *et al*., 2020). The specific surface area (SSA) influences the efficiency of a substrate, i.e., the smaller the SSA the higher the adsorption efficiency (Lyngsie *et al*., 2013).

Ion exchange is a reversible chemical reaction of exchanging ions from a solution for a charged ion attached to an immobile solid particle (Gupta, *et al.*, 2009). The cation exchange of substrate is a mechanism of nutrient transformation between flood and drain wetland systems. Using Fick's law, oxygen diffusion from the air-water interface and across biofilms (<100 μm) takes place within one or less than one second (Austin, 2006). The substrates effective for pollutant removal should have a high bonding affinity, adsorption capacity, and low desorption rates (Lyngsie *et al*., 2013). The exchanged ions are ammonium, phosphorus, and heavy metals (zinc, copper and lead) (Gupta, *et al*., 2009; Yang *et al*., 2018). Substrates with high adsorption capacity that have been used in CW are biochar, zeolite, activated carbon, limestone, vermiculite and agricultural by-products (Wang *et al.*, 2020).

2.4.7 Anaerobic Ammonia Oxidation

Anaerobic ammonium oxidation (anammox) is a nitrogen removal process that directly converts ammonium to nitrogen gas (Meng *et al.*, 2014; Saeed & Sun, 2012). Ammonia is partially oxidised to nitrite by ammonia oxidizing bacteria (AOB) and partly converted anaerobically by anammox bacteria (Winkler & Straka, 2019). The ammonium ion is the electron donor while nitrite is the electron acceptor under anaerobic conditions (Faulwetter *et al.*, 2009) (Eq. 9).

$$1NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.130H^+ \rightarrow 1.02N_2 + 0.260NO_3^- + 0.066CH_2O + {}_{0.5}N_{0.15} + 2.03H_2O \quad \text{(Eq. 9)}$$

Anammox microorganisms are of the order Planctomycetes with six genera of *Brocadia*, *Scalindua*, *Kuenenia*, *Jettenia*, *Anammoxoglobus* and *Anammoximicrobium* (Faulwetter *et al.*, 2009; Zhang *et al.*, 2020, 2021). Anammox bacteria are chemolithoautotrophs and do not need the addition of external carbon and have a low oxygen and energy demand (Faulwetter et al., 2009; Meng *et al.*, 2014; Ronan *et al.*, 2021). Anammox microorganisms do not produce nitrous oxide (N_2O) and have abilities to transform bicarbonate from the effluent into the cell, thus reducing the emission of greenhouse gases (Winkler & Straka, 2019; Sheng *et al*., 2020).

Anammox microorganisms have a slow growth rate of 0.04–0.06 d^{-1} with high sensitivity to changes in dissolved oxygen, temperature, pH, ammonia, nitrite, and nitrate concentrations (Saeed & Sun, 2012; Guo *et al*., 2020). The optimal temperature for growth is about 30°C and pH of 7.6, and dissolved oxygen concentrations above 0.2 mg L^{-1} inhibit anammox metabolic activities (Strous *et al.*, 1997; Lu *et al*., 2017). Coupling anammox systems with partial nitridation units improved the treatment of ammonia-rich effluent (Sheng *et al*., 2020; Ronan *et al*., 2021).

2.5 Advances in Biological Nitrogen Removal and Potential Application in Wastewater Treatment

Nitrogen removal in constructed wetlands occurs through a series of transformation steps of nitrification, denitrification, and anaerobic ammonia oxidation, performed under aerobic and anaerobic conditions (Seifi & Fazaelipoor, 2012; Meng *et al*., 2014). These removal processes are majorly influenced by the nature of wastewater, hydraulic operation conditions, substrate type and environmental factors (Truu *et al*., 2009; Wu *et al*., 2015). Nitrification and denitrification processes require energy for system aeration and space for the two-step compartments (Xi *et al.*, 2022; Yang *et al.*, 2019). The requirement of a specific carbon supplement for denitrification is a limitation that increases the cost of nitrogen removal (Winkler & Straka, 2019; Ronan *et al*., 2021). Achieving the aerobic anaerobic condition is expensive, time-consuming and requires a large area with separated compartments thus a limitation on the practical perspective (Wang 2019; Xi 2022). The growth and survival of *Nitrosomonas* and *Nitrobacter* species are compromised when wastewater has a high , thus reducing nitrification in wastewater treatment (Kim *et al.*, 2008).

Current studies in wastewater treatment focuses on reducing energy costs, greenhouse gas (GHG) emissions, and nutrient recovery (Winkler & Straka, 2019; Munasinghe-Arachchige *et al*., 2020; Han & Zhou, 2022). Recently, the discovery of heterotrophic nitrification, aerobic denitrification and phosphorus-accumulating organisms has simplified the removal of nitrogen and phosphorus from wastewater (Hao *et al.*, 2022; Shukla *et al*., 2020). These interventions in wastewater treatment have become a hotspot for research to interlude the traditional nitrification and denitrification concepts (Qiao *et al.*, 2020; Xi *et al*., 2022). The discoveries have overcome the limitation of controlling an anaerobic environment since a single compartment simultaneously removes multiple nutrients at reduced aeration costs and space requirements (Yang *et al.*, 2019). The discovery of metabolically adaptable microorganisms able to perform heterotrophic nitrification and aerobic denitrification (HN-AD) achieves high nitrogen removal.

Heterotrophic nitrification (HN) occurs through an oxidation sequence of enzymes; ammonia monooxygenase (AMO), and hydroxylamine oxidase (HAO) in converting ammonia to nitrogen gas (Chen & Ni, 2011; Zhao *et al.,* 2012). Nitrate reductase (Nar) and nitrite reductase (Nir) simultaneously reduce nitrate and nitrite to nitrogen gas under aerobic conditions (Robertson *et al*., 1988; Zhao *et al.,* 2012). Aerobic denitrification (AD) is performed by microorganisms of the phylum α-, β- and γ-Proteobacteria using oxygen and nitrate as electron acceptors (Ji *et al.,* 2015; Yang *et al.,* 2020). Oxygen is the most preferred electron acceptor over nitrate due to more energy gain, hence the possibility of AD process (Hao *et al.,* 2022). Aerobic denitrification occurs in the cell periplasm in a sequential enzymatic reduction of nitrate reductase, (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos) (Zhao *et al*., 2010; Ji *et al.*, 2015). There are two metabolic pathways for ammonia oxidation; sequential enzymatic reduction reactions and the hydroxylamine pathway which directly converts ammonia to nitrogen gas (Joo *et al*., 2005; Zhao *et al*., 2012).

Coupling heterotrophic nitrification and aerobic denitrification has been identified to improve the removal efficiency of pollutants (Third *et al.,* 2005; Rout *et al.*, 2017; Fan *et al.,* 2022; Janka *et al*., 2022). The optimisation of tidal wetland operation design and the use of combined substrate can create varying dissolved oxygen conditions to favour the growth and metabolic activities of HN-AD microorganisms for enhanced treatment efficiency.

The HN-AD microbial families consist the *Comamonadaceae*, *Microbacteriaceae*, *Cloacamonaceae*, *Alcaligenaceae*, and *Anaerolinaceae* (Luo *et al*., 2017; Rout *et al.,* 2017; Hao *et al*., 2022; Janka *et al.,* 2022). *Alcaligenaceae* dominates in the sequential moving bed biofilm reactors while Cloacomonaceae are common in anaerobic digesters (Lai *et al.*, 2018; Janka *et al.,* 2022). The strains of HN-AD have been isolated and identified in various habitats with special properties for potential application in commercial wastewater treatment (Table 2). Some of the features identified are the high growth rate of some strains, multiple pollutant removal, tolerance to high ammonia and salinity and metal-resistant (Li *et al*., 2017; Rout *et al*., 2017; Shukla *et al.,* 2020; Fan *et al.,* 2022). Screening and bio-augmentation of strains with adaptable features will play remarkable roles in commercial wastewater treatment applications. Further research is necessary to understand the ecology of diverse strains, and metabolic activities under varied environmental conditions. Zhao *et al.* (2012) reported *Alcaligenes faecalis* to produce nitrous oxide (N_2O) greenhouse gas under aerobic conditions. Halophilic *Vibrio* spp. has been identified to assimilate nitrogen into the cell, without nitrous oxide emission (Li *et al*., 2017), thus the need to be examined in industrial wastewater treatment.

Table 2: The microorganism strains, the origin of isolation and the unique features of heterotrophic nitrification and aerobic denitrification.

Species	Origin	Unique characteristics	Reference
Thiosphaera pantotropha	sulfide-oxidizing wastewater	Similar enzyme to ammonia-oxidizing bacteria	(Robertson *et al*., 1988)
Zoogloea spp.	Landfill leachate	A distinct polyamine pattern	(Lukow & Diekmann, 1997)
Alcaligenes faecalis	Sludge system	Tolerant to high ammonia Use hydroxylamine pathway Lack of nitrite and nitrate reductase enzymes	(Joo *et al*., 2005)
Acinetobacter calcoaceticus	Membrane Bioreactor	Use hydroxylamine pathway Lack nitrite and nitrate reductase enzymes	(Zhao *et al*., 2010)
Agrobacterium spp.	Landfill leachate	Use hydroxylamine and nitrate reductase enzymes	(Chen & Ni, 2011)
Achromobacter spp.	Landfill leachate	Hydroxylamine and nitrate reductase enzymes	(Chen & Ni, 2011)
Comamonas spp.	Landfill leachate	Hydroxylamine and nitrate reductase enzymes	(Chen & Ni, 2011)
Pseudomonas stutzeri	Swine manure effluent	Ammonium removal under at low temperature	(Zhang *et al*., 2011)
Klebsiella pneumoniae	Domestic wastewater	Secrete extracellular polymeric substances for biofilm development and flocculation	(Padhi *et al*., 2013)
Zobellella taiwanensis	Landfill leachate	Tolerance to high ammonia	(Lei *et al*., 2016)
Vibrio sp.	Sea surface sediments	Tolerance to high ammonia Assimilation and storage of ammonia	(Li *et al*., 2017)
Pseudomonas putida	Activated sludge	Simultaneous removal of nitrogen and phosphorus Resistance to heavy metal and nanoparticles	(Yang *et al*., 2019)

2.6 Factors affecting Heterotrophic Denitrification and Aerobic Denitrification (HN-AD) on Pollutant Removal

The transformations and efficiency of aerobic denitrifiers are influenced by dissolved oxygen concentration, C: N ratio, temperature, and pH (Ji *et al*., 2015). The performance of aerobic denitrifiers is effective at 3 – 5 mg L^{-1} dissolved oxygen concentration, temperature range of 20 – 40°C, and 7 – 8.5 pH range, with a C:N load ratio of 5 – 10 and varies depending on the aerobic denitrifier strain (Ji 2015; Rout *et al.,* 2017; Zhang *et al.,* 2019). Extreme temperatures inhibit the metabolic activities of most AD strains, and the dissolved oxygen threshold is also species-specific (Xi *et al*., 2022). Some strains tolerate high and low temperatures and pH for their growth and metabolism (Hao *et al*., 2022). *Pseudomonas stutzeri* removed ammonium at a low-temperature range of 4 – 10°C, and high dissolved oxygen of about 175.6 mg L^{-1} (Zhang *et al*., 2011).

The primary source of energy for denitrification in wastewater treatment is inorganic carbon (Wang et al., 2015; Ni et al., 2017). External carbon sources such as methanol and glucose have been used as supplements to inorganic carbon which is usually low in industrial effluents, limiting microbial metabolism in the traditional denitrification (Hao *et al.,* 2022). The carbon sources used are acetate, glucose, citrate, succinic acid, sucrose, formic acid, and tartrate (Chen & Ni, 2011; Zhou *et al.,* 2017; Chen *et al*., 2018). The HN-AD process requires high organic carbon, which is species-specific for their metabolic activities (Shukla *et al.,* 2020; Ronan *et al*., 2021; Xi *et al.*, 2022). The organic and inorganic wetland substrates are rich carbon sources that supplement the limiting labile carbon in wastewater.

2.6.1 New Directions for Biological Nitrogen Removal in Wastewater Treatment

Effective nitrification requires energy for system aeration and space for the two steps, i.e., ammonia and nitrite oxidation (Xi *et al.,* 2022; Yang *et al*., 2019). The requirement of a specific carbon source supplement is a limitation that increases the cost of nitrogen removal (Winkler & Straka, 2019; Ronan *et al*., 2021). Highly polluted effluents also compromise the growth and metabolic activities of nitrifying bacteria due to the toxicity of high ammonia, and other pollutants such as cyanide and phenols (Kim *et al*., 2011). Further research should explore innovative and low-energy and cost mechanisms for treating high-strength wastewater.

Recent research focused on reducing energy costs, greenhouse gas emissions and nutrient recovery (Winkler & Straka, 2019; Munasinghe-Arachchige *et al*., 2020; Han & Zhou, 2022). It has been revealed that two-step nitrification and denitrification can simultaneously occur in the same compartment (Janka et al., 2022; Shukla *et al.,* 2020). The discovery of metabolically adaptable microorganisms performing simultaneous HN-AD may simplify the removal of pollutants from wastewater (Shukla *et al.,* 2020; Hao *et al*., 2022). These interventions are gaining momentum for further research to augment the commonly used nitrification and denitrification (Qiao *et al*., 2020; Xi *et al*., 2022).

Simultaneous nitrification and denitrification combine the activities of nitrifiers and denitrifiers to remove nitrogenous compounds (Seifi & Fazaelipoor, 2012). This technology reduces the cost and space of using two compartments and consumes less energy due to reduced aeration. This technology maintains a neutral pH when alkalinity produced during denitrification is utilised in nitrification. Some microorganism strains with resistance to high ammonia, salinity, heavy metals and antibiotics have been discovered in

HN-AD technology (Guo *et al*., 2017; Shukla *et al*., 2020; Yang *et al.*, 2019). With these remarkable potentials, further studies should focus on augmenting the bacterial strains effective for HN-AD and identify limitations for commercial treatment settings.

2.6.2 Future Considerations on Biological Nitrogen Removal in Constructed Wetlands

The evolution of HN-AD microorganisms indicates a possibility of remediating nitrogen in industrial effluent. The discovery of metabolically adaptable microorganisms with multiple pollutant removal abilities has improved wastewater treatment. Simultaneous removal of pollutants in high-strength effluent is a promising technique for industrial application. The multiple pollutant removal on small footprints has economic benefits in wastewater treatment. Future attempts should examine the potential of HN-AD consortiums so that their special properties complement each other in a tidal constructed wetland design.

Some HN-AD strains thrive well under fluctuating oxygen concentrations which can be manipulated in tidal wetland design through intermittent operation to enrich the growth of these specific strains. HN-AD strains require a high organic carbon level as an energy source for metabolism which is expensive and unavailable hence limiting nitrogen removal efficiency. Future research should investigate the potential of substrates rich in natural carbon to reduce treatment costs.

Further research should investigate strains that can convert nitrogen into the cell biomass and nitrogen recovery techniques to minimise nitrous oxide (N_2O) greenhouse gas emissions. Many lab-scale and pilot studies have pointed out the efficiency of HN-AD strains on nitrogen removal, which should be translated into full-scale industrial wastewater applications.

2.6.3 Phosphorus removal in Constructed Wetlands

Phosphorus (P) occurs as either organic/inorganic or soluble/insoluble complexes with a sedimentary cycle (i.e., binding on soil) rather than gaseous states (Scholz & Lee, 2005). Dissolved inorganic phosphorus is considered bioavailable, while organic phosphorous must be transformed into inorganic forms for bioavailability (Reddy *et al.*, 1999). Inorganic phosphorus are polyphosphates and orthophosphates (soluble reactive phosphorus) which are not bound to carbon and hydrogen (Hauda *et al.*, 2020).

High phosphorus concentration increases eutrophication in freshwater ecosystems and is harmful to human health (Reddy *et al.*, 1999; Hauda *et al.*, 2020). Phosphorus removal is low in CW (Luo *et al*., 2017). Low-cost adsorbents from natural industrial and manmade products have been used for phosphorus removal (Gupta *et al.*, 2009; Cheng *et al*., 2018; Wang *et al.*, 2020). The removal efficiency of phosphorus in SSF CWs depends on the physicochemical and hydrological properties of the substrate material. Substrates rich in metal cations such as iron, aluminium, and calcium and can effectively remove phosphorus through adsorption, chemical precipitation and ligand exchange (Gupta, *et al*., 2009; García *et al.*, 2010; Yang *et al.*, 2018).

Chemical precipitation occurs when inorganic phosphate anions (PO_4^{3-}-P) in wastewater react with cations of aluminium (Al^{3+}), calcium (Ca^{2+}), iron (Fe^{3+}), magnesium (Mg^{2+}) and ammonium (NH_4^+) to form insoluble compounds which can be removed as precipitates (Morse et al., 1998; Rittmann et al., 2011). Precipitates of iron and aluminium can be formed at low pH while alkaline conditions favour calcium precipitation (Reddy *et al*., 1999; Arias *et al*., 2001). Calcium (Ca) content in sand is an important characteristic determining its capacity for P removal (Arias *et al*., 2001). Calcium-rich adsorbents can be improved by heating substrate at high temperatures to form calcium oxide (CaO), which has more reactive binding sites compared to calcium carbonate ($CaCO_3$) (Vohla *et al*., 2011). Hydraulic conductivity and phosphorus adsorption are important considerations in effluent treatment since substrate normally gets saturated over time (Westholm, 2006; Vohla *et al*., 2011).

Regular plant harvest and assimilation by phosphorus-accumulating organisms (PAOs) improve phosphorus removal in wetlands (Vymazal, 2010; Shukla *et al.*, 2020). Denitrifying polyphosphate accumulating organisms (dPAOs) can store phosphorus in the cells under alternate aerobic and anaerobic operating conditions (Kim, 2013; Winkler & Straka, 2019; Shukla *et al.*, 2020). Phosphorus accumulating organisms The roots and rhizomes offer surfacesgrow slowly and can be enriched under aerobic and anaerobic conditions (De-Bashan & Bashan, 2004; Winkler & Straka, 2019). The identification of the strains of phosphorus accumulating organisms with abilities to remove phosphorus in high-strength effluent should be considered in future studies of ebb-and-flow operations.

2.7 The potential use of alternative wetland substrates on nutrient removal in constructed wetlands

Constructed wetlands have been used as a low-cost alternative to conventional wastewater treatment to enable re-use and minimize environmental pollution (Lee & Fletcher, 2009; Götz et al., 2014). Wetland components comprising substrates, microorganism, plants and their interactive functions purify wastewater through chemical, physical, and biological processes (Faulwetter *et al.*, 2009; Vymazal, 2011; Dordio & Carvalho, 2013). Wetland design and operation have necessitated further research on substrates to enhance nutrient removal (Yang *et al.*, 2018; Wang *et al.*, 2020). The substrate particle size and shape determine the porosity and the surface area for biofilm growth to support microorganism activities for wastewater treatment (Gregory *et al.*, 2012, Almuktar *et al.*, 2018).

Advanced research has led to the evolution of traditional substrates (sand, soil, rocks, and gravel) to alternative substrates encompassing natural materials, industrial/ agricultural by-products and artificial materials. Natural, industrial, and man-made products such as zeolite, slag, alum, and clay aggregates have shown potential for pollutant removal (Cheng *et al.*, 2018; Wang *et al.*, 2020). Examples of artificial substrate materials are activated carbon and ceramsite, synthetic fibre, modified clays cement clinker and recycled concrete (Vohla *et al.*, 2011; Cheng *et al*., 2018; Wang *et al*., 2020).

Traditional substrates require a large area of land to treat high-strength wastewater while materials with large surface areas and high cation exchange capacity can achieve high efficiency on smaller footprints (Vohla *et al.*, 2011; Kizito *et al*., 2017). Activated carbon is a stable substrate with a large surface area and a high adsorption capacity for pollutants (Fu *et al.*, 2020; Marlena & Nowicki, 2022). However, the cost of processing activated carbon is high, and thus not viable media for sustainable wastewater treatment (Fu *et al.*, 2020). Substrates have different properties associated with pollutant removal, and a combination can improve treatment efficiency (Dordio & Carvalho, 2013; Wu *et al*., 2019).

Combining inexpensive locally available and expensive conventional substrates is economical to achieve desirable properties for multiple pollutant removal (Liu *et al.*, 2020; Zhang *et al.,* 2021). Kizito *et al.* (2017) compared biochar, and gravel media in tidal operations treating post-anaerobic digester effluent. The biochar-packed media removed 70% – 83% while gravel had 52– 62% for ammonia, COD, and orthophosphate, respectively. High removal efficiency in biochar media was attributed to the higher adsorption capacity and the large surface area for microbial colonisation. Lima *et al.* (2018) reported 82 % and 87 % phosphorus removal on clay bricks CW with and without *E. crassipes* respectively, in treating synthetic sewage effluent. The author reports no phosphorus desorption after 296 days of operation, implying that the substrate was not yet saturated. The high removal efficiency was related to the strong binding capacities of the media. Zhang *et al*. (2021) reported a higher ammonia removal of 55.5%−96.7% in mixed zeolite and gravel than in gravel alone (35.5% − 61 %). Zeolite addition enhanced ammonia adsorption and the development of nitrifying microorganisms. The various media have different physicochemical properties that increase microorganism diversity and improve nutrient removal.

Recent studies explored the physicochemical properties of various substrates, i.e., porosity, stability, and synergistic impacts of the combined substrate on the success of wastewater treatment (Cheng *et al.*, 2018; Fu *et al.*, 2020). Wu *et al*. (2019) reported a high potential of layered combined substrates of zeolite, anthracite, and bio-ceramic with removal efficiencies of 83–89.9% for ammonia-N, 88.3–91.5 % for total nitrogen and 93.8–98.6% for soluble reactive phosphorus in vertical flow CWs. These findings are attributed to the synergistic relationship between substrates, microorganisms, and plants in pollutant removal. Fu *et al*. (2020) reported high removal efficiencies of 97.4 % ammonia-N and 96.2% total nitrogen in combined sand, activated carbon, and ceramsite for treating saline wastewater in CWs. The authors concluded that porous substrates create microhabitats with varied dissolved oxygen levels, favouring the existence of diverse microbial consortiums for enhanced nitrification, denitrification, and anaerobic ammonia oxidation.

The nutrient removal efficiency decreases over time as substrate binding sites gradually saturate (Liu & Lee, 2014). Liu *et al*. (2014) reported a saturation of media adsorption sites in long-term operation, relating to the reduced efficiency over time in this study. Screening and layering of high removal efficiency substrates have been identified as anti-blockage mechanisms for enhanced spatiotemporal treatment (Wu *et al.*, 2019; Liu *et al.*, 2020). Therefore, the appropriate selection of wetland media considers the nature of effluent to be treated, treatment purpose cost of media and re-use of media.

2.8 The potential use of the pretreated brewery effluent as a nutrient and water source for plant growth

Plants are an important component of wetland design and have several properties related to wastewater treatment (Vymazal, 011). Studies have indicated that CW design with plants achieved higher treatment efficiencies (Fan *et al*., 2013; Li 2015; Jesus *et al*., 2018; Zhang *et al*., 2021). Plants play major roles in removing nutrients such as nitrogen and phosphorus and degradation of organic matter through uptake at the plant roots (Vymazal, 2007, 2011). Root exudates supply carbon as an energy source for microbial activity, while oxygen produced through the roots can enhance their activities in wastewater treatment (Chaudhary *et al*., 2003; Vymazal, 2013).

The microorganisms attached to plant roots stimulate the uptake and degradation of organic matter. Organics can be further degraded through plant tissues enzymatic activities (Vymazal, 207, 2011; Dordio & Carvalho, 2013). Dordio & Carvalho, (2013) explained the ability of plants to metabolise organic xenobiotics in CWs through abiotic and biotic processes. Shelef *et al.* (2013) reported that *Bassia indica*, a halophytic plant, can phytoremediate salt by accumulating up to 10% of its dry weight, increasing the potential use of plants in constructed wetlands for pollutant removal. Plants have been reported to accumulate heavy metals in the tissues. Saeed *et al.* (2021) reported the Phragmite and Vetiver plants to remove heavy metals such as Zinc, Chromium, Nickel and Lead removal from landfill leachate by using two-hybrid subsurface flow CWs. Therefore, the ability of plants' properties of phytoremediation and phytoindicators are essential in selecting wetland plants to remove specific or multiple pollutants depending on the plant's tolerance to pollutant and growth conditions (Dordio & Carvalho, 2013; Shelef *et al.,* 2020). Evaluation of plants' contribution is necessary for selecting a wetland design to improve the treatment performance. There is a debate on the role of plants in wastewater treatment due to the low amount of nutrient uptake (Stottmeister *et al.*, 2003; Shelef, 2013; Jesus *et al.*, 2018). Multiple plant harvests have been reported to improve nitrogen and phosphorus removal in the above-ground biomass (Jones *et al*., 2016; Luo *et al.*, 2017). Plant harvest timing is crucial because nutrients can be transferred between above and below ground and released back during plant decomposition (Wallace, 2009; Dotro *et al*., 2017).

The effluent composition is an important parameter determining the choice of wetland plant, growth and survival. Recent studies explored the potential of using constructed wetlands to produce food crops and ornamental plants, as an added benefit of improving food production and income (Shelef *et al*., 2013). Industrial wastewater are rich sources of organic and inorganic nutrients essential for plant growth (Jones *et al*., 2016; Taylor *et al*., 2018). Adjusting brewery effluent pH improved nutrient availability and successful production of tomatoes, lettuce and cabbages. Power & Jones (2016) reported a mean dry weight of 42.3 g of tomato grown for 49 days, while Taylor *et al.* (2018) achieved 478 g of mean cabbage biomass after 12 weeks in pH-adjusted brewery effluent (Table 3).

Halophyte plants have been considered as phytoremediators through the uptake of salts from wastewater. Salt-tolerant plants have various mechanisms of adapting to salt environments such as regulating plant cell osmotic pressure, mediating the hormone signalling and osmotic stress pathways and controlling the changes in the cell wall composition (Jouyban *et al.*, 2012; Zhao 2020). Brewery effluent has been used as a nutrient and water source for salt-tolerant crops. Mabasa et al., (2021) reported a mean wet weight of 8173 g m^{-2} of a salt-tolerant Swiss chard grown for 19 weeks. Other salt-tolerant plants such as maize, sunflower, sesame, lucerne, and saltbush have been grown using brewery effluent (Senthilraja et al., 2013; DeJong, 2019; Mabasa *et al*., 2021) (Table 3). A better understanding of the plants' biochemical and physiological responses to saline growth conditions can provide valuable strategies to improve crop production in CWs.

Table 3: The system design and examples of plant crop and biomass produced using brewery effluent

System design	**Effluent nature**	**Crop type**	**Irrigation treatment**	**Plant density**	**Growth period**	**Chlorophyll content**	**Biomass produced**	**Proline content (mg g^{-1})**	**Source**
Plastic pot (20.5 × 24.0 cm) filled with soil	Brewery effluent	*Zea mays* (maize)	Control 25 % brewery effluent 50 % brewery effluent 75 % brewery effluent 100% brewery effluent	2 per pot	45 days	2.9 ± 0.06 mg g^{-1}	-	94 ± 1.9	(Senthilraja *et al*., 2013)
Plastic pot (20.5 × 24.0 cm) filled with soil	Brewery effluent	*Helianthus annus* (sunflower)	Control 25 % brewery effluent 50 % brewery effluent 75 % brewery effluent 100 % brewery effluent	2 per pot	45 days	2.9 ± 0.1 mg g^{-1}	-	112 ± 2.3	
Plastic pot (20.5 × 24.0 cm) filled with soil	Brewery effluent	*Sesamum indicum* (sesame)	Control 25 % brewery effluent 50 % brewery effluent 75 % brewery effluent 100 % brewery effluent	2 per pot	45 days	3.2 ± 0.1 mg g^{-1}	-	75 ±1.51	

Tubular channel (377 m^2) with 120-mm gravel pots	Post-primary facultative pond	*Lycopersicum escolentum*	pH adjusted with 80% phosphoric acid		49 days	>25	42.3 ± 2.8 g $plant^{-1}$ dry weight	-	(Power & Jones, 2016)
Pot culture 23 L bucket	Aerobic digester	*Brassica oleracea* (cabbage)	pH adjusted with 98 % sulphuric acid	1 plant per pot	12 weeks	>50	478 ± 17 g wet weight	-	(Taylor *et al.*, 2018)
Gravel bed (0.64 m^3)	Post anaerobic digester	*Spinacia oleracea* (Spinach)	Tidal Aerated Un-aerated	15 plants per bed	19 weeks	14.51 ± 0.77 9.84 ± 0.80 6.71 ± 0.89	23970 ± 2.57 g 6410 ± 183 g 6040 ±244 g	-	(DeJong, 2019)
1 m^3 raised bed	Post-primary facultative pond	*Beta vulgaris* (Swiss chard) *Atriplex nummularia* (Saltbush) *Salicornia meyeriana* *Sorghum bicolor* L.	Crop rotation Crop rotation Crop rotation Crop rotation	8 m^{-3} 8 m^{-3} 8 m^{-3} 8 m^{-3}	16 weeks	61 50.70 - 9.79	11647 g m^{-2} 4550 g m^{-2} 422 g m^{-2} 3004 g m^{-2}	-	(Mabasa *et al.*, 2021)

2.9 Celery Growth in a Hydroponic System

Celery (*Apium graveolens* L.) is a perennial food crop of the family Umbelliferae (Kooti & Daraei, 2017; Ashmawi, 2019). The demand for celery stalks, leaves, and seeds is rising as people become health-conscious (Ashmawi, 2019; Stephen *et al.*, 2020). Celery has bioactive compounds comprising flavonoids, alkaloids, phenolic acids, terpenoids, and tannins, all with medicinal properties (Stephen *et al*., 2020). Celery is a halophyte plant and can regulate the salts in the tissues to enable the uptake of essential macro and micronutrients through adaptive strategies. For instance, celery synthesizes mannitol, a compatible solute that protects the cell structure by increasing osmotic potential (Everard *et al*., 1994; Pardossi et al., 1999; Noiraud *et al*., 2000). Kotzen & Appelbaum (2010) reported good celery growth in an aquaponic system using brackish water of 4500 µS cm^{-1} electrical conductivity. Ashmawi (2019) examined 1000 to 4000 mg L^{-1} of saline water on celery growth and chemical composition and recommended 1000 mg L^{-1} for good celery growth. Therefore, further studies are necessary for a better understanding of celery growth and how it copes with high salinity from a brewery effluent. The present study compared the unmixed and mixed media on celery growth, health, survival and chemical composition in the ebb-and-flow constructed wetlands.

Plate 1: Celery (*Apium graveolens*) plant crop (Source: Author)

2.10 Clogging of the Substrate in Constructed Wetlands

Wetland clogging is the primary operational and maintenance factor determining the performance and life span of a CW (Knowles *et al.*, 2011; Wang *et al*., 2021; Zhou *et al.*, 2020). Clogging is described by a decrease in the infiltration rate, hydraulic conductivity and porosity of the filter matrix (Pucher, 2019). Hydraulic malfunction creates dead zones that reduce oxygen supply and impair wetland performance and life span (Pedescoll *et al.*, 2009) (Figure 7). Clogging occurs through solid build-up, excess biofilm growth, plant litter and deposits of chemical precipitates on wetland media over time. During metabolism, microbes produce extracellular polymeric substances (EPS) that facilitate biofilm formation and in excess, contribute to substrate clogging (Zhou *et al*., 2020). Two types of cumulative solids that cause blockages: interstitial solids trapped in the pore space of the media and adhered solids that are tightly adsorbed in the media (Caselles-Osorio & Garcia, 2007b).

Non-uniform influent distribution, high loading rates of the influent and substrate size all influence clogging (Wang *et al.*, 2021). Clogging in SSF CWs can be identified with the appearance of water on the surface (ponding) of the substrate and at high influent loading, water floods on the wetland surface (Knowles *et al*., 2011; Wallace, 2009).

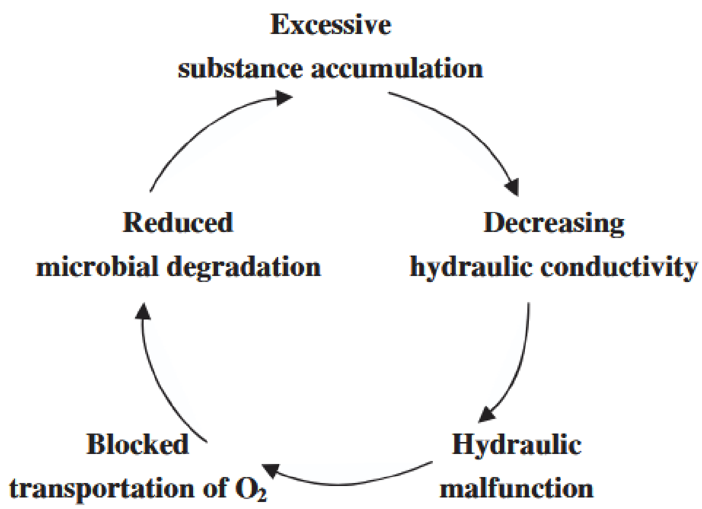


Figure 7: Clogging Process in Constructed Wetland (Meng *et al.*, 2014)

Vertical flow constructed wetlands with intermittent loading have been proven to have the potential to minimise clogging (Knowles *et al*., 2011; Vymazal, 2018; Zhou *et al.*, 2017). Incidences of clogging can be minimised when using a suitable porous substrate material, appropriate influent loading and best management practices of pretreatment are applied in CWs (Vymazal, 2018).

2.11 Methods Used to Assess and Minimise Clogging in Constructed Wetlands

Methods for assessing clogging in CWs involve measurements of saturated hydraulic conductivity (in situ constant and falling head permeameter) that determine the intensity and distribution of clogging. Tracer tests characterize the clogged matter (Caselles-Osorio

& Garcia, 2007; Pedescoll *et al.*, 2009; Knowles *et al*., 2011). Recently, microbial fuel cell (MFC) technology has been used to assess for wetland clogging (Corbella *et al*., 2016). MFC involves producing electric current proportional to the amount of organic matter in a wetland system (Corbella *et al.*, 2016).

2.11.1 Primary Treatment

Primary treatment is the best management practice that reduces accumulation of solids on wetland substrates (Wang *et al.,* 2021). Pretreatment methods such as physico-chemical, clarification, membrane filtration, coagulation and flocculation, lowering the risk of clogging in SSFCWs (Meng *et al*., 2014). Caselles-Osorio & Garcia, (2007b) tested the effect of physico-chemical pretreatment on pollutant removal operated in three hydraulic retention times. The use of anaerobic digester (AD) as primary pretreatment not only removes organic pollutants but also produces energy in the form of methane (Tauseef *et al*., 2013). De la Varga et al. (2013) report a reduction of 76% of suspended solids in up-flow anaerobic digesters for treating winery wastewater. The study further reports a low TSS of 4.6 kg TSS/m^2 in the pretreated effluent after two years of operation.

2.11.2 Management Strategies of a Clogged Wetland

Management and recovery of clogged systems can be achieved using preventive and restorative strategies. This involves adjustment of influent loading, changing hydraulic operating conditions (using intermittent feeding, reversing the direction of flow/ backwashing) and best management practices (Knowles *et al*., 2011; Jaime Nivala *et al.*, 2012). Restorative strategies include partial replacement of media, washing of gravel media, application of chemicals (hydrogen peroxide, Sodium hydroxide, sodium hypochlorite) to the gravel bed and introducing earthworms to the system (Nivala *et al*., 2012; Pedescoll *et al*., 2009; Vymazal, 2018). The use of appropriate particle size, multilayer substrates, and backwashing helps slow down the clogging process. Alternatively, it is more cost-effective to introduce earthworms to break down organic matter in CWs than to replace gravel media and perform backwashing (Wang *et al*., 2021). Pucher (2019) summarized treatment methods used to remediate a clogged wetland as shown in Table 4.

Table 4: Remediation strategies for clogged wetland

Category	Method	Time until Recovery
Destructive Method	Excavation and replacement using new media, washing and reuse of media	Within one or several days
Active treatment	Application of oxidizing agent, Addition of solubilisation agent, Enzyme treatment The addition of earthworms to break down organic matter within the substrate bed	Several hours to one week Around 10 days
Passive treatment	The resting period for the treatment process in the wetland system	10-20 days

2.12 Conclusions of the literature review

Brewing industries have increased the consumption and release of large volumes of effluents, requiring appropriate treatment before discharge to water bodies. The strict effluent discharge regulations justify the recycling and reuse of brewery effluent. Conventional wastewater treatment accounts for high energy consumption and treatment costs. This has prompted onsite effluent treatment using a low-cost alternative of a constructed wetland.

Dissolved oxygen is a limiting factor for the oxidation and degradation of organic and inorganic pollutants in wastewater treatment. Artificial aeration enhances oxygen supply when treating high organic-load wastewater (Dong *et al.*, 2012; Boog *et al*., 2016). However, the high costs associated with conventional blowers and activated sludge increase treatment expenses and are thus unsustainable for wastewater treatment. This highlights the need for further research into alternative mechanisms of oxygen supply and wastewater treatment system design that operate at lower costs.

The evolution of wetland operation has prompted further design optimisation to achieve higher nitrogen removal. The transformation steps in nitrogen removal have led to a combination of vertical flow (VF) and horizontal flow (HF) designs to provide aerobic and anaerobic conditions essential for multiple nutrient removal. A tidal system is an innovative wetland design bridging the gap between highly engineered and capital-intensive conventional wastewater treatment systems. This technology encompasses a single compartment that reduces space requirements and aeration costs.

Hydraulic loading rate/ retention time is a principal parameter determining the land area required for wetland operation. The availability of land is a limitation for low hydraulic loading for effluent treatment in CWs. Therefore, a high hydraulic loading points to a smaller land area requirement. The tidal wetland fill-and-drain cycles determine the hydraulic loading rate of an effluent and can be optimised for high treatment efficiency.

Substrate is a key component in wetland design, and its properties, such as particle size, surface area, porosity and ion exchange capacity, determine the treatment efficiency. Materials with large surface area and high cation exchange capacity achieve high treatment efficiency on small footprints. Substrate combination can achieve desirable properties for specific or multiple pollutant removal with an economic impact on wastewater treatment. An appropriate substrate choice for a sustainable operation considers the cost, availability, saturation period, and recyclability.

Plant growth in constructed wetland systems is a sustainable practice for crop production, recovery of nutrients and recycling of water. This strategy has the potential for large-scale application by planting diverse crops to maximise nutrient uptake, increase food production and reduce treatment costs. Microorganisms transform, degrade and remove pollutants under aerobic and anaerobic conditions. The metabolic activities of microorganisms are influenced by oxygen, pH, temperature, salinity, and organic carbon availability. The manipulation of aerobic and anaerobic conditions in CW promotes the establishment of diverse microbial consortia for improved pollutant removal. Therefore, concerted actions of CW components play interactive roles in nutrient removal and can be optimised for high treatment efficiency, long-term operation and sustainable wastewater treatment.

CHAPTER THREE

METHODOLOGY

3.1 Study Area

Two experiments were conducted at Ibhayi Brewery (33.8378° S, 25.5419° E) in Port Elizabeth, Eastern Cape, South Africa. The climate of Port Elizabeth is subtropical with warm summers and moderate winters. Experiment 1 was conducted for 12 weeks between August and October, 2022 while the second experiment was also run for twelve weeks from November to January 2023.

3.2 Wastewater Treatment Process as an Influent Source for the Experiment

The wastewater treatment at Ibhayi Brewery employs physical, chemical and biological methods (Simate *et al.*, 2011). The treatment begins with physical methods to remove solid wastes through screening in the drum filters, followed by flow equalisation and sedimentation to settle solid wastes (Plate 1, steps 1, 2 and 3). Steps 4 and 5 are the biological treatment methods of anaerobic digester and activated sludge units. The screened effluent is delivered to an equalisation basin, which controls water flow into the anaerobic digester (Plate 1, step 5). The AD breaks down dissolved solid wastes without oxygen to produce energy (biogas), carbon dioxide and traces of hydrogen sulphide (Cilliers, 2012; Dejong, 2019). About 1041 m^3 of the effluent is treated in the anaerobic digester per day, while 670 m^3 is treated in the activated sludge unit, totalling 65% of the effluent treated. The remaining 500 m^3 (35%) is sent to a municipal sewer (Simate *et al.*, 2011; Cilliers, 2012). The effluent is further treated by the chemical methods of disinfection, chlorination and chemical precipitation for storage and reuse in the factory (Plate 1, steps 7-10). A portion of post-anaerobically digested effluent is further treated onsite at an experimental scale at the Eden project research facility, using low-cost alternative methods of primary facultative ponds, high-rate algal ponds and constructed wetlands (Cilliers, 2012; Power & Jones, 2016).

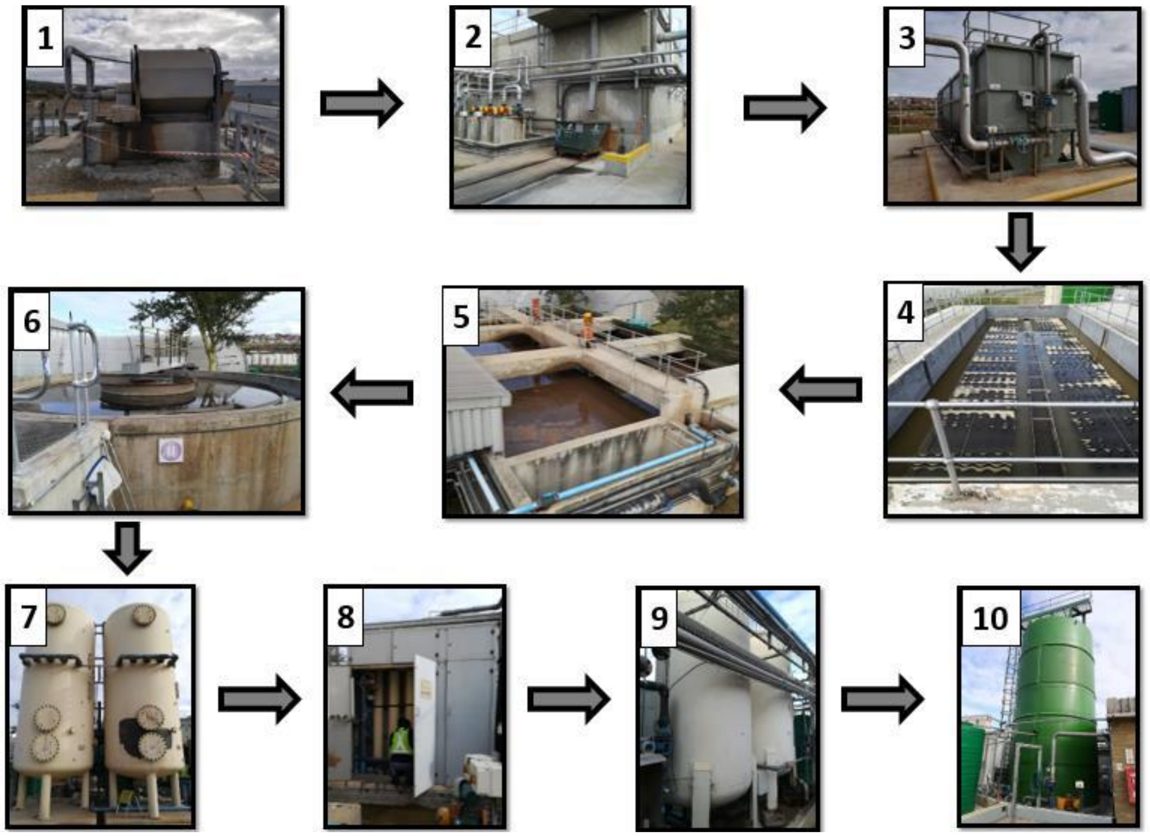


Plate 2: Wastewater treatment process at South African Ibhayi Brewery. The figures labelled 1-10 illustrate the sequence of different treatment units: 1-drum filter; 2-equalisation basin; 3-pre-clarifier; 4-anaerobic digester; 5-activated sludge; 6-clarifier; 7-multimedia-filters; 8 ultra-filters; 9-carbon-filters; 10-storage tank. The arrow indicates the flow of water through the treatment process (Cilliers 2012; De Jong, 2019).

3.3 Preparation of the experimental materials

3.3.1 Acquisition of gravel, substrate types and celery plant crop

Three hundred celery seedlings of similar size were purchased from Moorland Seedlings Pty Ltd, Humansdorp, a commercial seedling nursery in the Eastern Cape of South Africa. Three graded sizes of crushed gravel (7, 13, and 19 mm) were purchased from a quarry while other substrate types were locally sourced from industries in Port Elizabeth, Eastern Cape, South Africa. The gravel sizes were chosen based on the range of pebble sizes used as filter media in CWs. The substrate types were selected based on availability in the area, cost and physicochemical characteristics (Table 5).

Table 5: Substrate types, sizes and physicochemical characteristics used in this study

Nature	Type	Size (mm)	Physicochemical properties	Reference
Clays	Clay pebble	8−20	Highly porous, large surface area, rich in Si_4^+, $Al^{3+,}$ and Mg^{2+} cations	Lima *et al*. (2018)
	Clay bricks	15−25	Irregular surface with micropores	Gu *et al*. 2019*)*
Plastics	Bioballs	10	Large surface area, light-weight, made of polyethene terephthalate, rough surfaces	Sandoval *et al*. (2019)
	Recycled plastics	10−20		
Stones	Gravel	7,13, 19	Large surface area, highly stable	Yang *et al.* (2018)
	Silica sand	1.05–2	Coarse, large surface area, rich in Ca^{2+} and SiO_2	Arias (2001)
Carbon supplements	Activated carbon	3	Light-weight, large surface area, high adsorption capacity	Sanjrani *et al.* (2019); Fu *et al*. (2020)
	Pine bark	25	Light-weight, a fluffy texture, rich in organic carbon	Liu and Zhang (2009); Liu *et al*. (2014)

3.3.2 System preparation for the experiments

3.3.2.1 System setup for experiment 1

The experiment was set on top of a wetland cell filled with gravel (Plate 2). Twenty-seven ebb-and-flow systems were built using 1000-L plastic tanks (1 m x 1 m x 1 m). Each tank was fitted with an overflow outlet pipe (1 m height) to regulate the water level during flood cycles. A 75-mm PVC pipe was installed on top of the systems as a water supply line, with 50-mm-diameter PVC water taps to allow effluent to trickle down through the gravel-filled systems (Plate 2). The effluent from the post-anaerobic digester was pumped into the ebb-and-flow units using a submersible water pump (AquaDrive 670-SPECK Pump-6452NTL-A12X). The water flow from the supply line into each unit was uniformly controlled using a 75-mm PVC water valve. All units were covered with shade cloth (50% shade factor) to create uniform lighting (Plate 2).

Plate 3: The ebb-and-flow experimental units filled with different sizes of gravel and covered with shade cloth on top of a constructed wetland cell (Source: Author)

3.3.2.2 Ebb-and-flow system components and operation

A bell siphon is a mechanical device that automatically regulates water flow using pressure and gravity. The media guard polyvinyl chloride (PVC) (110-mm diameter) prevents pebbles from clogging the siphon. A standpipe (diameter 75 mm) was uniformly perforated at the bottom to regulate the maximum water level in the substrate bed. A siphon pipe of 50-mm diameter PVC was fitted with end caps to create low pressure, enabling the water level to rise within the substrate bed. At the bottom of the siphon pipe was a 50-mm elbow joint connected to the drainage pipe (Figure 10). During the filling phase, water is forced via the perforations of the media guard and standpipe. As its level reaches the height of the standpipe, low pressure is created in the bell cap, allowing water to drain. Air is then sucked at the perforated tip, and the pressure difference created breaks the siphon. The substrate bed is refilled with water in repeated cycles (Figure 8).

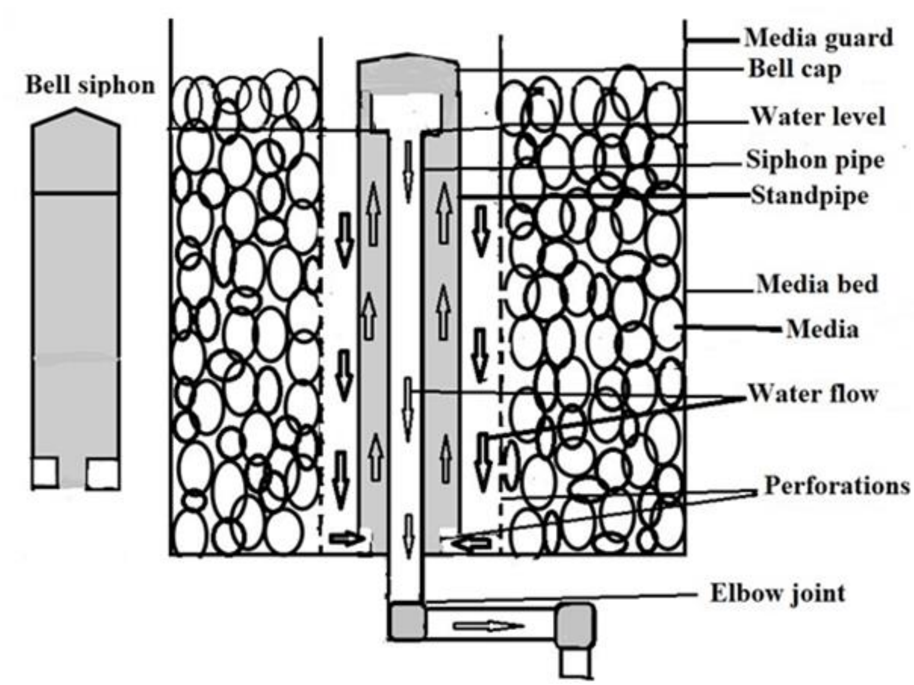


Figure 8: Cross-section and the operation of a bell siphon

3.3.2.3 System setup and operation for experiment 2

Forty-eight ebb-and-flow systems were positioned on a tidal wetland cell (320 m^2) with gravel. The systems were built using 100-L circular plastic tanks (0.45 m diameter and 0.55 m height). A water supply line made of a 50-mm diameter PVC pipe was installed on top of the systems, along with 25-mm water taps for each unit to allow the effluent to trickle through the substrates, Plate 3. Post-anaerobic digester effluent was pumped into the units using a submersible water pump (AquaDrive 670-SPECK Pump-6452NTL-A12X).

Plate 4: Ebb-and-flow system filled with various media used in the experiment (Source: Author)

3.4 Study design

3.4.1 Study design for experiment 1

The two main factors, gravel size (7, 13, 19 mm) and retention time (10, 20 and 40 minutes), were allocated to treatment combinations and replicated three times in a randomised block design (Figure 9). The hydraulic retention time (HRT) for each treatment was set using a stopwatch to measure the water flow rate using equation [1].

$$\text{Hydraulic Retention time (L minute}^{-1}\text{)} = \frac{\text{Volume of container}}{\text{flow rate(Q)}} \qquad [1]$$

$$\text{Where, flow rate (Q)} = \frac{\text{Volume (L)}}{\text{time (sec)}}$$

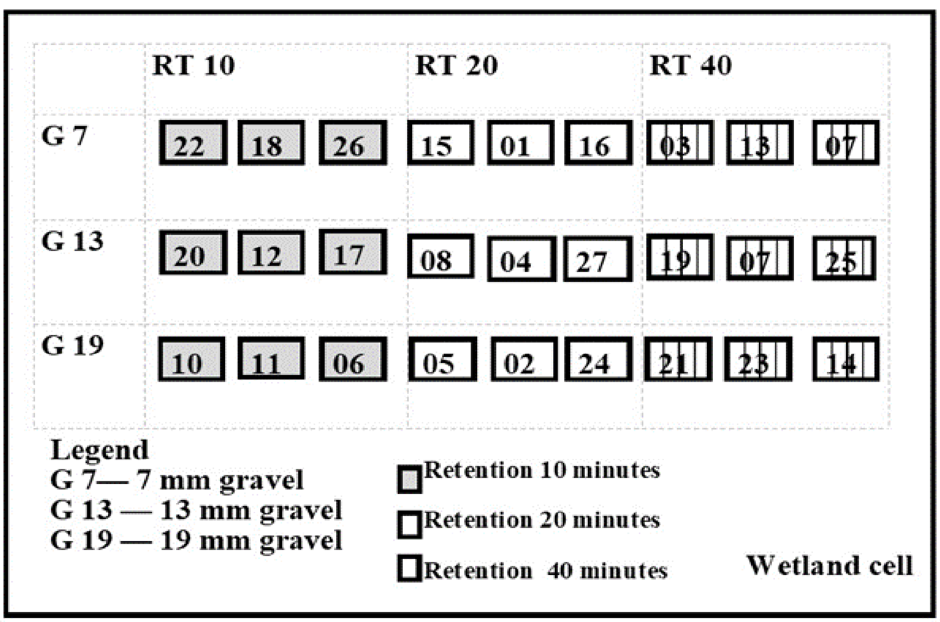


Figure 9: The layout of the experimental ebb-and-flow system (not drawn to scale). The boxes represent gravel sizes and retention time treatment combinations; each block has a distinct colour of boxes: grey for 10 minutes, plain for 20 minutes and striped for 40 minutes of RT treatments.

3.4.2 Study design for experiment 2

Eight media comprising clay pebbles, clay bricks, bioballs, recycled plastics, sand and gravel pebble sizes (7, 13, 19 mm) (Table 2), either alone or mixed, were tested in 100-L experimental units. A 2:1:1 ratio of each media, pine bark and granular activated carbon, respectively, was used for the mixed treatments. A 12-minute retention time (RT) was used as an extension for the recommended RT in the previous study, which treated anaerobic digester brewery effluent at Ibhayi Brewery (De Jong, 2019). A single-pass ebb-and-flow design was used, and the outflow from the system was periodically drained into the tidal wetland. Healthy celery seedlings with an average height of 4.64 ± 0.06 cm and a weight of 5.39 ± 0.60 g were planted at a uniform density of eight plants m^2. All treatments were in triplicate, a completely randomised design.

Table 2: Media type treatments in the ebb-and-flow constructed wetlands

Media type treatments		
Media	**Un-mixed**	**Mixed with pine bark and GAC at (2:1:1)**
Bioballs	T1	T2
Gravel	T3	T4
Recycled plastic	T5	T6
Clay pebbles	T7	T8
Clay bricks	T9	T10
Coarse Sand	T11	T12

3.4.3 System acclimatisation

At the beginning of the experiment, gravel media in the ebb-and-flow systems were acclimatised for two weeks with post-AD effluent at a uniform flow rate of 0.5 L per minute. The unmixed and mixed media treatments in the ebb-and-flow units of the second experiment were not acclimatised.

3.5 Data collection

3.5.1 Water quality sampling and laboratory analysis

Water temperature (°C) and dissolved oxygen (mg L^{-1} O_2) were measured daily using a portable Lutron PDO-519 DO meter. Water pH and electrical conductivity (mS cm^{-1}) were measured weekly at the inlet and outlet of the systems using a Lutron conductivity meter, code PCD-432 and a pH Lutron-electrode meter, Model: PH-220, respectively. Water samples were collected at the inlet and outlet every week and analysed for chemical oxygen demand (COD), ammonia-N, nitrite-N, nitrate-N and orthophosphate using a spectrophotometer (DR2800-01B1, Hach (Pty) Ltd, USA). The concentrations of water quality parameters were analyzed according to the standard procedures outlined in the commercial test kit manual (Hach (Pty) Ltd, product numbers as follows;

- Chemical oxygen demand cell test (200 to 15,000 mg Product no. 21259- 15, Merck (Pty) Ltd, Germany)
- High-range ammonia-nitrogen test (Range 0.4 to 50.0 mg L^{-1}, product no. 26069-45, Hach (Pty) Ltd, USA)

- Nitrite-Nitrogen test (Range 0.002 to 0.300 mg L^{-1}, product no. 21075-69, Hach (Pty) Ltd, USA)
- Nitrate-Nitrogen test (Range 0.3 to 30.0 mg L^{-1}, product no. 21061-69, Hach (Pty) Ltd, USA)
- Phosphorus, Reactive (Orthophosphate) test (Range 0.23 to 30.00 mg L^{-1}, product no. 22441-00, Hach (Pty) Ltd, USA)
- Chloride test, Mercuric Thiocyanate Method (Range 0.1 to 25.0 mg L^{-1}, product 23198-00, Hach (Pty) Ltd, USA).

The nutrient removal efficiency was calculated as weekly values using Equation 2.

$$\text{Nutrient removal (\%)} = \frac{Ci - Ce}{C_i} \times 100 \qquad [2]$$

Where: C_i: inflow concentration, C_e: outflow concentration

Total inorganic nitrogen removal was determined by adding up the inorganic nitrogen species using Equation 3;

$$\text{Total inorganic nitrogen} = [NH_4^+\text{-N}] + [NO_2^-\text{-N}] + [NO_3^-\text{-N}] \qquad [3]$$

3.5.2 Sampling of Celery Plant Tissue Samples

The initial weight of the seedlings was measured in grams (to the nearest 0.01) using a Radwag weighing balance (WLC 6/A/A2/C/2, code WL-217-0014, LLC, Poland). Plant height was measured weekly using a measuring tape (cm). The chlorophyll concentration index (CCI) was measured at the start and end of the experiment on the topmost leaves using a chlorophyll content meter (CCM-200, Opti-Sciences Inc., USA), Plate 4.

Plants were monitored for stress symptoms and observations were captured in photographs. At the end of the trial, three plants from each replicate were harvested and weighed as wet-weight (g). The weighed samples were oven-dried at 70˚C for three days and recorded as dry weight, as described by Reuben et al. (2016) (equation 3).

$$\text{The gross weight of dry biomass} = \frac{\text{Dry sample weight}}{\text{Fresh sample weight}} \times \text{Gross fresh weight} \quad [3]$$

Plate 5: Measurements of plant height and chlorophyll concentration on celery planted in unmixed and mixed media in the ebb-and-flow systems (Source: Author)

3.5.3 Chemical analysis of celery leaf samples

Celery leaves were sampled from each replicate at the start and end of the experiment for elemental analysis. The leaves were analysed for concentrations of nitrogen, potassium, sodium, phosphorus, aluminium, calcium, copper, iron, manganese, magnesium and zinc, at Elsenburg, commercial laboratory in the Department of Agriculture, in South Africa. Celery leaves were analysed using the standardized procedures by the Agricultural Laboratory Association of Southern Africa (ALASA, 1998). Nitrogen was determined using the standard Kjeldahl method. Leaf samples were ashed in a Neytech Muffle Furnace (Vulcan 3-1750A) overnight at 460 ^{o}C to determine the concentration of leaf potassium, sodium, phosphorus, aluminium, calcium, copper, iron, manganese, magnesium and zinc. The samples were heated for 30 minutes in a sample-to-acid ratio of 1:1 consisting of 32% hydrochloric acid. Distilled water of 50 ml was added to the mixture and filtered to determine the element concentrations using inductively coupled plasma atomic emission spectroscopy (ICP-OES, Vista MPX, Varian Inc., USA; Sithole, pers. comm.).

3.6 Statistical Analyses

All data were checked for equality of variance and normality of residuals using Levene's and Shapiro-Wilk's tests, respectively. If the assumptions were not met, data were log-transformed. The means of the weekly data for water quality variables, celery height, dry and wet biomass were compared between treatments using analysis of variance (ANOVA). The inflow and outflow physicochemical variables and nutrient removal efficiencies were compared over time using repeated measures ANOVA at an alpha level of $p \leq 0.05$. The significantly different means were distinguished using Tukey’s post-hoc test. The Kruskal-Wallis test compared the percentage of plant mortality between treatments.

The second-order polynomial regression models described the relationship between percentage nutrient removal and time. A second-order polynomial regression models are a non-linear relationship between a dependent variable and independent variables by fitting a quadratic equation to the data. The maximum value at a given time was calculated using the second derivative of the regression equation. The inflection point (*i*) was calculated using the formula $i = b_2 / (2 \times b_3)$ and substituting *(i)* into the polynomial model; $y = a + b_1 x + b_2 x^2$ to determine the number of weeks by which a model reached a maximum value. Here, b_2 and b_3 are the regression coefficients on the changes of the independent variable (weeks). One-way ANOVA compared the peak values between treatments or treatment combinations. Principal Component Analysis (PCA) was used to identify the treatment performance patterns using PAST ver. 2.17 software. Other statistical analyses were performed using Statistica (Version 14).

CHAPTER FOUR

RESULTS

4.1 The effect of retention time and gravel sizes on nutrient removal

The combined effect of retention time and gravel size had an effect on nutrient removal from the post-anaerobic digester effluent ($p \leq 0.05$) for the 12-week operation in the ebb-and-flow system. The mean inflow of the water variables is presented in Table 6.

Table 6: The mean (± SE) inflow and outflow variables of the post-anaerobically digested brewery effluent into the ebb-and-flow system (n= 324)

Water parameter	Inflow values	Outflow values
Temperature ˚C	22.47 ± 0.10	21.22 ± 0.08
Dissolved oxygen (mg L^{-1})	1.55 ± 0.01	3.22 ± 0.04
Chemical oxygen demand (mg L^{-1})	109.7 ± 1.09	103.4 ± 1.02
Ammonia-N (mg L^{-1})	21.27 ± 0.45	16.03 ± 0.33
Nitrite-N (mg L^{-1})	0.04 ± 0.01	0.01 ± 0.01
Nitrate-N (mg L^{-1})	3.70 ± 0.11	0.73 ± 0.02
Orthophosphate (mg L^{-1})	16.41 ± 0.17	16.24 ± 0.17
Conductivity (mS cm^{-1})	2.92 ± 0.16	2.87 ± 0.02
pH range	8.06 – 8.4	8.1 ± 0.02

Retention time and gravel particle size influenced the physicochemical water variables at the outflow of the ebb-and-flow CWs ($p < 0.05$). Water temperature range was from 22 – 26 ˚C (Figure 10). The interaction between weeks, RT and gravel size did not affect the changes in dissolved oxygen concentration ($F_{(44, 198)} = 0.6$, $p = 0.99$, Figure 10). However, the outflow dissolved oxygen (DO) differed significantly between treatments ($p < 0.01$,

Figure 10). The 10-minute RTs had the highest mean DO of 3.07 ± 0.03 mg/L, followed by 2.63 ± 0.03 mg/L and 2.52 ± 0.02 mg/L at 20 and 40-minute RT, respectively. Gravel sizes 19 mm and 13 mm had higher mean oxygen concentrations than the 7 mm particles (Figure 10). The outflow means for the conductivity range were 3.05 ± 0.01 to 3.12 ± 0.01, while pH was 8.07 ± 0.01 and 8.09 ± 0.01 in the treatment combinations, Figure 10.

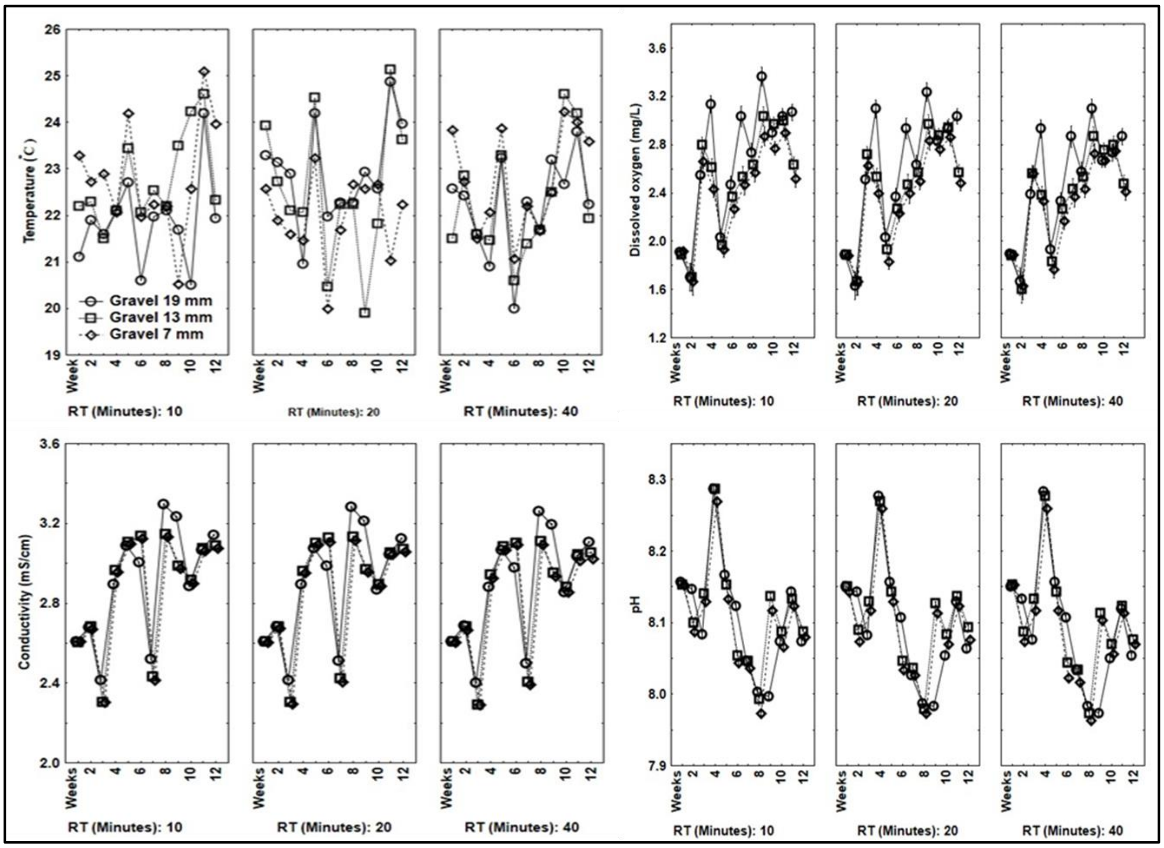


Figure 10: The mean (± standard error) of the outflow concentration of dissolved oxygen, temperature levels (upper row), conductivity and pH (lower row) of the media and retention time treatment combination in an ebb-and-flow system for 12 weeks. The treatment combinations are designated by dashed, dotted and continuous lines.

4.2 The efficiency of nutrient removal in the gravel size and retention time treatment combinations

The interaction between RT and gravel size was not significant for the reduction of COD and ammonia-N ($F_{(4, 18)} = 0.69$ $p = 0.61$), Figure 11 (i, ii). The reduction efficiency for COD and ammonia-N decreased with increasing RT and gravel surface area, with higher mean removal at an RT of 10-minutes than at 20 and 40-minutes. A RT of 10-minutes with 19-mm gravel had the highest mean percentage reduction for both COD (8.16 ± 0.35 %) and ammonia-N (7.66 ± 0.05 %). The lowest reduction of COD (6.03 ± 0.35 %) and ammonia-N (5.58 ± 0.18 %) was at an RT of 40-minutes with 7-mm gravel (Figure 11 i –ii).

The mean removal for nitrite-N, nitrate-N and orthophosphate was influenced by an interaction between RT and gravel size ($p < 0.001$, Figure 11 iii – v). Nitrite-N removal decreased with increasing retention time and gravel surface area, with the highest mean removal of 14.23 ± 0.22 % at an RT of 10-minutes with 19-mm gravel and the lowest removal of 10.53 ± 0.13 % at 40-minutes RT with 7-mm gravel (Figure 11 iii).

Nitrate-N removal increased with both increasing RT and gravel surface area (Figure 11 iv-vi). The 40 and 20-minutes RT treatments had higher mean nitrate-N removal than the 10-minutes RT. The highest mean nitrate-N removal was 18.63 ± 0.09 % at an RT of 40 minutes of 7-mm gravel and the lowest mean was 12.20 ± 0.02 % at 10-minutes RT at 19-mm gravel size.

Orthophosphate removal was the low (< 5 %) in all the treatment combinations. The interaction between gravel size and RT was not significant for total inorganic nitrogen removal ($F_{(4. 18)} = 2.48$, $p = 0.08$, Figure 11 vii). The 10- and 20-minutes RTs treatment combinations had an average TIN removal of 38 %, which was higher than 36.17 ± 0.21 % at 40 minutes of 7-mm gravel (Figure 11 iv).

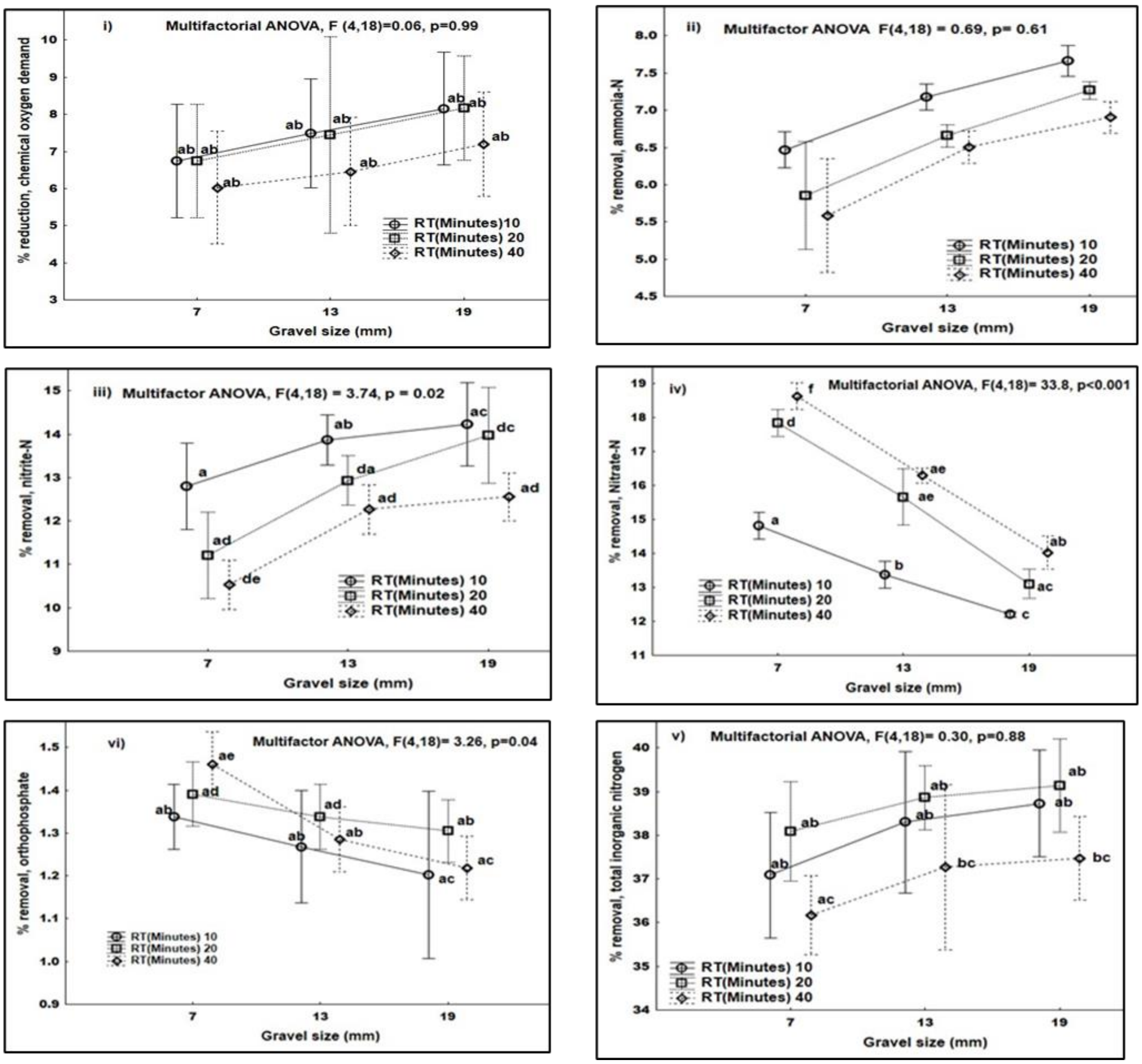


Figure 11: The mean percentages nutrient removal at 95 % confidence interval, (i – v) for ammonia-N, nitrite-N, and nitrate-N, total inorganic nitrogen and orthophosphate in the ebb-and-flow system with gravel size and retention time treatments. F-statistics and p-value refer to the test for interaction between main effects.

4.3 The efficiency of nutrient removal in the retention time and gravel size combinations over time

Figures 12 and 13 are the polynomial regression models illustrating the estimated number of weeks and the peak values of percentage nutrient removal. Total inorganic nitrogen removal increased to a maximum value for the retention time and gravel size treatment combinations. followed by a decline over time. The peaks for TIN removal significantly differed between the respective treatment combinations. One-way ANOVA $F_{(2, 24)} = 10.37$, $p<0.001$. The 10- and 20-minute RTs treatment combinations had an average TIN removal of 38.26 ± 0.36% and 38.70 ± 0.20%, respectively, which was higher than 36.17 ± 0.21 % at 40 minutes of 7-mm gravel. All the RT treatment combinations had peaks between weeks 10 and 11 ($p <0.001$, Figure 12).

The peaks for ammonia-N removal significantly differed between the RT treatment combinations One way ANOVA $F_{(2, 24)} = 3.97$, $p =0.03$. Ammonia-N removal was highest at10 minutes RT of 19-mm gravel, achieving a maximum removal of 7.11± 0.18 %. The lowest maximum mean ammonia-N removal was at 40 minutes RT of 7 mm gravel with 6.33 ± 0.20%. The 19 mm gravel at 10 minutes RT peaked after 8 weeks, while other treatment combinations peaked between weeks 7 and 8 (Figure 12).

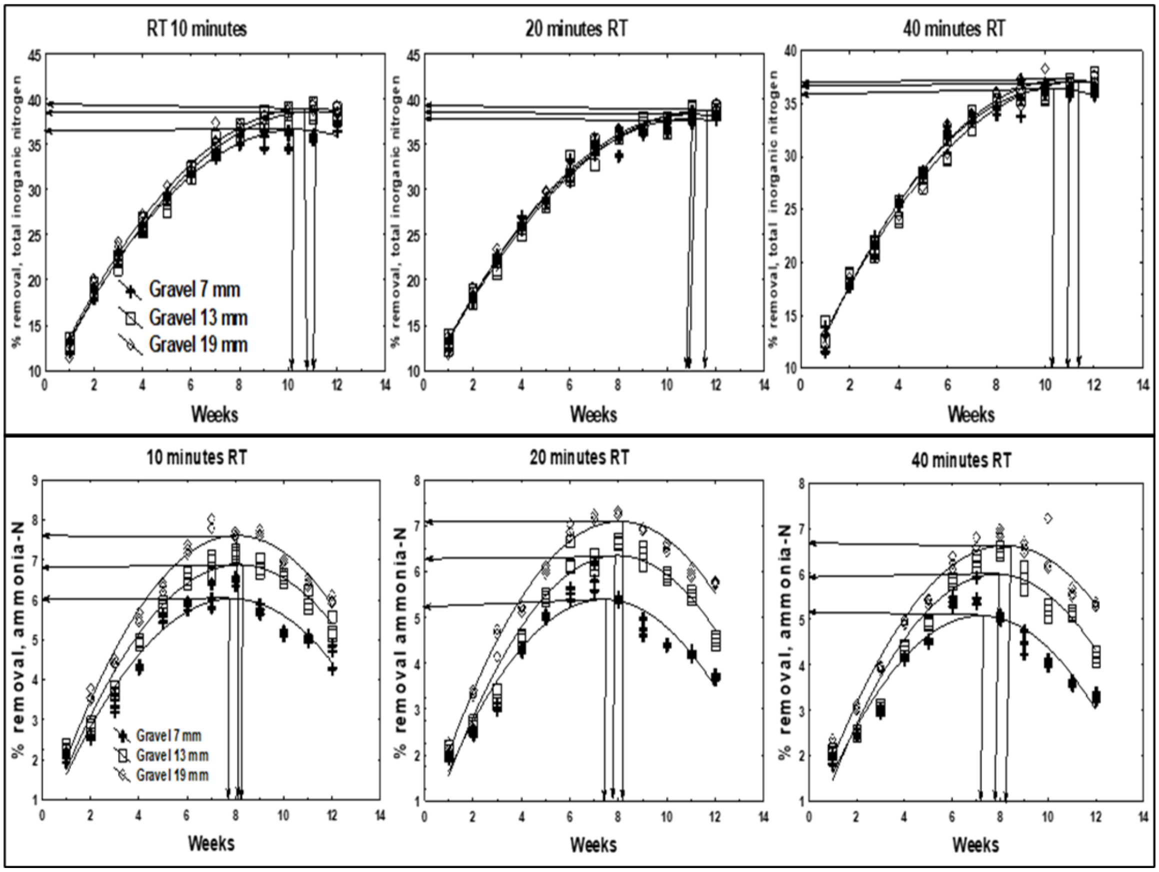


Figure 12: The polynomial regression models illustrating the efficiency of nutrient removal over time (weeks) for total inorganic nitrogen (upper row) and ammonia-N (lower row) as a function of retention time (10, 20, 40 minutes) and gravel size (7, 13, 19 mm) treatment combinations in the ebb-and-flow systems.

Nitrite-N and nitrate-N removal increased as a function of time, with maximum values at week 12, with different peaks between treatments ($p \leq 0.001$, Figure 13). The 10 and 20-minute treatment combinations had significantly higher peaks of 13.62 ± 0.24 % and 12.70 ± 0.42 % respectively, higher than 11.79 ± 0.32 % at 40 min RTs. The 19-mm gravel treatment combinations had the highest maximum values for nitrite-N removal, while the 7-mm gravel had the highest peak nitrate-N and orthophosphate removal in all the treatment combinations (Figure 13).

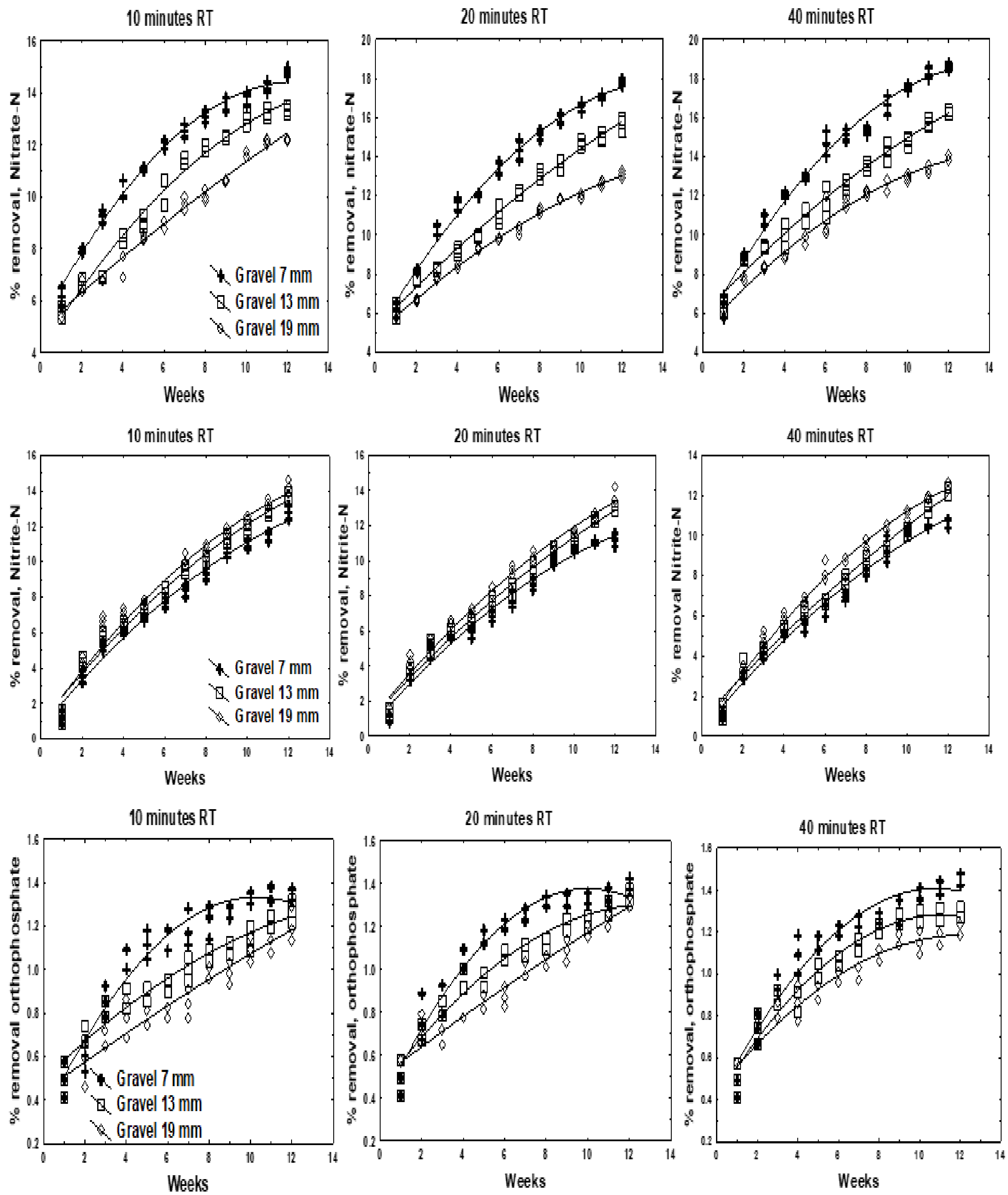


Figure 13: The polynomial regression models illustrating nutrient removal over time (12 weeks) in the treatment combinations of gravel size and retention time of ebb-and-flow systems; Nitrite-N (upper row), Nitrate-N (middle row) and orthophosphate (bottom row).

Figure 14 illustrates the principal component based on the retention time and gravel sizes, indicating a clear separation between treatment patterns in the PCA 1 and 2. Along PCA 1, the treatments are separated by color codes, which are different between treatments. The 67.8% variance of water variables is explained in Component 1, while the second component explains 25.6 % of the variation. Dissolved oxygen concentration, ammonia-N, and chemical oxygen demand had a stronger positive association in PCA 1, while nitrate-N, orthophosphate were inversely associated in the same component, as shown in Figure 14.

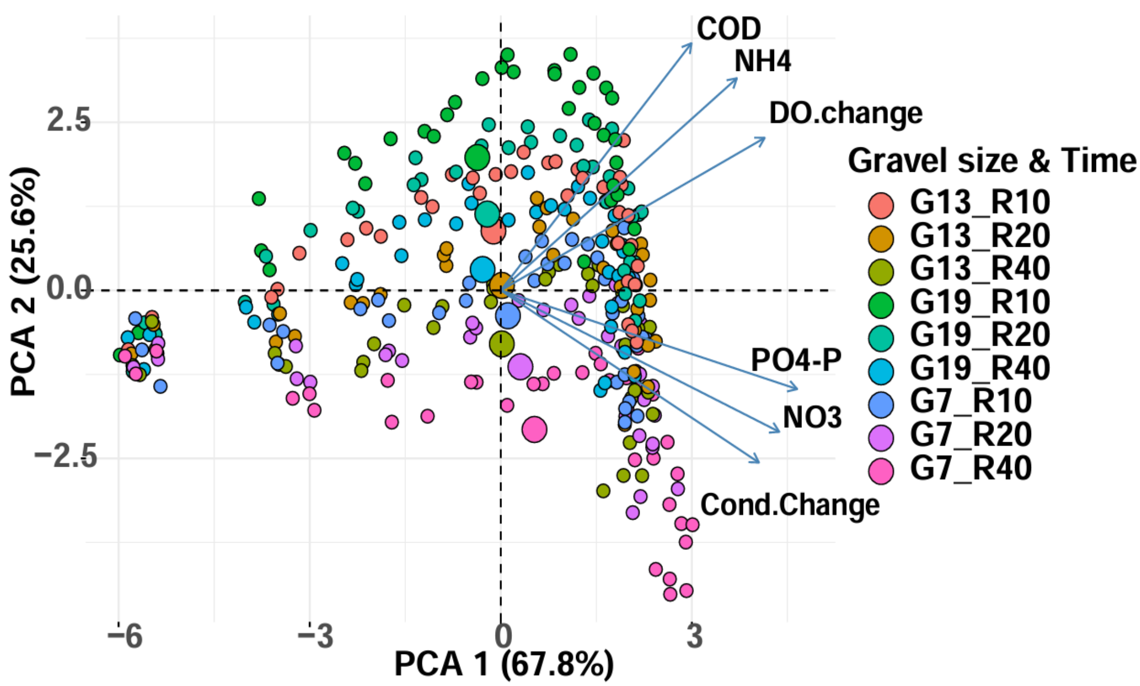


Figure 14: A two-dimensional principal component analysis for retention time and gravel size treatment combinations and loading vectors for selected variables

4.4 The effect of unmixed and mixed media on nutrient removal in the ebb-and-flow wetland

4.4.1. The physicochemical water variables in the ebb-and-flow systems with unmixed and mixed media

The mean inflow concentration of water quality parameters from post-anaerobic digester effluent into the ebb-and-flow system is presented in Table 8.

Table 7: The mean inflow concentrations of the post-anaerobically digested effluent water variables into the ebb-and-flow system (n= 624)

Water parameter	Inflow values	Outflow values Unmixed	Outflow values mixed
Temperature ˚C	23.3 ± 0.12	22.4 ± 0.31	23.1 ± 0.12
Dissolved oxygen (mg L^{-1})	1.05 ± 0.01	2.89 ± 0.23	2.14 ± 0.18
Chemical oxygen demand (mg L^{-1})	109.7 ± 1.09	91.69 ± 0.51	92.36 ± 0.79
Ammonia-N (mg L^{-1})	24.91 ± 0.25	19.23 ± 0.60	19.38 ± 78
Nitrite-N (mg L^{-1})	0.06 ± 0.11	5.01 ± 0.13	4.94 ± 0.12
Nitrate-N (mg L^{-1})	0.04 ± 0.11	0.03 ± 0.01	0.26 ± 0.01
Orthophosphate (mg L^{-1})	16.05 ± 0.14	14.64 ± 0.14	13.68 ± 0.12
Conductivity (mS cm^{-1})	2.92 ± 0.16	2.88 ± 0.61	2.84 ± 0.48
pH range	8.06 – 8.4	8.03 ± 0.21	8.01 ± 0.38

The overall dissolved oxygen (DO) levels increased in the outflow of the ebb-and-flow systems with different media, with ranges of 1.6–3.2 mgL^{-1}. The interaction between weeks, media and mixed did not affect the outflow DO concentration ($F_{(77, 352)} = 0.9$, $p = 0.61$). The highest mean outflow DO was in unmixed clay pebbles (2.33 ± 0.15 mg L^{-1}) and bioballs (2.32 ± 0.13 mg L^{-1}), followed by 19 mm gravel (2.31 ± 0.03 mg L^{-1}), while the lowest was in sand (2.08 ± 0.07 mg L^{-1}). Clay bricks, gravel (7 and 13 mm) and recycled plastic media had a mean DO concentration range of 2.20 ± 0.11 –2.30 ± 0.14 mg L^{-1}. The temperature range was 18.1–28.5 ^{o}C. There were slight differences between the inflow and outflow conductivity and pH levels.

4.4.2 The efficiency of nutrient removal in the unmixed and mixed media of the ebb-and-flow system

Media types significantly differed in reducing COD and ammonia-N removal ($p < 0.01$, Figure 15 i and ii). Unmixed media had higher means of 6.55 ± 0.13 % and 7.22 ± 0.14 % than mixed treatments, with 5.86 ± 0.10 % and 6.47 ± 0.11% for COD and ammonia-N removal, respectively (Figure 15 i and ii). The unmixed clay pebbles had the highest mean COD reduction of 7.51 ± 0.22 % and ammonia 8.13 ± 0.23 %, followed by bioballs and 19 mm gravel media and lowest in sand media with 5.03 ± 0.12 % and 5.35 ± 0.16 %, respectively.

An interaction between media and mixing influenced nitrite-N, nitrate-N, total inorganic nitrogen and orthophosphate removal ($p <0.001$, Figure 15 iii – vi). The mixed media had significantly higher overall mean nitrite-N removal of 7.53 ± 0.05 % than the unmixed with 6.48 ± 7.03 %. Mixed bioballs (7.93 ± 0.33 %) had the highest mean nitrite-N removal, followed by 7 mm gravel (7.81 ± 0.21 %). All unmixed media treatments had the lowest nitrite-N removal, which did not differ between treatments, $p >0.05$, Figure 15 iii.

The mean nitrate-N and TIN removal significantly differed between media treatments $p < 0.001$. Mixed media had a mean removal of 15.89 ± 0.1 % and 29.93 ± 0.83 % higher than unmixed with 13.35 ± 0.09 % and 26.59 ± 0.22 % for nitrate-N and TIN, respectively. Nitrate-N and TIN removal did not differ between mixed media, $p > 0.05$, Figure 15 iv–v. The unmixed clay pebbles mean nitrate and TIN removal were 15.26 ± 0.47 % and 28.68 ± 1.06 %, respectively. The lowest means were in unmixed sand with 12.62 ± 0.58 % and 25.11± 0.94 %, respectively. The mean orthophosphate removal differed between media and mixing, $F_{(1, 32)} = 12608.2$, $p < 0.001$, Figure 15 vi, with higher means in mixed (12.90 ± 0.11 %) than unmixed media (8.61 ± 0.21 %). Mixed media of clay pebbles, clay bricks, and sand had the highest mean orthophosphate removal of 13 %, while mixed gravel and plastic media had a mean range of 12.53 –12.74 %. Orthophosphate removal was lowest in the unmixed plastic and gravel media with 7.6 ± 0.13 – 9.7 ± 1.18 % (Figure 15 vi).

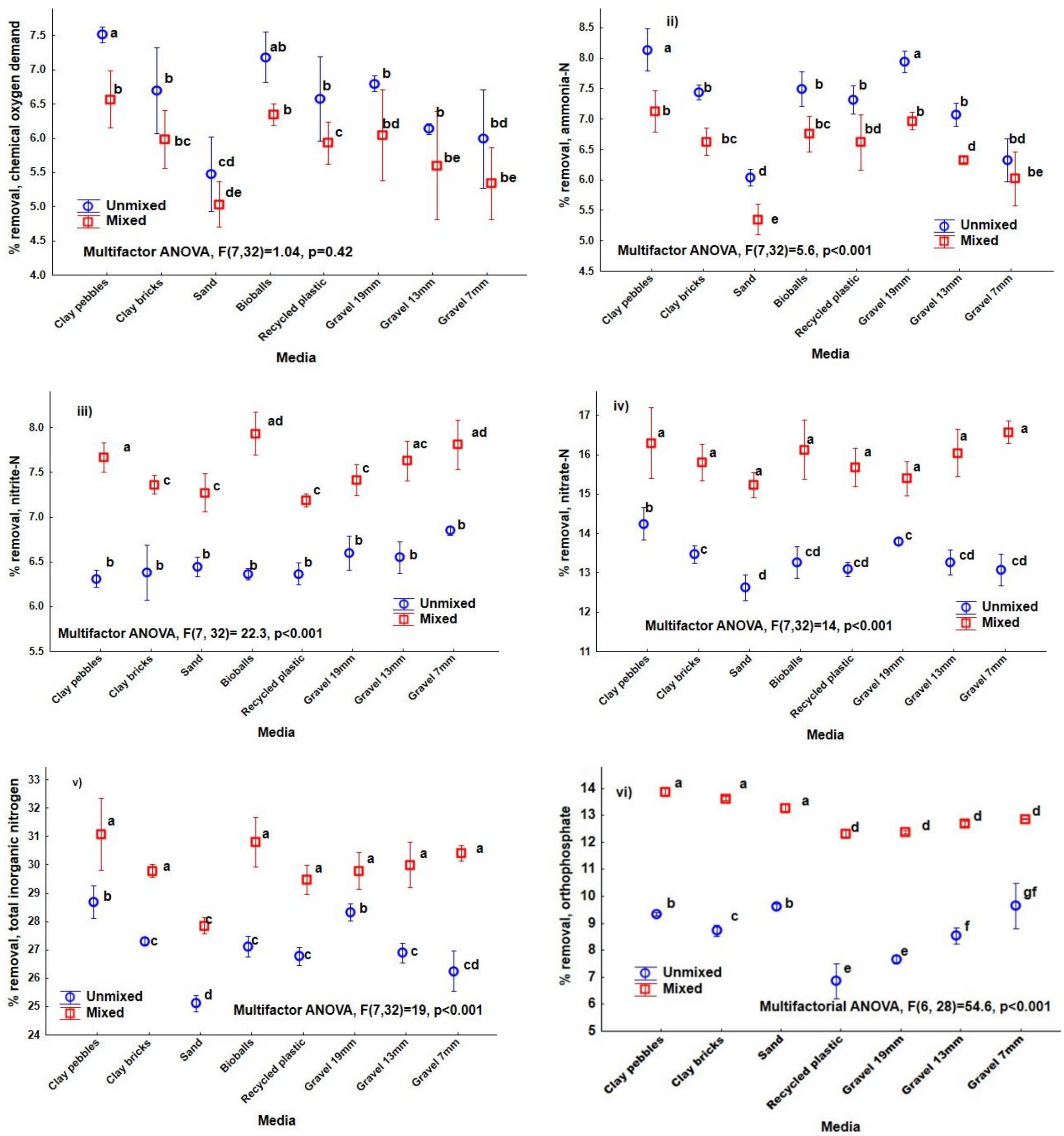


Figure 15: Percentage mean nutrient removal at 95 % confidence interval between the unmixed and mixed media in the ebb-and-flow system. i–vi is ammonia-N, nitrite-N, nitrate-N and orthophosphate removal. Treatments with letters a–f next to the mean were significantly different (p < 0.001).

4.4.3. The efficiency of nutrient removal between unmixed and mixed media over time in the ebb-and-flow wetland

The efficiency of media in removing nutrients increased with similar trends to maximum values, after which it decreased (Figure 16 i –vi). The unmixed media had higher mean reduction efficiency for COD and ammonia-N removal than the mixed media (repeated measures ANOVA, $p < 0.001$, Figure 16 i, ii). Mixed media had higher removal efficiencies of nitrite-N, nitrate-N, total inorganic nitrogen and orthophosphate ($p < 0.001$, Figure 16 iii –vi).

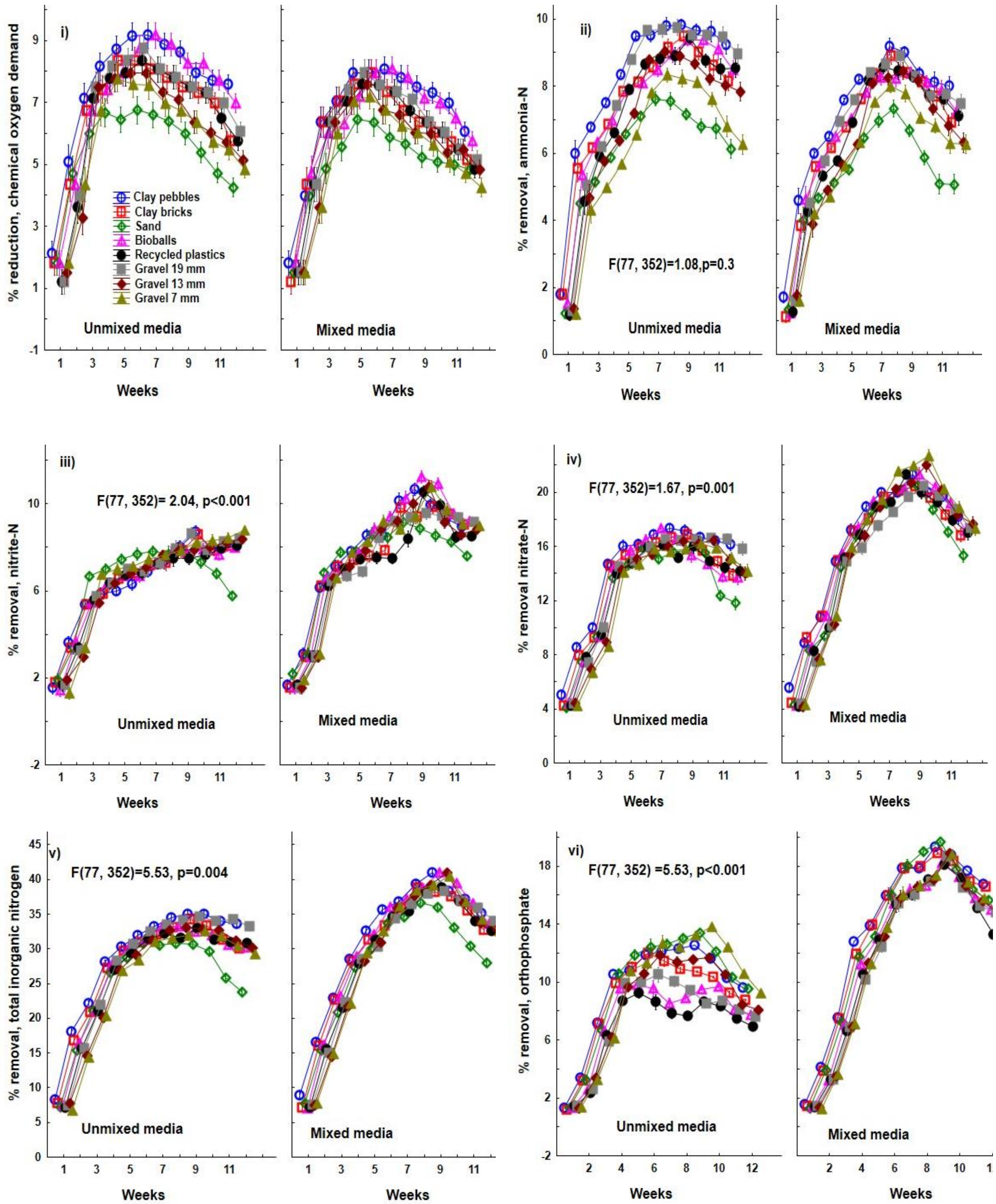


Figure 16: The percentage nutrient removal for the unmixed and mixed media over time in the ebb-and-flow system for 12 weeks. Numbering (i –vi) is the reduction of chemical oxygen demand and removal efficiencies for ammonia-N, nitrite-N, nitrate-N, total inorganic nitrogen and orthophosphate. (Repeated measures ANOVA, F-statistics for the interactions of media, mixing and time are shown in the graphs).

4.5 The effect of unmixed and mixed media on celery growth parameters and chemical composition in an ebb-and-flow wetland

4.5.1 The height and chlorophyll index of celery grown in unmixed and mixed media of the ebb-and-flow system

The celery height and chlorophyll concentration index differed between media ($p < 0.05$, Figure 18 i, ii). Celery grown in bioballs media died four weeks after planting, and no growth parameters were recorded. The longest mean celery was in mixed (62.11 ± 0.70 cm) than unmixed media (52.72 ± 1.34 cm), and lowest in unmixed recycled plastic (38.76 ± 3.69 cm), Figure 18 (i). The sand and 7 mm gravel media did not differ in mean celery height. All other unmixed media had a mean height of 55.3–57.6 cm (Figure 18i).

The mixed media had a higher chlorophyll concentration index (CCI) (70.23 ± 0.58) than the unmixed (63.45 ± 1.44) (Figure 18 ii). The mixed 7 mm gravel (75.73 ± 1.53) had the highest mean chlorophyll index, followed by clay pebbles, clay bricks, sand, gravel 13 and 19 mm, with a mean range of 70.76± 0.54 – 71.73± 0.79. The lowest was in unmixed recycled plastic media (48.33 ± 4.45). Unmixed clay pebbles, clay bricks and gravel (7, 13 and 19 mm) had a CCI range of 65.96 ± 0.70 – 68.31± 0.90 (Figure 18 ii).

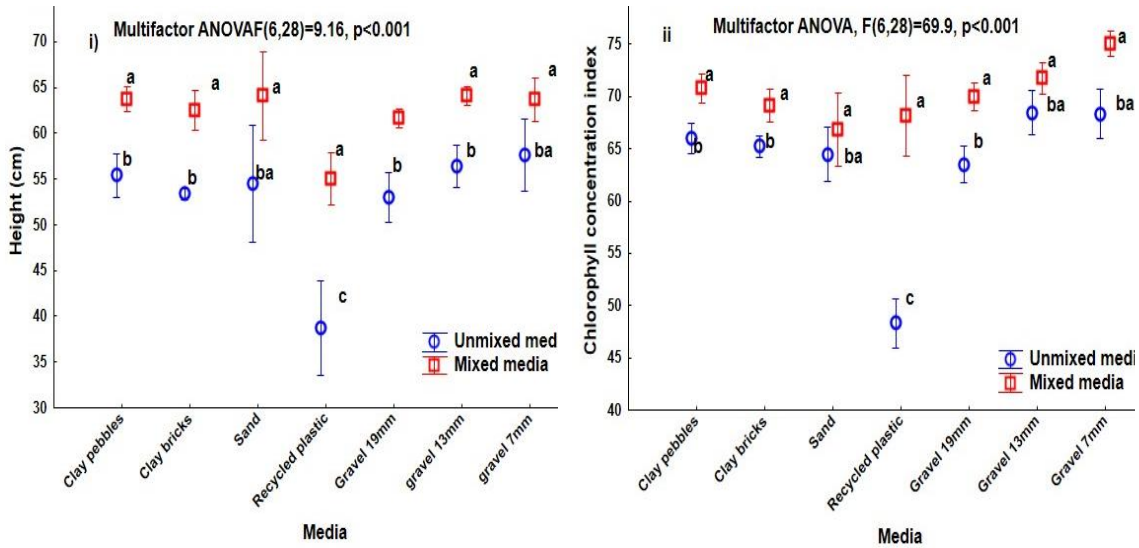


Figure 17: Means at 95 % confidence interval of celery height and chlorophyll concentration index (i– ii) grown in the unmixed and mixed media and irrigated with post-anaerobic digester brewery effluent for 12 weeks in the ebb-and-flow systems. Treatments with similar letters next to the mean are not significantly different p >0.05.

4.5.2 Celery plant biomass grown in the unmixed and mixed media in the ebb-and-flow system

The celery biomass increased from seedlings (5.85 ± 0.03 gm^{-2}) to final weight (9229.43 ± 383.38 gm^{-2}) in all media. Media types produced celery biomass that significantly differed between treatments ($p < 0.001$, Figure 19 i, ii). The mixed media had 11158.48 ± 411gm^{-2} and 1157.14 ± 45.05 gm^{-2} higher than unmixed (7300.38± 248.05 gm^{-2}) and (751 ± 24.46 gm^{-2}) wet and dry biomass, respectively. The mean wet biomass did not differ between mixed clay pebbles, clay bricks and sand, with a mean range of 13241.3 ± 423.70 gm^{-2} – 13458.3 ± 336 gm^{-2}. Mixed gravel media biomass range was 8350.6±160.20 gm^{-2} –

10685.3 ± 452 gm^{-2}. The unmixed recycled plastic had the lowest mean wet biomass of 64841± 423.70 gm^{-2}, corresponding to the dry biomass, respectively (Figure 19 i, ii).

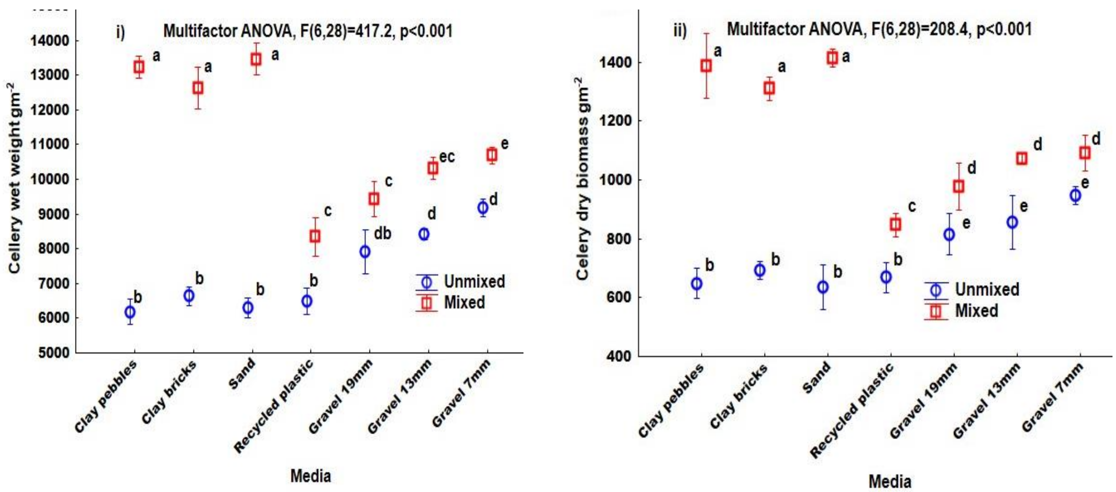


Figure 18: The means at 95% confidence interval of wet and dry biomass gained per treatment of celery grown in unmixed and mixed media and irrigated by post-anaerobic digester brewery effluent for 12 weeks in ebb-and-flow systems.

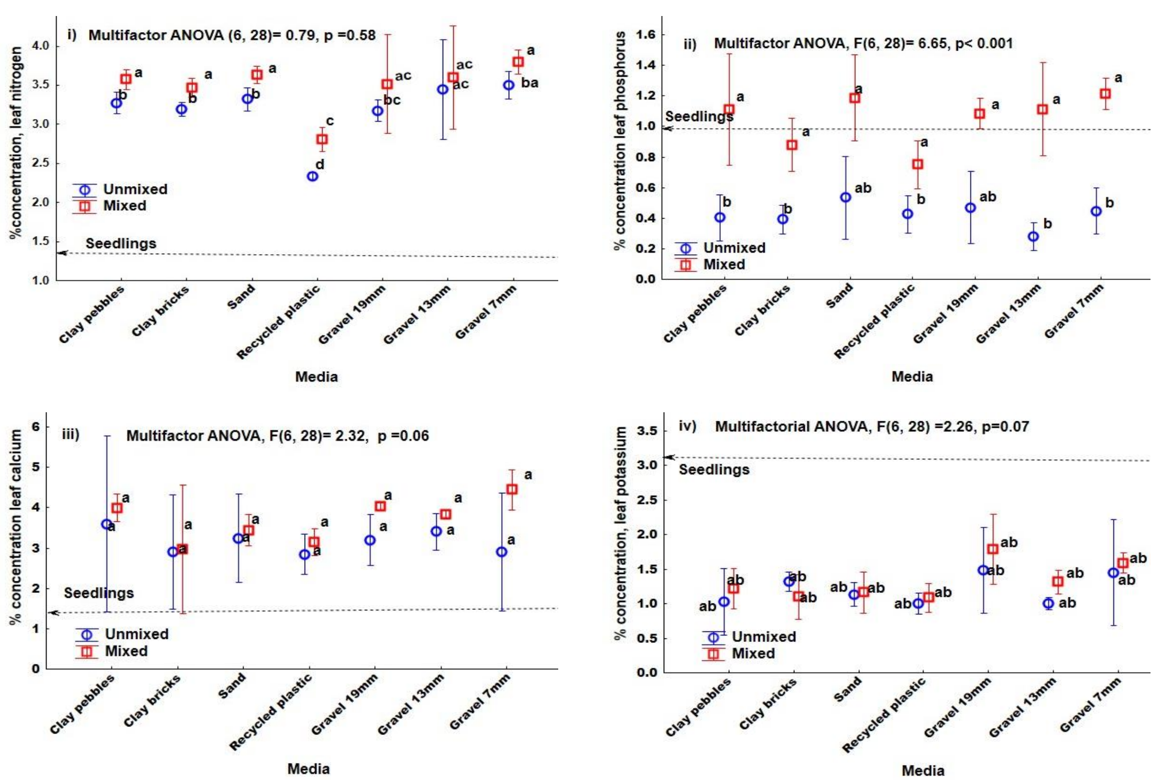
4.5.3. Leaf chemical analysis of celery grown in unmixed and mixed media in the ebb-and-flow system

The seedlings' composition and elemental uptake of celery grown in the media treatments in an ebb-and-flow system for 12 weeks are illustrated in Figure 20 i –xi. The percentage uptake of nitrogen, calcium, zinc, manganese, and sodium increased from seedlings in celery leaves grown in all media treatments. Leaf potassium, copper, iron and aluminium decreased in the media, except for unmixed sand with increased leaf iron (Figure 20 vi). The mean leaf sodium concentration increased from seedlings 13040 ± 62.5 mg kg^{-1} to

37824.3 ± 2121.7 mg kg^{-1} and 41047.6 ± 1822.9 mg kg^{-1} in unmixed and mixed media, respectively (Figure 20 ix).

An interaction between media and mixing did not affect the uptake of leaf nitrogen, calcium, potassium, manganese and sodium ($p > 0.05$, Figure 20 i, iii, iv, vii, ix). The mean leaf nitrogen was lowest in unmixed recycled plastic media (2.33 ± 0.01 %). Leaf nitrogen did not differ in clay pebbles, clay bricks and sand media treatments (Figure 20 i). Leaf calcium, potassium, manganese and sodium concentrations did not differ between treatments (Figure 20 iii, iv, vii, ix).

Leaf phosphorus, potassium and magnesium were below 5% in all media. Unmixed sand had the highest mean leaf iron of 247.63 ± 11.8 mg kg^{-1} (Figure 20 vi). The leaf zinc, copper and aluminium concentrations were similar in all media (Figure 20 vii, x, xi).



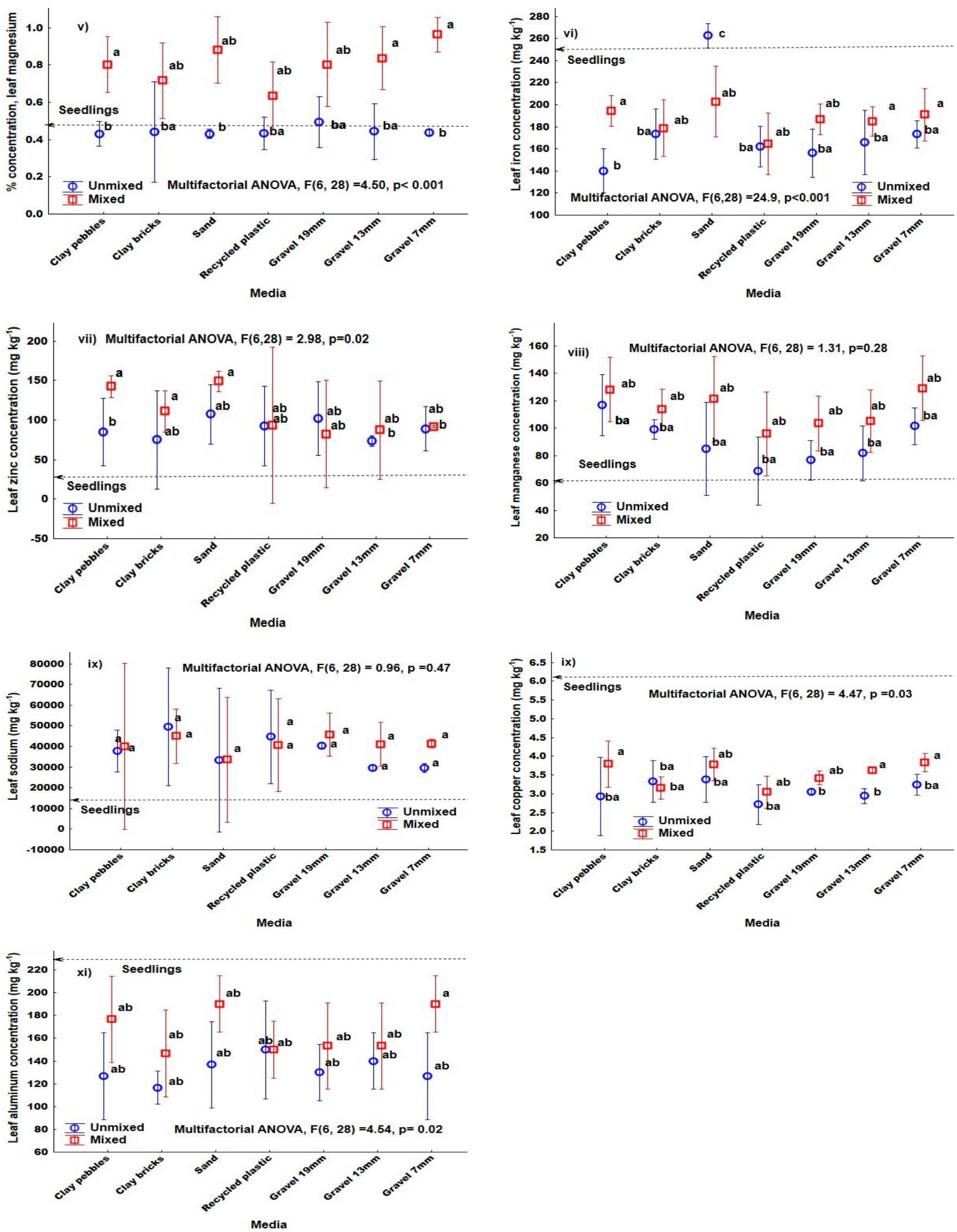


Figure 19: Means at 95 % confidence interval of (i–xi) element uptake in celery leaves grown in the mixed and unmixed media irrigated by post-anaerobic digester brewery effluent in the ebb-and-flow system for 12 weeks. The dotted line designates the seedlings' element concentration.

4.5.4 The health and stress symptoms of celery grown in the unmixed and mixed media of the ebb-and-flow system

The mortality of seedlings in the first month of planting was significantly higher in unmixed than mixed media (H (1, N =48, =12.1, p <0.05). There was unequal variation in percentage mortality between media (H (7, N =48, = 8.7, p=0.28). The pale-yellow top leaves were observed among all media, Plate 1 (a–d). Plants grown in recycled plastics had short stature, chlorosis, shrink and hard leaves with burnt tips. Plate 1 (b, e), and pale yellow in the unmixed clay bricks and gravel media, Plate 1 (a, c, d).

Plate 6: Stress symptoms and visual indicators of celery grown in unmixed and mixed media and irrigated with post-anaerobic digester effluent in the ebb-and-flow system for 12 weeks. Plates (a-c) are leaf chlorosis in clay bricks, recycled plastics, and gravel, (d–f) are pale yellow leaves in gravel, shrink leaves in recycled plastic and dead celery in bioballs media (Source: Author)

CHAPTER FIVE

DISCUSSION

5.1 Overview

This study examined the operation and design characteristics of ebb-and-flow constructed wetland components by varying the retention time on different gravel particle sizes, and a range of unmixed and mixed media to treat a unique post-anaerobic digester brewery effluent. These variations influenced nutrient removal that changed over time.

5.2 The effect of retention time and gravel sizes on physicochemical water variables and nutrient removal

Both retention time and gravel size influenced the nutrient removal efficiency for the 12-week operation period of the ebb-and-flow systems. The low dissolved oxygen levels at the inflow facilitated the need to increase O_2 concentration for further treatment of the post-anaerobic digester effluent by using an ebb-and-flow system. The higher concentration at the outflow may be caused by the ebb-and-flow operation that diffuses atmospheric oxygen into the system, to occupy voids left between the gravel particles in the subsequent drain phases (Austin et al., 2006; Li et al., 2015).

An increase in oxygen concentration within the substrate of larger gravel particle size could be attributed to differences in the interstitial spaces between the gravel particles, and to the duration of effluent retention within the beds. The differences in DO concentration between retention time and gravel size treatments indicate varied aerobic conditions that influence nutrient transformation and removal through nitrification, denitrification.

anaerobic ammonia oxidation (annamox) and decomposition of organic matter (Li *et al.*, 2015; Guo *et al*., 2020; Hao *et al*., 2022).

In this study, short effluent RT had more drain cycles, translating to rapid oxidation and degradation of nutrients. Some studies revealed that short effluent retention reduced the contact time between microorganisms and nutrients, limiting system performance (Saeed & Sun, 2012; Norton, 2014; Wu *et al*., 2015). This may primarily depend on the system design, type of effluent and the treatment purpose. However, according to Fick's law, rapid oxygen diffusion at the air-water interface and across biofilms (<100 μm) occurs within a few seconds in the wetland system (USEPA, 2000; Austin, 2006)..

Temperature changes did not differ during the experimental period, with ranges of 22–28 °C, which are within ranges for effective microbial growth rate and metabolism for most microorganisms. Truu *et al*. (2009) reported the optimal temperature for nitrification activity at 28–36 °C. Meng *et al*. (2014) reported fast growth of ammonia-oxidizing bacteria at temperatures above 15 °C.

The removal efficiencies of ammonia-N, nitrite-N and COD decreased as RT increased. The shortest RT of 10 minutes had rapid oxygen transfer, and more ammonia was transformed into nitrite-N and nitrate-N. Similarly, De Jong (2019) reported high DO profiles at shorter RTs of 15- and 30 minutes with higher ammonia oxidation than the 60-minute RT. Rodríguez-Gómez *et al*. (2021), stated that the oxidation of nitrite is always faster than ammonia when there is an adequate supply of oxygen, hence preventing its accumulation in the large gravel particles. These findings demonstrate that ebb-and-flow system operation could effectively reduce organic matter and ammonia at short effluent RT and large gravel particle size.

The 40-minute RT and 7-mm gravel treatments may have restricted oxygen supply, which could cause partial oxidation of organic matter, ammonia-N and nitrite-N. At longer effluent retention periods, dissolved oxygen in the wetland bed is depleted (Muda *et al*., 2011; Li *et al*., 2015). Substrate particle with large surface area creates varied DO microenvironments that influence nutrient transformation and removal processes in tidal wetland systems (Li *et al*., 2019; Fu *et al.,* 2020; Hao *et al.,* 2022). In addition, the metabolic activities of nitrifying bacteria in oxidizing ammonia are suppressed at low DO conditions (Seifi & Fazaelipoor, 2012).

High nitrate-N removal at 40-minute RT and 7-mm gravel could be attributed to a prolonged flooding phase that depleted DO within the small interstitial spaces of gravel media, creating a conducive environment for denitrification and anaerobic ammonia oxidation (anammox). The activities of heterotrophic denitrifying and anammox bacteria are enhanced at low oxygen conditions where nitrate-N is used as an electron acceptor (Ni *et al*., 2017; Sheng et al., 2020). The partial oxidation of ammonia forms the primary reactant to remove nitrogen through the anammox process. In this case, nitrite (NO_2^-) is reduced to nitrous oxide (NO), which combines with ammonium ions (NH_4^+), forming hydrazine (N_2H_4) that is converted to nitrogen gas (N_2) (Winkler & Straka, 2019; Sheng *et al*., 2020; Ronan et al., 2021). Therefore, nitrogen removal in the ebb-and-flow system occurs through denitrification and anaerobic ammonia oxidation under low DO conditions. Effective denitrification is not only influenced by oxygen concentration but also by adequate inorganic carbon in the effluent (Wang *et al*., 2015; Winkler & Straka, 2019). Determining the organic carbon to nitrogen ratio (C: N) of the post-AD brewery effluent can determine denitrification in these filter systems.

Low nitrate-N removal at 10 minutes RT and 19-mm gravel size could be due to high oxygen concentration within the substrate bed. The rapid oxidation of ammonia that builds up nitrate-N in the effluent limits denitrification. Denitrifying and anammox microorganisms are effective under anaerobic conditions (Seifi & Fazaelipoor, 2012).

Orthophosphate removal was below 5% in all treatments; the inflow concentration of orthophosphate was high, with a range of 10.96 – 21.82 mg/L. Phosphoric acid used as a cleaning detergent in upstream activities could contribute to high phosphorus concentration in the effluent. Similarly, Jong (2019) compared tidal, aerated and un-aerated post-treatment efficiency and reported low phosphate removal. Gravel used as the substrate in the study has unreactive surfaces for phosphorus adsorption. Xu *et al*. (2006) compared gravel and iron slag-filled wetlands at an hydraulic loading rate of 0.100 md^{-1} for a pre-treated domestic effluent and reported 44 % and 1% orthophosphate removal, respectively, consistent with the low P removal in gravel substrate used in the present study. High-adsorption capacity substrates of clay pebbles, alum sludge, and slag, containing cations of iron, aluminum, calcium, and magnesium, improve phosphorus removal (Vohla et al., 2011; Wang et al., 2020).

5.3 The efficiency of nutrient removal in the retention time and gravel size combinations over time

Recent studies have mainly focused on the efficiency of tidal wetlands in nutrient removal, while the period of maximum efficiency during operation was not reported. This study contributes to effluent treatment by estimating the period for maximum nutrient removal in the ebb-and-flow filters. The 10 and 20-minute RT treatment combinations achieved 38

% total inorganic nitrogen removal between weeks 10 and 11. This could be attributed to rapid aeration that enhanced nitrification while suppressing denitrification. The inflowing post-anaerobic digester effluent could be limited to organic carbon to facilitate denitrification. Kizito *et al.* (2017) reported a low total nitrogen removal of 22 % in gravel media and 47 % in corn cob biochar in the tidal systems treating anaerobically digested effluent. The biochars' carbon content and large surface area for attachment of heterotrophic denitrifying bacteria improved nitrogen removal. Zhang *et al*. (2021) compared lab-scale tidal systems of gravel and a mixture of gravel and zeolite media, operated at 2, 3, and 4 m d^{-1} hydraulic loading rates. The total nitrogen removal range (5.4 % –11.4 %) for combined gravel and zeolite (24 % – 43 %) was attributed to the inflowing water's low carbon: nitrogen ratio. The present study presented similar findings regarding the low nitrogen removal on gravel media. Therefore, effective nitrogen removal in tidal systems requires sequential nitrification and denitrification, with an optimal carbon supply.

A decline in ammonia-N removal efficiency could be attributed to a high concentration of 21.68 mg L^{-1} – 32.6 mg L^{-1} at the inflow. Rapid biofilm formation on gravel surfaces over time could also limit oxygen transfer for ammonia oxidation. Microorganisms produce extracellular polymeric substances, and in excess, could reduce the interstitial spaces between gravel particles (Zhou *et al*., 2020). Dense biofilm loses stability and may detach aggregates of slow-growing nitrifying bacteria through sloughing (Chaudhary *et al.*, 2003; Knowles *et al*., 2011), thus affecting the removal efficiency.

The progressive trends for nitrite-N and nitrate-N removal could be associated with reduced oxygen transfer into the substrate, which could favour the growth of denitrifying and anammox microorganisms in removing these nutrients, and the nitrite-N and nitrate-N are likely to be used as electron acceptors in denitrification (Ni *et al*., 2017; Guo *et al*., 2020). In addition, anammox bacteria do not need external carbon for metabolism (Winkler & Straka, 2019; Guo *et al*., 2020), which may have improved the removal efficiencies.

A study by De Jong (2019) on the effluent retention time of 15, 30, and 60 minutes in tidal CW treating anaerobically digested brewery effluent demonstrated increased dissolved oxygen at shorter RTs of 15 minutes and 30 minutes, treating ammonia-N and reduction of COD to discharge standards. An extension of the shorter RTs was done in this study to improve ammonia removal and COD reduction on a large gravel size of 19 mm, while the orthophosphate removal was not improved. The overall treatment efficiency differed in these studies and could be attributed to system design and the study period. A five-day batch cycle tidal design was used over five months, while the current study used a single-pass tidal design to examine the combined effect of gravel sizes and RTs over three months.

The anaerobic digester effluent is a unique effluent with varied compositions of nutrients, depending on the season of the anaerobic digester malfunction. The high concentration of ammonia-N, phosphorus, conductivity, and high pH contributed to the ebb-and-flow CW's performance in nutrient removal in this study. Therefore, the recycling of effluent and incorporation of plants could improve nutrient removal in the systems.

5.4 The efficiency of nutrient removal in the unmixed and mixed media of the ebb-and-flow system

The ebb-and-flow wetland operation strategy and substrate types influenced nutrient removal from a post-anaerobic digester effluent. The higher outflow dissolved oxygen concentration than the inflow was attributed to subsequent system drain cycles, contributing to oxidation, transformation and degradation of nutrients in the ebb-and-flow CWs. Austin (2006) and Behrends (2000) reported that the draining phase of a tidal CW rapidly diffuses atmospheric oxygen in the biofilm of the substrate. The rate of diffusion varies depending on media porosity and surface area (Akratos & Tsihrintzis, 2007; Almuktar *et al.*, 2018; Fu *et al.*, 2020).

The highest COD reduction and ammonia removal in unmixed clay pebbles, followed by bioballs and 19 mm gravel, could be attributed to the physical properties of these media. Clay pebbles and bioballs are lightweight and less compacted. Clay pebbles have air spaces that trap more oxygen, for the oxidation of chemical oxygen demand and ammonia-N. The 19-mm gravel is dense with large interstitial spaces and better effluent drainage during the ebb-and-flow operation. Gravel media porosity and effluent drainage decreased with increased pebble surface area. Sand is compacted with limited air spaces and poor effluent drainage. Media with bigger air spaces are linked to better aeration, while compacted media are related to low O_2 levels. Aerobic and anaerobic conditions created by the ebb-and-flow operations in the substrate transform ammonia-N to nitrite-N and nitrate-N through adsorption and desorption processes (Austin, 2006; Gregory *et al.*, 2012). Moreover, oxygen distribution within the substrate is stratified; the surface and middle layers are highly aerated, while the bottom layer can be anoxic (Saeed & Sun, 2012; Huang *et al*., 2013), creating a range of environmental conditions for enhanced nutrient removal.

The superior performance of mixed media in removing nitrite, nitrate, total inorganic nitrogen, and orthophosphate is due to varied porosities and surface area, creating varied DO conditions facilitating oxidation, transformation and nutrient removal. A combination of substrates' physical and chemical properties has complementary benefits. For example, granular activated carbon has a large surface area, creating more adsorption sites, thus contributing to nutrient removal (Wu *et al*., 2019; Fu *et al.*, 2020). The supplemented pine bark media is a carbon source for denitrification (Saeed and Sun, 2013; Hua *et al*., 2016). Saeed & Sun (2011) reported 97.8 % total nitrogen removal in combined gravel and organic wood mulch in vertical wetlands with *Phragmites australis* plants. Fan *et al*. (2013) reported a simultaneous removal of 99 % ammonia-N and 90 % total nitrogen with intermittent aeration at an influent COD: N ratio of 1:10 in vertical CWs. The difference between these studies could be that synthetic wastewater was treated on a laboratory scale, while the current study used a unique brewery effluent with varied effluent concentrations over time.

The low orthophosphate removal in unmixed media could be associated with high competition for nutrients on the limited adsorption sites. The mixed media of clay and sand media have aluminium (Al^{3+}), calcium (Ca^{2+}), iron (Fe^{3+}), and magnesium (Mg^{2+}) exchangeable cations, while granular activated carbon has more adsorption sites (Rittmann *et al*., 2011; Fu *et al.*, 2020; Marlena & Nowicki, 2022). Recently, studies have indicated that denitrifying polyphosphate-accumulating organisms (dPAO) can store phosphorus in the cells under alternate aerobic and anaerobic conditions (Winkler & Straka, 2019;

Shukla *et al*., 2020). Therefore, the identification and isolation of dPAO strains should be considered in future studies of ebb-and-flow operations.

5.5 The efficiency of nutrient removal between unmixed and mixed media over time in the ebb-and-flow wetland

Nutrient removal increased with similar trends in all media, followed by a phase of reduced efficiency. The increased nutrient removal phase could be due to progressive biofilm formation on the substrate, improving microorganism activity. The alternate aerobic and anaerobic conditions favour the growth of diverse microorganisms depending on the media type used (Dordio & Carvalho, 2013; Liu *et al*., 2020). Zhang *et al.* (2021) reported a higher ammonia removal of 55.5%–96.7% in mixed zeolite and gravel than in gravel alone (35.5% – 61 %). Zeolite addition enhanced ammonia adsorption and the development of nitrifying microorganisms. The various media used in this study had different physicochemical properties, which could increase microorganism diversity and improve nutrient removal.

A decreased nutrient removal efficiency after 8 weeks could be associated with excessive biofilm formation and pollutants concentrating on the adsorption sites over time, blocking and saturating the media (Hua *et al*., 2013; Vymazal, 2018; Zhou *et al*., 2020). Liu *et al*. (2014) reported media saturation in a long-term operation of tidal flow CWs treating high ammonium effluent, consistent with this study. The brewery's effluent composition varies over time depending on the season of beer production. Moreover, the high conductivity and pH of the post-AD effluent can influence the performance of the ebb-and-flow in this study. Therefore, screening and layering of substrates have been identified as anti-blockage mechanisms for enhanced spatiotemporal wastewater treatment (Wu *et al*., 2019; Liu *et al.*, 2020).

5.6 Celery growth parameters in unmixed and mixed media of the ebb-and-flow system

At the end of the study, celery growth in height, chlorophyll content and biomass were exhibited in all media used in the ebb-and-flow system. This indicates that celery adapted to post-aerobic digester irrigating effluent, media types and ebb-and-flow operation. However, celery grown in bioballs media died four weeks after planting and recycled plastic media had the lowest mean height. The plastic media are light-weight with irregular spaces that could not support celery roots in the ebb-and-flow systems. The similar celery height in sand and 7-mm gravel media can be related to their density and stability, supporting celery roots. The large surface area of these media increased the surface contact, enhancing nutrient retention capacity for celery uptake (Carlheiros *et al*., 2008; Ge *et al.*, 2015).

The chlorophyll concentration index indicates plant health and productivity of the celery plant (Liu *et al*., 2019). A higher chlorophyll concentration and above-ground biomass of celery grown in mixed media could be attributed to the irrigating effluent nutrient composition and the supplemented media properties. The unmixed media relied on the nutrients from the irrigating effluent, which could be deficient in organic carbon, an essential plant growth promoter. Wu *et al.* (2015) reported that industrial effluents have varied organic matter content and limited labile carbon depending on the raw materials and pretreatment processes. Therefore, post-AD effluent supplemented with organic carbon can improve celery productivity.

5.7 Celery leaf chemical composition

At the end of the study, leaf nitrogen, calcium, zinc, manganese and sodium concentrations were higher than in the seedlings in all media. The increased leaf nitrogen content could be attributed to ammonia uptake from the inflowing wastewater and nitrate as transformed ammonia products by the ebb-and-flow operation. The forms of nitrogen for plant uptake are ammonia and nitrate-N, with ammonia-N being preferred (Vymazal, 2007). An increase in nutrient uptake corresponded to celery height, chlorophyll concentration index and above-ground biomass in mixed media treatments.

The increased leaf sodium and calcium uptake is expected due to high concentrations of dissolved salts from the effluent source. Sodium hydroxide, sodium chloride, caustic soda, and calcium sulphate are used as pH buffers and cleaning detergents in brewing activities, thereby increasing the effluent salinity (Simate *et al.*, 2011; Cilliers, 2012). Celery is a halophyte adapted to saline conditions by synthesizing mannitol, a photosynthetic product that increases the osmotic potential of the cell wall (Everard *et al.*, 1994; Noiraud *et al.*, 2000). High salinity levels increase the uptake of calcium and sodium ions in the plant cell (Zhao *et al.*, 2021). Similarly, Pardossi *et al*. (1999) reported a high salt tolerance in celery, by comparing salinity levels of 50, 100, and 300 mM sodium chloride in celery growth and mineral content. Therefore, the high sodium and calcium contents of celery grown in this study increase celery's potential use as a bio-indicator and phytoremediator of a saline effluent quality.

The high leaf iron content in unmixed sand could be due to the composition of ferric iron an exchangeable cation increasing the uptake (Arias *et al*., 2001). The decreased P content in celery leaves from the seedlings stage could be due to competition between plant uptake, microorganisms' activities and adsorption on the media. Phosphorus binding properties could increase adsorption on surfaces with high cation exchange in clay pebbles, sand and GAC (Vohla *et al.*, 2011; Lima *et al.*, 2018).

A decrease in the content of potassium, copper and aluminium in the leaves could be related to the high salinity of irrigating effluent hindering the uptake of these microelements. Sodium and potassium are monovalent cations, and at high salinity sodium ions limit the uptake of potassium ions at plant roots (Pardossi *et al.*,1999; Jouyban, 2012). Potassium ions are essential for enzyme activities and maintaining cell turgidity. A deficiency in potassium limits plant growth and metabolism (Jouyban, 2012). This is the first study of celery grown in a brewery effluent and adjusting the post-AD effluent pH and salinity could improve bioavailability and nutrient uptake.

5.8 Celery plant health and stress symptoms

Plant health and stress symptoms exhibited by visual indicators relate to plant productivity. Celery grown in bioballs media died, while those grown in recycled plastic showed stress symptoms. The bioballs and recycled plastic are lightweight media limiting the support of plant roots during ebb-and-flow operation (Yang *et al.*, 2018). The top leaves chlorosis observed in the celery grown in these media could be due to low phosphorus, potassium and iron uptake.

The pale-yellow celery leaves observed in celery grown in unmixed clay bricks could be caused by excessive iron uptake. Clay bricks are rich in ferric iron and are susceptible to cation exchange (Gu *et al*., 2019). Aslam & Chouhan (2023) reported iron toxicity in soils high in pH to limit the uptake of manganese, zinc and copper thus, causing chlorosis, necrosis and slow growth. This can be mitigated by adjusting soil pH, using iron-tolerant plants and chelation.

A decrease in leaf copper, iron and aluminium concentrations could be due to low content in the irrigating effluent. Rakocy *et al.* (2006) recommended supplementing the limiting nutrients to meet plant requirements in a hydroponic system. Overall, this study demonstrated the removal efficiency of comparable media and celery growth on nutrient removal as a potential for treating post-AD brewery effluent.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The ebb-and-flow operation, effluent retention time and gravel particle size influenced nutrient removal from a post-anaerobic digester effluent. A 10-minute RT in a CW with 19-mm gravel achieved the removal efficiencies for 7.7 % ammonia-N and 8.2 % COD reduction. The 40-minute RT at 7-mm gravel had the highest nitrate-N removal of 18.6 %. Orthophosphate removal was below 5%. The peak removal efficiency for ammonia-N was after 8 weeks, while nitrite-N and nitrate-N removal efficiency was progressive over time. Therefore, the efficiency of ebb-and-flow design on nutrient removal from a brewery effluent is time-dependent.

Media, mixing and celery growth influenced the treatment of post-anaerobic digester effluent in a single-pass ebb-and-flow constructed wetland design. The unmixed media of clay pebbles had the highest mean COD reduction of 7.51 % and ammonia at 8.13 %, followed by bioballs and 19 mm gravel. Mixing improved the removal efficiency of nitrite-N (7.6 %), nitrate-N (15.3 %), TIN (29.9 %), orthophosphate (12.7 %) and plant biomass (11158.48 gm^{-2}).

Media porosity, surface area, substrate composition and celery growth enhance nutrient removal through synergistic interactions. The post-anaerobic digester effluent has macro and micronutrients that can support celery growth in the ebb-and-flow system; however, the high salinity and pH of post-anaerobic digester effluent influenced the uptake of phosphorus, magnesium, iron, copper and aluminium, thus causing stress symptoms and impaired plant health.

6.2 Recommendations

The study recommends a short effluent retention time of 10 minutes on 19-mm gravel to improve nitrogen removal and reduction of COD in a single-pass ebb-and-flow filter. When maximum nutrient removal is reached, a restoration strategy of backwashing the gravel should be considered for continued operation. Ebb-and-flow filter design characteristics in this study can be applied in treating other industrial and aquaculture effluents, depending on the nature and strength of the effluent.

Further optimisation is required to examine substrates with high cation exchange capacity, and the incorporation of plants and earthworms to degrade clogged organic matter on the wetland substrate. Future studies should consider testing layered gravel in the ebb-and-flow design, to utilise small space, stratify dissolved oxygen distribution and enhance treatment efficiency. Further studies should identify and characterize microorganisms colonizing the substrate in the ebb-and-flow constructed wetlands.

The study recommends unmixed clay pebbles, bioballs and 19-mm gravel for improved ammonia-N removal and mixed media for multiple pollutant removal and celery productivity. Further studies should explore the ebb-and-flow system's performance under a wider range of environmental conditions. Future studies should examine the spatiotemporal effects of alternative substrate layering as an anti-blockage mechanism for nutrient removal in the ebb-and-flow constructed wetlands. Celery growth in the anaerobically digested effluent indicates its potential use as a phyto-remediator and a bio-indicator of effluent quality, and as a good candidate for sustainable practice and solution for high salinity effluents. The ability of celery to accumulate salt in the tissues can be detrimental to human consumption and health. Further studies should investigate the optimal sodium content in the celery fit for human consumption. The quality of post-anaerobic digester effluent can be further improved using diverse salt-tolerant crops to maximize salt uptake from the effluent.

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APPENDICES

Appendix I: The effect of system design, effluent type, hydraulic retention time, inflow concentration, substrate type, and plant species on nitrogen removal in tidal wetland system

System Design	Effluent type	Scale	HRT or HLR	System size	Inflow rate	Inflow concentration ($mg\ L^{-1}$)
Vertical flow	Dairy	Commercial	1–4 days	992 L	300 Ld^{-1}, 150 Ld^{-1}, 100 Ld^{-1} 75 Ld^{-1} for 1–4 days HRT	NH_4^+:34.1–46.1 Total kjedahl nitrogen; 18.6-24.5
Non-aerated (NA) Intermittently aerated (IA) Continuously aerated (CA)	River water	Lab	19, 38 and 76 cm day^{-1} 19, 38 and 76 cm day^{-1} 19, 38 and 76 cm day^{-1}	0.2 m^2	-	NH_4^+; 3.5–10.6 Total nitrogen; 5.8–12.7
Intermittent aeration VFCWs COD/N ratio 2.5:1–20:1	Synthetic wastewater	Lab	0.21 $m^3\ m^{-2}\ batch^{-1}$	13 m^2	-	NH_4^+: 39.8–40.2 Total kjedahl nitrogen: 45
Tidal flow with media types	Synthetic wastewater	Lab	207 L $m^{-2}\ d^{-1}$	22 m^2	Flood/drain ratio of 16 h/8 h	NH_4^+:100 Total kjedahl: 33.3 ± 4.9
Reciprocating flow	Synthetic wastewater	Lab	0.33 $m^3\ m^{-2}\ d^{-1}$	40 L	Wet phase 24 h Dry phase 12 h	NH_4: 30 TN: -
Constrained flooded time	Synthetic wastewater	Lab	12-48 h effluent retention	8.8 L	-	NH_4^+:32.8–37.5 TN: -

Media types	Anaerobic digested swine effluent	Lab	Flood/drain ratio Phase1; 4:8 h Phase 2; 8:4 h	10.4 L	30 mL/min	COD; 1500 mg L^{-1} NH_4^+: 500 mg L^{-1} TN: -
Intermittent aeration (IA) and tidal flow(TF)	Synthetic wastewater	Lab	Flood/drain ratio; 4:8 h and 8:4 h	3.5 L	Airflow rate 0.5 L /min or 4 h (hours 0-1, 6-7, 12-13 and 18-19)	COD:210 NH_4^+; 42.09 TN: -
Intermittent flow	Synthetic saline water	Lab	0.32 m/m^2.d	96 L	Flood / drainFlood/drain 24 h	NH_4^+: 1.5 TN: -
Tidal wetland Up-flow Down-flow mode	Synthetic mariculture effluent	Lab	0.33 $m^3/m^2 \cdot d$.	36.7 m^2	Flood and drain cycle: 24 h	NH_4^+: 8 TN: -

Appendix II: The effect of system design, effluent type, hydraulic retention time, inflow concentration, substrate type, and plant species on nitrogen removal in tidal wetland system

Period (days)	Substrate type	Plant species per m^2	Plant density	NH_4^+-N removal	TN removal	Reference
112	Layers Gravel 1 (1.5 cm) Gravel 2 (2.5 cm)	*Typha angustata*	235 per m^2 275 per m^2 386 per m^2 447 per m^2	81 ± 6.1 % 92.9 ± 1.4 % 97.4 ± 0.3 % 99.9 ± 0.01 %	35.4±4.5 % 80.5±3.1 % 84.6±2.4 % 94.5±7.8 %	(Ghosh & Gopal, 2010)
150	Gravel layers: (7–15 mm) (18–32 mm) (inoculated)	-	-	40 % 65 % 54 %	- 29–57 %	(Dong *et al*., 2012)
150	Phragmites	*Phragmite australis*	*6–8 per unit*	99 %	90 %	(Fan *et al*., 2013)
200	Zeolite (2–4 mm) Sand (2 –4 mm) Ceramsite (3–5 mm) Volcanic rock (5–8 mm)	–	–	97 % 15–34 % 15–34 % 15 –34 %	85 % (12–31 %) (12–31 %) (12–31 %)	(Liu *et al*., 2014)
245	Gravel layers 1,2 10–20 mm 8–10 mm	*Iris pseudacorus*	125 per m^2.	76 ± 3.9 %	81-93 %	(Zhi *et al*., 2015)
188	Layers Volcanic rock 8–10mm	*Juncus effusus*	-	55-82 %	60-84 %	(Li *et al*., 2015)
300	Gravel 10–20 mm Corn cob wood biochars (2–10 mm) Gravel (50mm)	-	-	76 %	>37 % 22 – 49 %	(Kizito *et al*., 2017)

150	IA + gravel IA+ biochar TF + gravel TF + biochar	*Iris pseudoacorus*	6 rhizomes per unit	85.8 % 87.9 % 96.2 % 98.4 %	56.9–72.2 %	(Li *et al*., 2019)
457	Layer Coarse gravel (8–12 mm) lava rock (5–10 mm)	*Phragmite australis* *Spartina alterniflora*	100 per m^2	84.7 %	22.5 –87.5 %	(Zhang *et al*., 2021)
90	Layers Gravel (5–10 cm) Sand (1–4 mm), Burn slag (2–10 mm) Fine gravel (5–20 mm)	*Suaede salsa*	2 per unit	70.42–73.05 % 70.4–73.0 %	81.5 % 61.8 %	(Liu *et al*., 2022)

Appendix III: Effects of effluent recirculation and hydraulic loading rate and substrate surface area on the removal efficiency of nitrogen in subsurface flow wetlands

System design	Scale	Effluent	Recirculation	Flow rate	Hydraulic loading rate
Down-flow and up-flow stages in in vertical subsurface flow	Pilot	Pretreated effluent from swine farming	25, 50, 100, 150 %	$0.4\ m^{-1}\ d^{-1}$	$6.25, 7.5, 10, 15\ cm\ d^{-1}$
High-load VSSF with intermittent recirculation (R-VSSF)	Pilot	Raw domestic effluent	6 intermittent aerations per cycle	$0.56\ m^{-1}\ h^{-1}$	$168 \pm 44\ L\ m^{-2}\ d^{-1}$ $158 \pm 17\ L\ m^{-2}\ d^{-1}$
High-load VSSF with intermittent aeration (A-VSSF)			12 intermittent aerations per cycle	$3.5\ m^{-1}\ h^{-1}$	$179 \pm 5\ L\ m^{-2}\ d^{-1}$
High-load VSSF with intermittent recirculation and aeration (AR-VSSF)			-	-	-
Re-circulated hybrid tidal flow	Lab	Septic tank effluent	1 time 2 times 1 time 2 times	-	$0.5\text{-}1.5\ m^{-2}\ d^{-1}$ $0.5\text{-}1.5\ m^{-2}\ d^{-1}$
2-stage VSSF Stage 1 Recirculation Stage 2 Intermittent feeding	Full	Raw effluent Raw waste-water +recirculation	3 times	$2.16\ m^{-3}\ d^{-1}$	
2-stage VSSF Modifications (M) Step feeding Carbon addition)	Full	High-strength domestic waste-water	100% recirculation	$25\ m\text{-}^{3}d^{-1}$ raw effluent and $25\ m\text{-}^{3}d^{-1}$ recirculated effluent	$0.135\ m^{-1}\ d^{-1}$

Appendix IV: Effects of effluent recirculation and hydraulic loading rate and substrate surface area on the removal efficiency of nitrogen in subsurface flow wetlands.

Surface area	Substrate	NH_4^+ removal	Total Nitrogen removal	Reference
	Multilayers			(Lian-sheng *et al*., 2006)
4 m^2	Zeolite (5–8 mm)	61.7 %	66.6 %	
	Cinder (12–20 mm)			
	Gravel (10–40 mm)			
2.25 m^2	Drainage layer			(Foladori *et al*., 2013)
depth: 0.8 m	Gravel 15–30 mm	72 %	44 %	
	Gravel 7–15 mm	69 %	49 %	
	Sand 1–6 mm	71 %	-	
2.25 m^2 depth: 0.6 m	Blast furnace + coarse sand	47.4–64.5 %	24.3–48.8 %	(Cui *et al*., 2012)
		54.5–69.9 %	26.4–52.2 %	
1000 L	Blast furnace alone			
		52.8–61.6 %	23.3–39.3 %	
		66.9–76 %	24.5–53.2 %	

	Volcanic rock 4–8 mm Modified with plastic conduit sacks each	99 %		(Nivala *et al.*, 2019)
20 m^2	Layers: Zeotuff 2–4 mm Zeotuff 4–8 mm Zeotuff 10–25 mm	59 %	0.6–11.8 % reduced performance by 8 mg L^{-1}	
995 m^2	Sand 1– 4 mm Fine gravel 2–8 mm Sand 1–4 mm	99.5 %	82.9 %	Al-wahaibi *et al.*, (2021)

Appendix V: Similarity Report


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