CONSTRUCTED WETLANDS FOR DEGRADING INDUSTRIAL EFFLUENTS



UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

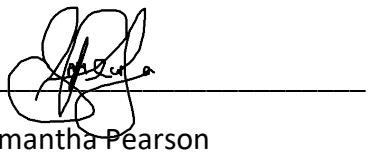
Samantha Pearson

A dissertation submitted to the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Engineering.

December 2020

Declaration

I declare, that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science in Engineering to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.



Samantha Pearson

8th day of DECEMBER, 2020

Summary

Human and environmental exposure to chemical compounds, specifically aquatic environments are becoming more of a concern as environmental degradation continues. The release of polyphenolic compounds into the environment is of major concern as they are not easily broken down and persist for long periods of time exerting toxic effects on both human and wildlife. Constructed wetlands are being considered in South Africa, as an environmentally friendly alternative industrial wastewater treatment technology and are low in capital costs and require less maintenance and infrastructure than other treatment technologies.

The pulp and paper industry is one of the biggest industries in the world, producing an effluent that is phenol-rich. This study looks at pulp and paper mill (phenol-rich) effluent remediation by constructed wetlands on a laboratory-scale.

There are three main parts to this study on the use of constructed wetlands for phenol rich remediation: batch operation, continuous operation and phenol remediation kinetics. Batch operation investigated the most effective type of emergent macrophyte species as well as the effect pulp and paper mill effluent strength had on the phenol remediation efficiency. Continuous operation used a pure phenol solution to determine the most effective constructed wetland macrophyte configuration for effluent remediation and investigated the phenol and carbon oxygen demand concentration gradients along the length and depth of the constructed wetland bed for pure phenol solution and a full-strength pulp and paper mill effluent. The third phases, kinetics, investigated the approximate phenol remediation kinetics in both batch and continuous operation.

It was found that different emergent macrophytes species do have an influence on the effluent remediation process and a full-strength pulp and paper (phenol-rich) effluent can be remediated by constructed wetlands in both batch and continuous operation with phenol remediation kinetics favoring warmer temperatures.

Acknowledgements

I wish to thank the following people for their support and assistance:

- Professor Craig Sheridan for his outstanding support and supervision;
- Dr. Uwe Kappelmeyer for his guidance and support;
- The IMWaRU and CIWaRD team for their guidance and support; and
- The staff at the School of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg.

Contents

1. Introduction 1

1.1 Dissertation Structure 3

2. Background 4

2.1 Water scarcity 4

2.2 Pulp and paper industry 4

2.3 Effluent hazards 5

2.4 Component detection and discharge laws and regulations 6

2.5 Current pulp and paper effluent treatment technologies 8

2.6 Constructed wetlands 10

2.6.1 History of constructed wetlands 11

2.7 Types of constructed wetlands 11

2.7.1 Classification by macrophyte type 12

2.7.2 Classification by flow type 13

2.8 Uses of constructed wetlands 14

2.9 Advantages and limitations of constructed wetlands 15

2.10 How constructed wetlands work 17

2.10.1 Organic carbon removal by CWs 19

2.10.2 COD removal by CWs 20

2.10.3 Other processes not investigated in this work 20

2.10.4 The water balance 20

2.11 Kinetic remediation of phenolic compounds in a CW 23

2.11.1 Hydraulic equations describing subsurface CWs 23

2.11.2 Determining residence time distributions (RTDs) 23

2.12 Plants for constructed wetlands 26

2.12.1 *Phragmites australis* (Common Reed) 27

2.12.2 *Chondropetalum tectorum* (Cape Thatching Reed) 27

2.12.3 *Juncus effusus spiralis* (Common Spiral Rush) 27

2.12.4 Juncus effusus (Common Rush) 27

2.12.5 Zantedeschia aethiopica (Arum Lily) 28

2.13 Previous studies on pulp and paper (phenol-rich) effluent treatment 28

2.14 Research objectives 28

3. Materials and methods 29

3.1 Analyze the industrial effluent 29

3.2 Effluent strength influences on phenolic compound remediation 31

3.3 Determining the most effective CW macrophyte configuration and TPC and COD gradient along the length and depth of the CW bed 32

3.4 Measure the total phenolic content and chemical oxygen demand of the outlet for discharge purposes 34

3.5 Phenol remediation kinetics of batch and continuous constructed wetlands 34

4. Results and Discussion 36

4.1 Analysis of the industrial effluent 36

4.2 Effluent strength influence to phenolic compound remediation 37

4.3 CW macrophyte configurations and phenol concentration gradients along the length and depth of the CW bed 40

4.3.1 The most effective CW configuration 40

4.3.2 TPC and COD gradients along the length and depth of the CW bed – pure phenol solution 43

4.3.3 TPC and COD gradients along the length and depth of the CW bed – pulp and paper mill effluent 46

4.4 Outlet total phenolic content and carbon oxygen demand for discharge purposes 48

4.5 Phenol remediation kinetics for batch and continuous operation 51

4.5.1 Batch phenol remediation kinetics 51

4.5.2 Continuous flow phenol remediation kinetics 53

4.5.3 Batch vs continuous phenol remediation kinetics 55

5. Factors influencing pulp and paper mill effluent remediation using constructed wetlands 56

6. Conclusions 82

References 84

Appendices 90

Table of Figures

Figure 1: CW Batch Operation Setup 30
Figure 2: Laboratory-Scale Constructed Wetlands Setup 32
Figure 3: Irrigation Taps Used to Control Flow Rates 33
Figure 4: Phenol and COD Remediation Percentage Profiles for Each Macrophyte Species with Corresponding Effluent Strength 39
Figure 5: Phenol Remediation and COD Profiles for the Three CWs for Phenol Solution 42
Figure 6: TPC and COD Gradients Along the Length and Depth of CW C Using Pure Phenol Solution. 45
Figure 7: TPC and COD Gradients Along the Length and Depth of CW C Using Pulp and Paper Mill Effluent 47
Figure 8: TPC and COD Outlet Values for Pulp and Paper Mill Effluent 50
Figure 9: Batch Kinetics for Full Strength Effluent with J. effusus as the Macrophyte Species at 20-23˚C 52
Figure 10: Batch Kinetics for Dilute Strength Effluent with J. effusus as the Macrophyte Species at 20-23˚C 53
Figure 11: Reaction Rate Constant for Phenol Remediation in CW C at a Temperature Range of 15-18˚C 54

Table of Tables

Table 1: Summary of the Analytical Tests Performed on the Effluent 36
Table 2: Total Phenol Remediation and COD Removal Percentages for the Different Macrophyte Species after two weeks 37
Table 3: Phenol Remediation % and COD Removal % over the Three Different CW Macrophyte Configurations: 40
Table 4: TPC and COD Outlet Values for Pulp and Paper Mill Effluent per Day 48
Table 5: Approximate Batch Kinetic Rate Constants for Full Strength and Dilute Pulp and Paper Effluent Strengths Across all Macrophyte Species with units 1/h at 20-23˚C. 52

Nomenclature

φ	Channel Diameter
ρ	Density of Fluid
τ	Residence Time
μ	Viscosity of Fluid
$C_{i,in}$	Initial Concentration of Component *i*
$C_{i,out}$	Final Concentration of Component *i*
D	Dispersion Number
d	Average Depth
E_p	Monthly Potential Evapotranspiration
k	Rate Constant
K_{hyd}	Hydraulic Conductivity
n	Order of Reaction
N	Number of Tanks
Q	Flow Rate
r	Monthly Average Relative Humidity
Re	Reynolds Number
S	Slope of the Saturated Hydraulic Zone
t	Time
t_m	Mean Residence Time
T_{mon}	Monthly Average Temperature
v	Average Velocity
w	Average Width
X_i	Conversion of Component *i*
z	Length of Reactor

Acronyms

BOD	Biological Oxygen Demand
COD	Carbon Oxygen Demand
CWs	Constructed Wetlands
GAE	Gallic Acid Equivalent
TPC	Total Phenolic Content

1. Introduction

The Kraft process is the most commonly used process in the pulp and paper industry. Pulp and black liquor are the major products resulting from the Kraft process (Oliveira et al., 2018). Black liquor is a wastewater that contains lignin and hemicellulose, both of which contain many structures containing polyphenols (Cardoso et al., 2009). These components, high in polyphenol concentrations are highly toxic to the environment, even at low concentrations (Stefanakis et al., 2016) due their high chemical oxygen demand, high alkalinity and dark brown colour (Cao et al., 2011). Very little is known on the adverse effects and pollution of the pulp and paper effluent components and the effluent (phenol-rich) is being discharged into aquatic bodies, worldwide (Singh and Chandra, 2019).

Due to water availability in every sector becoming increasingly scarce, it is vital that all wastewaters are treated effectively and efficiently to ensure there is enough useable water to supply all the demands. The more effectively and efficiently water can be treated for different uses the more sustainable life and processes will become. This is of most importance to countries such as South Africa which is a water scarce country by nature (Gosling and Arnell, 2016; Oki and Kanae, 2006; Plessis, 2017).

Wastewater treatment is traditionally done through biological, chemical and/or physical processes supported by natural components such as microbial organisms (Sundaravadivel and Vigneswaran, 2001). These treatments require large amount of mechanical energy, are costly and lead to environmental degradation through improper disposal of their wastewaters (Sundaravadivel and Vigneswaran, 2001; Vymazal, 2014; Wu et al., 2015). For this reason, treatment methods that are cost-effective and sustainable are being actively sought out.

Phenol-rich effluent is commonly treated by a number of different technologies (anaerobic treatment such as sludge reactors, (Sokkanathan et al., 2018) and advanced oxidation processes such as direct contact thermal treatment (Brandão and Benachour, 2017), however these technologies are complex and expensive (Saeed and Sun, 2012). An alternative is to use constructed wetlands as they are considered a green option for wastewater treatments, are sustainable and have reduced construction, maintenance and operational costs (Stefanakis et al., 2016). Constructed wetlands (CWs) will be used in this research to remediate polyphenols from industrial phenol-rich wastewaters, as a sustainable treatment option that has technical, environmental and social advantages that include but not limited to:

- Possible reuse of treated water;
- Low maintenance system;
- Aesthetic quality of constructed wetlands; and
- Production of biomass in the form of grasses (thatching materials) and/or cut flowers.

Phenolic compounds are removed in CWs by means of microbial accumulation (bacteria in the biofilms around the root of the plants and gravel) and particles of clay through adsorption and organic matter (Rossmann et al., 2012). Few studies have been done on polyphenol treatment by CWs. However, the studies done previously, on similar organic compounds, such as benzene, show promising removal results (Stefanakis et al., 2016). Therefore, this study focuses on the use of horizontal sub-surface flow CWs as a possible cost saving and effective treatment method of phenol-rich effluent.

1.1 Dissertation Structure

This dissertation has been divided into 6 Chapters.

- **Chapter 1** - Introduction.
- **Chapter 2** – Background
    - An analysis of the literature relevant to work in this dissertation.
- **Chapter 3** – Materials and Methods
    - All materials and methods used throughout this work is discussed in this Chapter
- **Chapter 4** – Results and Discussion
    - All results are presented in this Chapter with the corresponding discussions.
- **Chapter 5** – Factors Influencing Pulp and Paper Mill Effluent Remediation using Constructed Wetlands.
    - This Chapter has been written in the form of a paper, with the intent to publish. For this reason, there is overlap between the previous Chapters and the paper's introduction, literature review and Results sections, we request the readers' indulgence for this.
- **Chapter 6** – Conclusion
    - Conclusions to the work presented in this dissertation

2. Background

2.1 Water scarcity

Water is a vital resource for all living organisms and most industrial processes. Demands for water from both sectors are increasing as the human population is increasing which in turn increases the demand for more industrial processes. Surface water (water found in lakes, rivers, dams and icecaps) is the most readily available for consumption. However, this fresh water only makes up three percent of the water on Earth (Oki and Kanae, 2006). Fresh water scarcity is a threat to human society and its sustainable development. According to the World Economic Forum, water crises is one of the leading global risks in terms of potential impact (Mekonnen and Hoekstra, 2016). Most industries utilise water in some form. This results in the production of various wastewater types, all requiring some form of treatment before being discharged or reused. Before being discharged the following need to be tested and all legislation requirements met: chemical oxygen demand (COD), total suspended solids (TSS), nitrogen compounds and absorbable organic halides (Ashrafi et al., 2015).

2.2 Pulp and paper industry

The pulp and paper industry is one of the world's largest industries; producing the third largest volume of wastewater in the industrial sector (Ashrafi et al., 2015) and ranked 6th in the world amongst the most polluting industries that produce hazardous effluent on a large scale (Pola et al., 2019; Samuelsson et al., 2016; Singh and Chandra, 2019). Approximately two billion m^3 per annum of pulp and paper industrial wastewater is generated worldwide (Purkarová et al., 2018). Depending on the type of paper required, one ton of paper produced, requires 5 to 100 m^3 of water, with some modern mills using between 10 and 50 m^3 of water

(Ashrafi et al., 2015; Toczyłowska-Mamińska, 2017). The Kraft process is most commonly used in the pulp and paper industry. Wood chips are converted to masses of fiber by reaction with sodium hydroxide and sodium sulfate at high temperature and pressures (Singh and Chandra, 2019). In the process lignocellulose biomass is separated into cellulose fibers, hemicellulose and lignin (Samuelsson et al., 2016). The process results in pulp (as the product) and so called black liquor as the major by-product. One ton of pulp produced, generates seven tons of black liquor (Oliveira et al., 2018). Black liquor contains water and cooking chemicals (such as sulfurous acid). It also contains lignin and hemicellulose which are polyphenol containing structures (Cardoso et al., 2009). The Kraft process results in an increased amount of phenolic hydroxyl groups, as a result of the pulping process where the cleavage of β-aryl bonds occur (Schmitt et al., 2017). This creates a phenol-rich effluent, that is discharged at a rate of 20-100 cubic metres per metric ton of product.

2.3 Effluent hazards

Several components of pulp and paper mill effluent have been identified as carcinogenic, mutagenic, clastogenic and/or as endocrine disruptors (Singh and Chandra, 2019). Toxic effects have been observed at the trophic level of aquatic life which directly impacts the environment and human health (Anku et al., 2017; Singh and Chandra, 2019; Yadav, 2006). Studies have shown the effects of discharging pulp and paper effluent into aquatic bodies result in slime growth, scum formation and low populations of aquatic habitat (Chandra et al., 2006). This has led to aquatic bodies becoming toxic and unsuitable for domestic and irrigation uses downstream (Singh and Chandra, 2019). This study will look at the phenolic components in the pulp and paper effluent.

Black liquor is one of the main by-products of the Kraft process in the pulp and paper industry. It is dark brown/black in color and has a very strong unpleasant smell (Kim et al., 2017). If discharged, without prior treatment, black liquor causes severe pollution in the environment due to its high COD, with values sometimes exceeding 70g/L (Pola et al., 2019) and high alkalinity with pH values between 11 and 13 (Purkarová et al., 2018). Due to a large percentage of the black liquor being phenolic compounds, phenol is a major component in the effluent.

Phenol is an organic compound that includes a hydroxyl functional group (-OH) directly bonded to an aromatic hydrocarbon (benzene) and is colorless. Polyphenolic compounds are complex aromatic structures with two or more hydroxyl groups (Stefanakis et al., 2014) and the solutions are generally dark brown/black in color. Phenol and polyphenolic compounds are the major organic component of black liquor. Due to this, the pulp and paper mill effluent is therefore phenol-rich. Phenol is a priority pollutant with a limit of 1mg/L in wastewater for discharge into natural water bodies (WHO Chronicle, 2011); and a characteristic odor is produced at a low level of 5mg/L (Rani and Shanker, 2018) and is therefore highly toxic, while aquatic life is threatened at 9-25mg/L.

2.4 Component detection and discharge laws and regulations

In this study the following parameters are investigated: chemical oxygen demand (COD), total phenol content and water color and odor as they are the most relevant parameters to the study of degradation of phenol-rich effluent.

Oxygen demand is commonly measured in two ways: chemical oxygen demand (COD) and/or biological oxygen demand (BOD). COD is a measure of the quantity of oxygen required (mg/L) to completely oxidize an effluent sample. BOD is a measure of the oxygen required for an

effluent to be biologically oxidized and is often expressed as a two or five-day value. Both measurements, COD and BOD, are somewhat limited as they are lumped parameters and do not differentiate between readily oxidized components and those that are not which may require more treatment (Burton et al., 2013). However, the COD test has the advantage of not being influenced by toxic metals and can be carried out it in a few hours, instead of the two to five-days required by the BOD test (Woodard & Curran, 2006). In South African legislation the oxygen demand parameter is measured as COD and therefore, COD is used in this study. Legislation states that "discharging of domestic and industrial wastewater into a water resource" COD must be 75 mg/L or less as stated in the General Authorizations in Terms of Section 39 of the National Water Act ("National Water Act No. 39 of 1999," 1999). The COD test uses potassium dichromate or potassium permanganate (strong oxidizing agents) to chemically oxidize organic material under heat and strong acidic conditions in the effluent sample (Woodard & Curran, 2006).

Total phenolic content was measured to determine the qualitative amount of phenols and polyphenolic compounds in the effluent. Phenol has a discharge limit of 1 mg/L into inland surface waters and 5 mg/L into public sewers (Hussain et al., 2015). The Folin-Ciocalteu method is included in the European Pharmacopeia (Europe's legal and scientific benchmark for pharmacopeia standards) and allows for total phenolic content (TPC) to be determined by measuring the total concentration of the phenolic hydroxyl groups present in the sample (Blainski et al., 2013; Struchkov et al., 2016). The reaction that occurs in this method is a colorimetric reaction and widely used with UV/VIS spectrophotometry. UV/Vis spectrophotometry is a common laboratory technique that is easy to perform, low cost and has fast results (Ramanjaneyulu et al., 2005). All colorimetric assays need a reference

substance; in the Folin-Ciocalteu gallic acid standards are used and the total phenolic content expresses as mg/L per gallic acid equivalents (Chen et al., 2015). Phenol and polyphenols react with the Folin-Ciocalteu reagent and forms a blue complex (phosphotungstic-phosphomolybdenum complex) that is quantified by UV/VIS spectrophotometry at a wavelength of 765nm (Blainski et al., 2013).

Water odour is controlled by human terms related more to the psychological stresses than the harm they actually cause, however, some odours can cause loss of appetite, impaired respiration and in some cases cause disruptions in socioeconomic activities (Burton et al., 2013). Phenols have a characteristic odour produced at a low level of 5mg/L (Rani and Shanker, 2018). Water colour is dependent on the type and quantity of the organic compounds in the effluent (Jørgensen et al., 2005). Pure phenol is colourless, however, polyphenols are brown in colour resulting in their wastewater being a brownish colour (Aghdam et al., 2016). Water colour is expected by the general public to look a certain way and if it does not it causes psychological stresses and causes sociotechnical issues (Burton et al., 2013). By legislation “discharging of domestic and industrial wastewater into a water resource” the wastewater release must prevent “nuisance conditions such as flies or mosquitoes, odour or secondary pollution” (“National Water Act No. 36 of 1998,” 1999), therefore, both odour and colour of phenol-rich, paper mill effluent need to be treated.

2.5 Current pulp and paper effluent treatment technologies

Currently, black liquor is treated using a variety of technologies. A common method used is the Timlinson recovery process. The process involves concentrating the black liquor in an evaporator and burning it in a recovery furnace. This allows the inorganic chemicals to be recovered as well as providing an energy and heat source. However, during the

burning process, air pollutants such as NO_x and SO_x are released (Oliveira et al., 2018). NO_x and SO_x emissions are harmful to human health and the environment. Biological treatment is an alternative option that utilizes bacteria that can break down the toxic and hazardous substances. Some of the biological treatment options are: anaerobic treatment, membrane biological reactors, aerobic biological reactors, aerated lagoons and activated sludge (Vashi et al., 2018). Physicochemical treatment methods include adsorption, filtration, membrane technology and wet oxidation. These methods have high operating costs due to their complexity (Kamali and Khodaparast, 2015). All the above methods generally require large amounts of energy – typically supplied by the combustion of fossil fuels, which leads to numerous other environmental issues (Saeed and Sun, 2012). Black liquor is also deposited in landfills. This leads to components such as phenols, leaching into groundwater and surface water – again causing harmful human and environmental impacts (Dan et al., 2017). A common trend in industry, is to reduce operating problems such as corrosion and environmental problems such as water and air pollution (Pola et al., 2019).

An alternative to the above methods is to use a passive method such as constructed wetlands (CWs). CWs were first used in the early 20^{th} century for the treatment of domestic sewage, with the first experiments conducted on CWs in the early 1950's (Sheridan, 2012). Since then, CWs have rapidly developed in the last few decades and been establish worldwide as a wastewater treatment option (S. Wu et al., 2014). CWs have been used to treat domestic sewage, urban runoff, industrial effluents, agricultural wastewater and storm water (Wu et al., 2015). Today CWs are considered a "green" option for wastewater treatment (Stefanakis et al., 2016), are natural and environmentally

friendly systems (Saeed and Sun, 2012). CWs mimic natural wetlands (Wu et al., 2015) utilizing the natural processes that occur in wetlands, but in a more controlled way. Natural processes involving the wetland vegetation, soils and the microorganisms within the system are utilized (Vymazal, 2014). CWs have advantages in that they produce high effluent quality with no need for fossil fuels – reducing operation costs (Saeed and Sun, 2012). CWs are a good alternative wastewater treatment method as they have lower costs once established than the above mentioned treatment methods, as well as less operational and maintenance requirements (Wu et al., 2015).

2.6 Constructed wetlands

Natural wetlands are a natural ecosystem that describes the integration between aquatic and terrestrial environments (Keddy, 2010; Stefanakis et al., 2014). They are complex systems that are able to change with time, depending on the environmental stresses (Vymazal, 2014). Wetlands can be partially or fully covered by water for extended periods of time (Stefanakis et al., 2014). In order for an area to be a wetland they must be submerged or waterlogged and have different types of soils to upland soils and vegetation that can withstand being saturated (Scholz and Lee, 2005). Wetlands are of environmental importance as they can naturally provide some or all of the following functions: trapping of solids, sediments and other substances, act as protective buffers, enrich groundwater aquifers and support for ecological systems (Stefanakis et al., 2014). These functions include chemical, physical and biological processes to improve the quality of ground and surface water. Using the same principles utilized by the natural wetlands; constructed wetlands can be made to perform the same functions in a variety of different, targeted applications.

Constructed wetlands (CWs) are areas of land that have been made into a wetland using specific saturated substances, vegetation and biotic life to exploit the natural wetland ecosystem processes under controlled conditions (Stefanakis et al., 2014) for one or more of the following functions (Scholz and Lee, 2005; Sundaravadivel and Vigneswaran, 2001):

- A flood control mechanism;
- A food and fire source;
- A wetland habitat to compensate the natural wetlands that have been converted into agricultural and urban developments;
- A conservation area to conserve native flora and fauna; and
- A wastewater treatment system that improves the quality of water.

2.6.1 History of constructed wetlands

CWs have been used for the remediation of pollution since the early 20th century. However, it was only in the early 1950's that experiments on wetlands plants for wastewater purposes were carried out by Dr. Käthe Seidel (Sheridan, 2012). The initial results were promising and resulted in further experiments throughout Europe, with attention reaching North America shortly after (Wolverton et al., 1975). From the growing research, different types of constructed wetlands were developed and are used for many purposes worldwide (Xu et al., 2019).

2.7 Types of constructed wetlands

CWs can be classified into different types depending on their characteristics or design parameters. The design parameters looked at are the hydrology of the system: open water-surface flow/free water surface flow and sub-surface flow, the macrophytic growth: emergent, free-floating, floating-leaved and submerged. The sub-surface flow

type can be further characterized by the type of flow path in the wetland: horizontal or vertical (Saeed and Sun, 2012; Stefanakis et al., 2014; Vymazal, 2014).

2.7.1 Classification by macrophyte type

The primary reason for using CWs as a wastewater treatment option is due to the ability of aquatic plants to translocate oxygen to their roots and the surrounding pore water (Sundaravadivel and Vigneswaran, 2001). A wide variety of aquatic plants can be used for the creation of constructed wetlands. Wetland plants can be classified under three categories: free-floating, submerged and emergent (Saeed and Sun, 2012).

2.7.1.1 Free-floating macrophytes

A free-floating macrophyte is classified as a plant body, that is exposed above the surface of the water (Headley and Tanner, 2012). They have the ability to remove nitrogen and phosphorus through nitrification and remove suspended solids (Almuktar et al., 2018). Common examples of free-floating macrophytes include *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce) (Wu et al., 2015).

2.7.1.2 Submerged macrophytes

A submerged macrophyte is classified as a plant body that is entirely covered by water and require aerated water for growth (Almuktar et al., 2018) . The entire plant plays a role in pollutant removal and are mainly used in secondary treatment plants (Saeed and Sun, 2012). Common examples of submerged macrophytes are *Potamogeton crispus* and *Ceratophyllum demersum* (Almuktar et al., 2018).

2.7.1.3 Emergent macrophytes

An emergent macrophyte is classified as a plant body that is fixed into the CW substrate but emerges to various heights above the surface of the water (Almuktar et al., 2018; Wu et al., 2015). The roots play a role in pollutant removal as well as surfaces for biofilms to form (Wu et al., 2015). Common examples of emergent macrophytes are *Phragmites australis* (Common Reed) and *Juncus Effusus* (Common Rush) (Almuktar et al., 2018).

2.7.2 Classification by flow type

Emergent macrophyte CWs can further be classified by the type of flow through the CW.

2.7.2.1 Free water surface flow

Free water surface flow constructed wetlands are classified by water that flows over the soil surface (Stefanakis et al., 2014). They are generally planted with emergent macrophytes and floating plants. Micro-organisms and macro-organisms are often found in these wetlands in high population numbers. Free water surface flow constructed wetlands are often used for refining secondary treatment processes effluents (Sheridan, 2012).

2.7.2.2 Sub-surface flow

Sub-surface flow constructed wetlands are classified by water that flows inside a porous media within the bed (Stefanakis et al., 2014)**.** This type is further characterized by their flow path, either horizontal or vertical (Saeed and Sun, 2012).

- Vertical sub-surface flow CWs are characterized by water flowing uniformly over the surface of the bed and allowed to percolate through the media and collected

by a drainage network. This type is often used for cases where either nitrification or denitrification is required (Sheridan, 2012; Yalcuk and Ugurlu, 2009).

- Horizontal sub-surface flow CWs are characterized by water flowing from the inlet on one side and removed from the other side (Saeed and Sun, 2012). The bed is generally filled with gravel or a similar type of matrix. Emergent macrophytes are generally used for this set-up (Sheridan, 2012; Yalcuk and Ugurlu, 2009).

Both the vertical and horizontal sub-surface flow types (in terms of mass pollutant removal per m^2 of the surface area of the system) are more effective than the surface flow systems (Saeed and Sun, 2012). A previous study found that horizontal sub-surface flow surfaces performed better in organic removal whereas vertical sub-surface flow performed better in ammonium removal (Vymazal, 2007; Yalcuk and Ugurlu, 2009). Wetlands can also be a combination of the different types. A hybrid wetland is a combination of vertical flow and horizontal flow sub-surface flow CW (Stefanakis et al., 2014; Vymazal, 2007). They are generally designed in several parallel CWs in series (Wu et al., 2015). Hybrids are often used for the optimization of organic and nitrogen removal (Saeed and Sun, 2012).

2.8 Uses of constructed wetlands

CWs have a wide range of treatment applications. They are most extensively used for domestic wastewater, storm water and sewerage overflow (Sheridan, 2012; Vymazal, 2014). More recently, they have attracted the attention of the industrial and mining industry to treat industrial and agricultural wastewaters, as well as acid mine drainage and landfill leachates (Scholz, 2011; Sundaravadivel and Vigneswaran, 2001). Other applications include treatments of aquaculture ponds (Li et al., 2007), removal of preservatives (Chen, 2011), heavy metal

removal (Carapeto and Purchase, 2000; Xu et al., 2019), diesel and petroleum wastewaters (Al-Isawi et al., 2016; Mustapha et al., 2018) and pharmaceutical wastewaters (Vo et al., 2018; Vystavna et al., 2017).

2.9 Advantages and limitations of constructed wetlands

As with all technologies, new developments and design specifications, CWs come with advantages and limitations. CWs designs are dependent on factors such as effluent strength, land availability, technology combinations and energy uses (Ilyas and Masih, 2017).

Some of the limitations experienced by CWs are: (Ilyas and Masih, 2017; Sheridan, 2012; Sundaravadivel and Vigneswaran, 2001; Vymazal, 2014)

- They require a large land area, depending on the treatment requirements, in order to achieve specific design objectives, which potentially makes them unsuitable for urban areas as well as making their performance susceptible to outdoor weather conditions such as high winds and floods;
- The vegetation requires time to grow and establish themselves before treatment can begin;
- Micro-organisms and plant interactions with the effluent and the hydraulic processes that occur within CWs and natural wetlands are not fully understood which sometimes leads to inaccurate design and operating conditions and criteria;
- The water body may be infested by pests such as mosquitos especially for surface water CW;
- The removal of nutrients, specifically nitrogen and phosphorus, may be poor; and
- oxygen transfer is limited.

Some of the advantages of CWs are (Ilyas and Masih, 2017; Scholz and Lee, 2005; Sundaravadivel and Vigneswaran, 2001; Vymazal, 2014):

- They require low external energy sources;
- They achieve high levels of treatment with low levels of maintenance, which makes them an attractive method of treatment for rural areas and areas that have little or no infrastructure;
- They offer high process stability as they are robust with a high buffer capacity for organic and load variabilities;
- They generally do not have a design life period as they tend to increase treatment capacity over time; and
- They provide an aesthetic appeal to the area as well as wildlife habitats and recreational uses.

Current treatment methods are energy intensive and have high energy costs as they use incineration which releases harmful gases such as NO_x and SO_x into the environment, CWs do not. The current treatment methods are also designed to a life period, after which, will need to be replaced or several of the equipment used in the method will need to be replaced, CWs do not need to be replaced only maintained. As demand capacity increases, current treatment methods will have to either be upgraded or entirely replaced to accommodate the capacity increases, CWs often tend to increase in capacity over time.

CWs will be used in this study as the main advantage of CWs is their simple operation once setup and pervious results have suggested promising phenol removal from effluent (Stefanakis et al., 2016).

2.10 How constructed wetlands work

CWs generally follow the following mechanisms: adsorption, sedimentation, filtration, precipitation, microbial biodegradation, plant uptake and volatilization (Dan et al., 2017; Schultze-Nobre L et al., 2017). The type of process is dependent on the type of pollutants that need to be removed (Tee et al., 2009). The wetland plants are oxygen suppliers and provide habitats for the micro-organisms in the root zone (rhizosphere), which both provide mechanisms for biodegradation to occur (Schultze-Nobre Luciana et al., 2017). Within the CWs, most regions are anaerobic. However, at the root surface, there is an oxidative protective film present providing an oxygen source and creating aerobic conditions. Wastewater in CWs is treated as wastewater passes through the wetland media and the plant rhizosphere (Tee et al., 2009) interacting with both aerobic and anaerobic zones. In summary, pollutants are removed in one or more of the following ways (Scholz and Lee, 2005; Stefanakis and Thullner, 2016; Sundaravadivel and Vigneswaran, 2001; Vymazal, 2007):

- Direct uptake by the vegetation;
- Filtering of particles through the vegetation's roots and stems;
- Vegetation and substrate media offer large surface area for microorganisms to survive and degrade pollutants;
- Adsorption of nutrients by the substrate media;
- Sedimentation of solids as the flowrate decreases through the CW; and
- UV radiation destroys pathogens.

The removal processes occur with the interactions between the CW vegetation, the effluent and the CW substrate and can be physical, chemical or biological processes.

Physical processes

Physical removal of pollutants in CWs occurs due to sorption at vegetation biomass and substrate media within the CW (Sundaravadivel and Vigneswaran, 2001). Vegetation physically slows down the flow causing sedimentation of suspended solids and the substrate media acts as a filter aiding physical removal of suspended solids (Saeed and Sun, 2012).

Chemical processes

Removal of chemical pollutant in CWs occur between different substances (Haberl et al., 2003; S. Wu et al., 2014). Exposure to UV light and atmospheric gases can lead to the breakdown of organic components and destroy pathogens. Antibacterial compounds released by vegetation can also aid in pathogen removal (Greenway, 2007; Scholz and Lee, 2005). Certain substances can cause heavy metals to precipitate out of solution under the right conditions (Carapeto and Purchase, 2000).

Biological processes

Plants adsorb and absorb dissolved nutrients and other pollutants from wastewaters, converting them into biomass. Microorganisms (including fungi and bacteria) present in the CW remove organic matter by converting them to new cell tissues and gases and by using pollutants for energy metabolism. CWs, themselves, are a biological remediation process that utilize one or more of the following biological reactions (Saeed and Sun, 2012; Scholz, 2011; Scholz and Lee, 2005; Sundaravadivel and Vigneswaran, 2001):

- Aerobic respiration results in oxygen-rich areas around the root zone of the plants which maintains partially aerobic condition promoting oxidations.

- Anaerobic respiration results in areas without dissolved oxygen but alternative electron acceptors like nitrate, nitrite, sulfate, CO2, etc.
- Fermentation results in anaerobic conditions - by the metabolic activities of microbial organisms present in the CW. This allows for the decomposition of organic carbon to energy-rich compounds; such as methane and volatile fatty acids.
- Nitrification and denitrification results in the removal of nitrogen from wastewaters by the microorganisms present in the CW.
- Phosphorus removal occurs within biofilms that develop on the vegetation substrates and microorganisms present in the CW.

For the purpose of this study, organic carbon in the form of COD and phenolic compounds are the main pollutants to be remediated and therefore the only pollutants considered. It is a recognized limitation of this study that there are other processes occurring with other chemical species which are not accounted for here (Section 2.10.3)

2.10.1 Organic carbon removal by CWs

Organic carbon is "transformed" by all processes mentioned above, within a CW. More specifically, phenol degradation is thought to occur in the rhizosphere of the CW. Plant-microbe interactions are complex due to the existence of possible microbial pathways involved in this transformation; however, mutual benefits are expected for both organisms (plants and microbes). Microbial populations are known to occur in the rhizosphere of the plant and their activity is stimulated by the oxygen, amino acids and aromatics excreted by the roots, they also protect the plant from toxins through biodegradation (Rocha et al., 2015). Plant-microbe systems in CWs are thought to control the removal of phenolic compounds (Dan et al., 2017; Poerschmann and Schultze-Nobre, 2014).

2.10.2 COD removal by CWs

COD removal in CWs occur in three possible ways: filtration, aerobic digestion and anaerobic digestion (Kadlec et al., 2000):

- Filtration facilitates the of removal process of the non-dissolved components that increase the COD.
- Aerobic digestion facilitates the conversion of carbohydrates to carbon dioxide and water. In the following chemical equation, if x, y and z are known, any organic component COD contribution can be calculated:

$$C_xH_yO_z + \frac{2x+\frac{1}{2}y-z}{2} O_2 \rightarrow xCO_2 + \frac{y}{2} H_2O$$

- Anaerobic digestion facilitates the conversion of organic carbon to energy-rich compounds.

2.10.3 Other processes not investigated in this work

A number of other processes occur within a CW. These include the removal of suspended solids, nitrogen components, phosphorus components and pathogen removal which have been previously briefly mentioned, metal removal, degradation of sulfate and acid mine drainage treatment. These have not been investigated in this study as the focus is phenol degradation, however these processes may be occurring simultaneously. A detailed description can be found in literature (Haberl et al., 2003; Kadlec et al., 2000; Scholz and Lee, 2005; Sundaravadivel and Vigneswaran, 2001; Vymazal, 2014).

2.10.4 The water balance

The mass balance over a CW is primarily a water balance and governed by the following equation (Novák, 2012):

$$Q_{in} + Q_{precipitation} + Q_{infiltration} - Q_{out} - Q_{evapotranspiration} = Accumulation$$ Equation 2.1

- In steady state conditions, accumulation is zero;
- Precipitation can be measured by rain gauge or meteorological records;
- Infiltration is generally zero, as CW are generally designed to be impermeable. However, infiltration can either add or decrease the volume in the CW if the has been designed to be impermeable.
- Evapotranspiration can be approximated using different correlations under different conditions (temperature, time period and vegetation coverage) (Novák, 2012) .

The Ivanov equation can be used to approximate evapotranspiration, where the input data are monthly air temperatures and air relative humidity (Novák, 2012).

$$E_p = 0.0018\,(25 + T_{mon})^2(100 - r)$$ Equation 2.2

- E_p is the monthly potential evapotranspiration in mm;
- T_{mon} is the monthly average temperature in ˚C; and
- r is the monthly average relative humidity in percentage

The characteristic flow within the CW is chosen by the designers of the CW. In order for the CW to affect any remedial action on an effluent, the residence time needs to be long enough for the subsurface flow. Therefore, flow within a CW ideally should be laminar (Economopoulou and Tsihrintzis, 2003). Different equations express flow including Darcy's law and the equations of motion and continuity.

Darcy's Law under laminar flow (Reynold's number < 2000) conditions is governed by the following equation (Coulson et al., 1999):

$$Q = K_{hyd} \cdot d \cdot w \cdot S$$ Equation 2.3

- Q is the volumetric flowrate;
- K_{hyd} is the hydraulic conductivity;
- d is the average depth of the CW;
- w is the average width of the CW; and
- S is the slope of the saturated hydraulic zone within the CW.

The Reynold's Number (Levenspiel, 1972)

$$Re = \frac{\varphi \cdot v \cdot \rho}{\mu} \qquad \text{Equation 2.4}$$

- ϕ is the channel diameter of the fluid flow through the media;
- v is the average velocity through the channel;
- ρ is the density of the fluid; and
- μ is the viscosity of the fluid.

The flow of fluid through a packed bed with reaction is governed by the following equation (Levenspiel, 1972):

$$D \cdot \frac{d^2 X_i}{d^2 z^2} - \frac{dX_i}{dz} + k_i \tau \cdot C_{i_0}^{n-1} \cdot (1 - X_i)^n = 0 \qquad \text{Equation 2.5}$$

- D is the dispersion number;
- X_i is the conversion of component i;
- C_i is the initial concentration of component i;
- z is the dimensionless length of the reactor;
- k_i is the rate constant for the conversion of component I
- τ is the ideal residence time of the system; and
- n is the order of the reaction.

2.11 Kinetic remediation of phenolic compounds in a CW

One of the parameters looked at in this study is the rate associated of remediation of phenolic compounds within the CW.

2.11.1 Hydraulic equations describing subsurface CWs

In order to determine the rate of degradation, the hydraulic processes need to be quantified and solved for a residence time. The kinetics of degradation within a CW follows one of the following hydraulic equations according to most publications in literature.

First order plug flow equation: $C_{i,out} = C_{i,in} \cdot e^{-k \cdot \tau}$ Equation 2.6

Assuming that there is a series on infinite "plugs" that move down the CW and that each "plug" experiences no lateral mixing nor diffusion in the flow direction (Fogler, 2006).

Perfectly mixed reactor: $C_{i,out} = \frac{C_{i,in}}{(1+k \cdot \tau)}$ Equation 2.7

Assuming the liquid is perfectly mixed throughout the CW bed (the exit concentration is the same as the concentration at any other point within the CW) (Fogler, 2006).

No CW follows the equation of a plug flow nor the perfectly mixed equation but have characteristics of both. In reality, all CWs experience dead zones, dispersion, diffusion and short circuiting of the fluid within the CW. In order to determine which hydraulic equation best suits the CW, an impulse-response or step-change tracer test needs to be conducted (Fogler, 2006; Levenspiel, 1972).

2.11.2 Determining residence time distributions (RTDs)

Two experimental methods can be used to calculate the RTD. The impulse-response test and the step-change test. Wastewater often does not have a concentration that is controllable and therefore the impulse-response test is used. Concentration vs time data is plotted,

mathematically transformed and the various moments of the resulting distribution provide different flow parameters. A full procedure is explained in (Fogler, 2006).

Residence time distribution function

The RTD function, E(*t*), describes quantitatively how much time different fluid elements have spent in the CW. A constant volumetric flowrate E(*t*) is defined as:

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)\,dt} \qquad \text{Equation 2.8}$$

Which can also be defined as:

$$\int_0^\infty E(t)\,dt = 1 \qquad \text{Equation 2.9}$$

As the fraction of all material has been inside CW for a time, t, between t = 0 and t = ∞ is 1. Where E(*t*) d*t* is the quantity of the fraction of fluid exiting the reactor has spent between time *t* and *t* + d*t* inside the CW.

The mean residence time

The mean residence time is defined as the first moment of E(*t*). The mean of the residence time distribution is defined as:

$$t_m = \frac{\int_0^\infty tE(t)\,dt}{\int_0^\infty E\,(t)\,dt} = \int_0^\infty tE(t)\,dt = \tau \qquad \text{Equation 2.10}$$

Other moments

The second moment of distribution is the variance, which is used to compare RTDs. The magnitude of the variance describes the spread of the distribution, the larger the moment the greater the distributions spread (Fogler, 2006). The variance is defined as:

$$\sigma^2 = \int_0^\infty (t - t_m)^2 E t(t) dt$$ Equation 2.11

By normalizing this moment, the dispersion number, D can be found:

$$\sigma_\theta^2 = \frac{\sigma^2}{t_m^2}$$ Equation 2.12

D can be found through iterations using the normalized second moment:

$$\sigma_\theta^2 = 2 \cdot D - 2 \cdot D^2 \cdot \left(1 - e^{-\frac{1}{D}}\right)$$ Equation 2.13

The third moment is the skewness relative to the mean. The greater the moment, the more skewed the distribution is.

$$s^3 = \frac{1}{\sigma^{\frac{3}{2}}} \int_0^\infty (t - t_m)^3 E(t) dt$$ Equation 2.14

Hydraulic processes that occur in the CW are not accounted for in the kinetic equations.

Tanks-in-series reactor models

The number of tanks, N, can be found using the data obtained from the moments of distribution:

$$N = \frac{1}{\sigma_\theta^2}$$ Equation 2.15

Or,

$$N = \frac{t_m^2}{\sigma^2}$$ Equation 2.16

If the value of $N \to \infty$ then the CW is best described as the plug flow equation (Equation 2.6) and if $N \to 0$ the CW is best described as the perfectly mixed reactor (Equation 2.7) and can be expressed as the Tanks-in-series equation (Fogler, 2006):

$$C_{i,N} = \frac{C_{i,0}}{(1+k_i \cdot \tau_j)^2}$$ Equation 2.17

Where k_i is the rate constant for component *i* and τ_j the mean residence time for reaction *j.*

2.12 Plants for constructed wetlands

Wetland plants occupy the central role of CWs. The following criteria need to be met for plants to be used in CWs (Scholz and Lee, 2005; Sundaravadivel and Vigneswaran, 2001; US EPA, 2000):

- Plants that are ecologically adaptable (no risk of disease or weed to surround ecosystems);
- Plants with a high nutrient load tolerance and high nutrient removal capacity;
- Plants with a high tolerance of pollutant concentration;
- Plants with a high tolerance to flooding conditions;
- Plants with a tolerance of local conditions (climate, pests and disease);
- Plants that are readily propagated, establish themselves quickly and that spread and grow quickly;
- Plants that are indigenous to the area;
- Plants that are non-invasive to the area;
- Plants that are perennial (reduces the need for yearly re-establishment);
- Plants that provide benefits to local wildlife; and
- Plants that use a variety of species for diversity (reduces monocultures that are susceptible to pests, insects and disease).

Five species that are commonly found in South African natural wetlands (Cowling et al., 2004) were used in this study as they all fit the above criteria; namely: *Phragmites australis*

(Common Reed), *Chondropetalum tectorum* (Cape Thatching Reed), *Juncus effusus spiralis* (Common Spiral Rush), *Zantedeschia aethiopica* (Arum Lily) and *Juncus effusus* (Common Rush).

2.12.1 *Phragmites australis* (Common Reed)

Phragmites australis, the common reed is a large perennial, grassy emergent macrophyte with a widespread geographical distribution; found all over Europe, Africa, Asia, America and Australia (Aysu and Esim, 2016; Ghassemzadeh et al., 2008). It is indigenous to Southern African and is naturally found in wetlands and nearby water bodies (Cowling et al., 2004). *Phragmites australis*, is long-lived and can grow to six meters high (Aysu and Esim, 2016). They have been found to be successful in the removal of heavy metals, acid mine drainage treatment (Arivoli et al., 2015; Haberl et al., 2003).

2.12.2 *Chondropetalum tectorum* (Cape Thatching Reed)

Chondropetalum tectorum, the cape thatching reed, is an emergent macrophyte that is commonly found in the western parts of Southern Africa (Bond et al., 1999). They have been found to be successful in the removal of phosphorus (Lambers et al., 2006).

2.12.3 *Juncus effusus spiralis* (Common Spiral Rush)

Juncus effusus spiralis, the common spiral rush or corkscrew rush, is a perennial emergent macrophyte that has green spiral stems. It can grow up to 1.5 metres high as well as spread 1 meter horizontally. It is commonly found throughout Southern Africa near water bodies (Cowling et al., 2004).

2.12.4 Juncus effusus (Common Rush)

Juncus effusus, commonly known as the common rush is a grass emergent macrophyte that is found across Southern Africa, America, Australia and in some parts of Europe (Zika, 2003).

They have been successful involved in the treatment of acid mine drainage wastewaters, nitrogen, phosphorus as well as heavy metal removal (Arivoli et al., 2015; Matthews et al., 2004; Vystavna et al., 2017).

2.12.5 Zantedeschia aethiopica (Arum Lily)

Zantedeschia aethiopica, commonly known as the arum lily in South Africa, also known as Calla lily, is a perennial emergent macrophyte. They are found throughout Southern Africa and have a white bulb flower and can grow up to a meter high (Cowling et al., 2004). They have been successful as a macrophyte involved in the treatment of petrochemical wastewaters (Belmont and Metcalfe, 2003).

2.13 Previous studies on pulp and paper (phenol-rich) effluent treatment

Previous studies on phenol degradation in CWs have only reported the total phenolic content of the wastewater and not the specific phenolic compounds (Schultze-Nobre L et al., 2017). Phenolic compounds are removed in CWs by means of microbial transformation (bacteria in the biofilms around the root of the plants and gravel) and particles of clay and organic matter (Rossmann et al., 2012). Studies done, on similar organic compounds, such as benzene, show promising removal results (Stefanakis et al., 2016). However, very few studies have been done on the degradation of polyphenols (lignin and hemicellulose) in black liquor by CWs, hence the purpose of this study.

2.14 Research objectives

Within the context presented above, there were five main research objectives for this study: These were:

- To chemically analyze the industrial effluent;

- To investigate how initial concentration influences phenolic compound remediation in CWs;
- To determine the concentration gradient of the phenolic compounds along the length and depth of the CW bed;
- To measure the chemical oxygen demand and total phenol content of the outlet for discharge purposes; and
- To determine the remediation kinetics of the system.

3. Materials and methods

This study was conducted in two phases, a batch operation phase and a continuous operation phase. The batch phase investigated the most effective macrophyte species involved in phenol remediation, the effect the effluent concentration has on the phenol remediation process and the remediation kinetics in batch phase. Results obtained in the batch phased were used in the continuous phase were phenol remediation, phenol concentration gradients and phenol remediation kinetics were investigated. Five specific research objectives were set out to achieve. Each objective was investigated in its own set of experiments. The materials and methods section follows that format.

3.1 Analyze the industrial effluent

Pulp and paper mill (phenol-rich) effluent was obtained from a pulp and paper mill on the east coast of South Africa. The effluent was analyzed for total organic content (TOC) (this analysis was outsourced), total phenolic content (TPC) and carbon oxygen demand (COD). (Photos of the effluent can be found in Appendix A).

Total organic content (TOC) was measured to determine the quantitative amount of carbon in organic compounds in the effluent (Cheung et al., 2003). TOC was determined by oxidizing the organic compounds in the effluent to carbon forms that can be quantified.

Total phenolic content was measured to determine the qualitative amount of phenols and polyphenolic compounds in the effluent (Cheung et al., 2003). Phenolic compounds are the primary pollutants and the most immediately toxic pollutant in the pulp and paper mill effluent. TPC was measured using the colorimetric Folin-Ciocalteu method, expressed as mg/L or ppm gallic acid equivalents (GAE). The method used followed the procedure set out by Faustino et al., (2010). A spectrophotometric calibration curve of absorbance vs concentration was constructed at a wavelength of 765nm using a Merck SQ-2800 UV-Vis spectrophotometer to determine changes in concentrations.



Figure 1: CW Batch Operation Setup

COD was determined by colorimetric method and spectrophotometrically, a calibration curve was constructed (absorbance vs concentration) at a wavelength of 605nm using a Merck SQ-2800 UV-Vis spectrophotometer to determine changes in concentrations.

The calibration curves were considered adequate and usable if the Pearson's product-moment correlation coefficient of at least 0.99 (r^2=0.99).

3.2 Effluent strength influences on phenolic compound remediation

Pulp and paper mill effluent concentration strength was investigated using CWs in batch operation using five different types of emergent macrophyte species: *Phragmites australis* (Common Reed), *Chondropetalum tectorum* (Cape Thatching Reed), *Juncus effusus spiralis* (Common Spiral Rush), *Juncus effusus* (Common Rush) and *Zantedeschia aethiopica* (Arum Lily). Figures of the macrophyte species can be found in Appendix B. Fifteen pot plant trays (length x width x depth = 0.50m x 0.20m x 0.20m) of 20L were used and placed on a roof of a building, exposed to the natural environment (Figure 1). Three trays per species were used with three plants of that species per tray. Each tray was topped with tap water to a predefined mark (0.16m) and kept topped with tap water to the mark throughout the experiments to ensure the water balanced remained closed. Evapotranspiration and rainfall were included in the mass balance. Plants were left to grow and stabilize for three months before any experiments were done.

The effect of effluent concentration of original black liquor from a paper mill upon the wetland plant species was investigated by using full strength effluent, diluted effluent (full strength effluent diluted by 2/3) and distilled water. The trays to the left in Figure 1 contained full strength effluent; the middle, diluted effluent and the right trays, distilled water. Distilled water was used as the control.

Experiment 1: Effluent (full strength, dilute and distilled water) was added to the assigned tray to the predetermined mark (0.16m). Distilled water was added if/when necessary needed to ensure the water balance was closed. Samples were taken once a week for two weeks to determine which macrophyte species was the most effective at pulp and paper remediation and which effluent strength gave the best remediation results. Each tray was sampled in two

locations (left and right) to provide average readings for each of the trays. Samples were tested for total phenolic content (TPC) and carbon oxygen demand (COD). TPC and COD were measured following the same procedure set out in Section 3.1. For statistical purposes a paired t-test was applied to all data with a 95% confidence level.

Experiment 2: A repeat of Experiment 1, except over a period of seven days to determine when phenol remediation reached completion.

3.3 Determining the most effective CW macrophyte configuration and TPC and COD gradient along the length and depth of the CW bed

Phenolic compound concentration along the length and depth of CWs was investigated in continuous operation using the most effective macrophyte species at phenol remediation found in Experiment 1 and 2. Three laboratory-scale constructed wetlands (Figure 2) were used, each with a volume of 12L of dimensions of length x width x depth = 1.00m x 0.185m x 0.20m.



Figure 2: Laboratory-Scale Constructed Wetlands Setup

Each CW was filled with commercial gravel with an average diameter of approximately 12mm (obtained from a local nursery) to a height of 0.16m. The water height of each CW was set to 0.15m. Flow rates into the CWs were controlled by irrigation taps (Figure 3). The macrophytes used in the continuous operation were based on results from the batch operation (A – *Zantedeschia aethiopica,* B – *Juncus effusus* and C - *Zantedeschia aethiopica* and *Juncus effusus).* All CWs were in operation for three weeks with water to allow for plant growth and biofilm development.



Figure 3: Irrigation Taps Used to Control Flow Rates

Expexriment 3: Pure phenol solution (made from phenol obtained from Merck and distilled water) of the same total phenolic content (concentration) as the pulp and paper mill effluent phenolic concentration, was used in all three CWs to determine the most effective CW macrophyte set-up. Phenol solution was used as a subsitutue to the industrial effluent due to the souce of the effluent being 800km away from the University. Artificial effluent was fed into the CWs for a period of six days. Samples, taken from the inlet, oulet and middle as well as the middle surface and middle bottom were taken daily and analyzed for TPC (using the Folin-Ciocllteau method as set out in Section 3.1) to invesigate the phenolic concentration gradients along the length and depth of the CW bed for phenol solution. For

statistical purposes a paired t-test was applied to all data with a 95% confidence level. The results from Experiment 2, determined the most effective CW set up to use in Experiment 3.

Experiment 4: Similarly, to Experiment 2, pulp and paper mill effluent was fed into one CW (CW C; planted with *Zantedeschia aethiopica* and *Juncus effusus*) for a period of six days. Samples were taken daily from the inlet, oulet and middle as well as the middle surface and middle bottom and analyzed for TPC (using the Folin-Ciocllteau method as set out in Section 3.1) to invesigate the phenolic concentration gradients along the length and depth of the CW bed for pulp and paper mill effluent. For statistical purposes a paired t-test was applied to all data with a 95% confidence level.

3.4 Measure the total phenolic content and chemical oxygen demand of the outlet for discharge purposes

Results from Experiment 4 were used as Experiment 5: to investigate the TPC and COD of the outlet effluent to determine if the CW treatment process could be used to treat pulp and paper mill effluent.

3.5 Phenol remediation kinetics of batch and continuous constructed wetlands

The phenol remediation approximate kinetics were investigated for both batch operation (and for different pulp and paper mill effluent strengths) and continuous operation. The temperature was recorded during these experiments as kinetics is temperature dependent (Akratos and Tsihrintzis, 2007).

Experiment 6: similarly, to Experiment 1 and 2, effluent (full strength, dilute and distilled water) was added to the assigned tray to the predetermined mark (0.16m). Distilled water was added if needed to the mark to ensure the water balance closed. Samples were taken

once a day for four days and analyzed for TPC (using the Folin-Ciocllteau method as set out in Section 3.1). For statistical purposes paired t-test was applied to all data with a 95% confidence level. Equation 2.6 (Section 2.11) was used for the batch kinetics (Fogler, 2006; Levenspiel, 1972):

$$C_{i,out} = C_{i,in} \cdot e^{-k \cdot \tau}$$ Equation 2.6

Experiment 7: similarly, to Experiment 3, pulp and paper mill effluent was fed into CW C. Samples were taken at different theoretical resident times by changing the inlet flowrate whilst ensuring it remained within the laminar flow region. This was done by adjusting the irrigation taps attached to the inlet pipe as seen in Figure 3. Samples were analyzed for TPC (using the Folin-Ciocllteau method as set out in Section 3.1). For statistical purposes a paired t-test was applied to all data with a 95% confidence level. To determine the continuous CW residence times and kinetics, the procedure set out in Section 2.11.2 was used using data from previous studies performed on the same laboratory-scale CWs (Fogler, 2006; Levenspiel, 1972).

4. Results and Discussion

This Chapter follows the same experiment order as presented in Chapter 3. The most widely used expression for data presentation is in percent concentration reduction and mass removal (Stefanakis et al., 2016) and therefore, the data found in this study is presented in this way.

4.1 Analysis of the industrial effluent

Table 1 is a summary of the analytical tests performed on the pulp and paper mill effluent:

Industrial Effluent	TOC (ppm)	TPC (ppm GAE)	COD (ppm)
Pulp and Paper Mill	1010	500	925

Table 1: Summary of the Analytical Tests Performed on the Effluent

Water discharge into an existing water body is required to have a COD value of no more than 75ppm ("National Water Act No. 36 of 1998," 1999) and a phenolic content of less than 1ppm (Hussain et al., 2015). All three analytical tests resulted in high values which indicate that the water will need be treated before being discharged into the environment. Different methods of CW operation were used to investigate the remediation of this pulp and paper mill effluent.

4.2 Effluent strength influence to phenolic compound remediation

Experiment 1 was conducted to determine the most effective macrophyte species (*P. australis, C. tectorum*, *J. spiralis*, *J. effusus* and *Z. aethiopica*), for pulp and paper mill (phenol-rich) effluent remediation. Each macrophyte species was exposed to full-strength pulp and paper mill effluent and a 2/3 diluted pulp and paper mill effluent. The TPC and COD absorbance readings can be found in Appendix C. A calibration curve using standards was constructed in order to convert the absorbance readings to concentration readings. The phenol remediation (by the Folin-Ciocalteu method) and COD removal are shown as percentages in Table 2:

	Phenol Remediation %		**COD Removal %**	
Effluent Type	**Full Strength**	**2/3 Diluted**	**Full Strength**	**2/3 Diluted**
Juncus effusus	100	58	73	17
Juncus effusus spiralis	100	96	78	36
Zantedeschia aethiopica	100	81	70	27
Phragmites australis	96	90	76	45
Chondropetalum tectorum	79	79	76	34

Table 2: Total Phenol Remediation and COD Removal Percentages for the Different Macrophyte Species after two weeks

The full-strength pulp and paper mill effluent showed greater phenol remediation % than the diluted pulp and paper mill effluent across all five macrophyte species. This result was not expected and no explanation for this is offered. Three of the macrophyte species: *J. effusus, J. effusus spiralis* and *Z. aethiopica,* all reached 100% phenol remediation for the full-strength effluent with the lowest remediation % at 79% for the *C. tectorum*. Previous studies of phenol remediation by CWs done have reported complete removal (Stefanakis et al., 2016 and A et al., (2017). Similar results can be seen with the COD %removal over the two-week period, with a maximum COD % removal of 78% for the *J. effusus spiralis* at a full-strength effluent. From the data in Table 2, it was concluded that effluent (phenol) remediation was most effective

on the full-strength pulp and paper mill effluent as three of the five species reached 100% phenol remediation with COD removal % above 70%.

To determine the most effective macrophyte species at pulp and paper mill effluent, paired t-tests were applied to the data (Appendix D). The t-test was applied to this data set to see if there was a statistical difference in the amount of phenol remediated for each macrophyte species. Statistically, *Chondropetalum tectorum* was the least effective at phenol remediation and was therefore not used in further experiments.

Experiment 2 was conducted to determine when the phenol remediation stopped in the batch operation process. The phenol remediation % and COD removal % profiles for full strength effluent and dilute effluent over seven days are shown in Figure 4, absorbance readings for Experiment 2 can be found in Appendix E.

Figure 4 shows that effluent (phenol) remediation occurs very rapidly in the first couple of days and then stabilizes for both pulp and paper mill effluent strengths. All macrophyte species reach stabilization in three days at 100% remediation, except for the *P. australis* which gradually tended towards 100% at 7 days. The same trend was observed for COD removal; it reached stabilization within in three days between 70-85% COD removal for the full-strength effluent. COD removal % fluctuated for the dilute strength effluent. Again, this is unexpected and no explanation can be offered for this behavior.

Phenol remediation occurred within the first four days at which point the phenol remediation stayed constant.

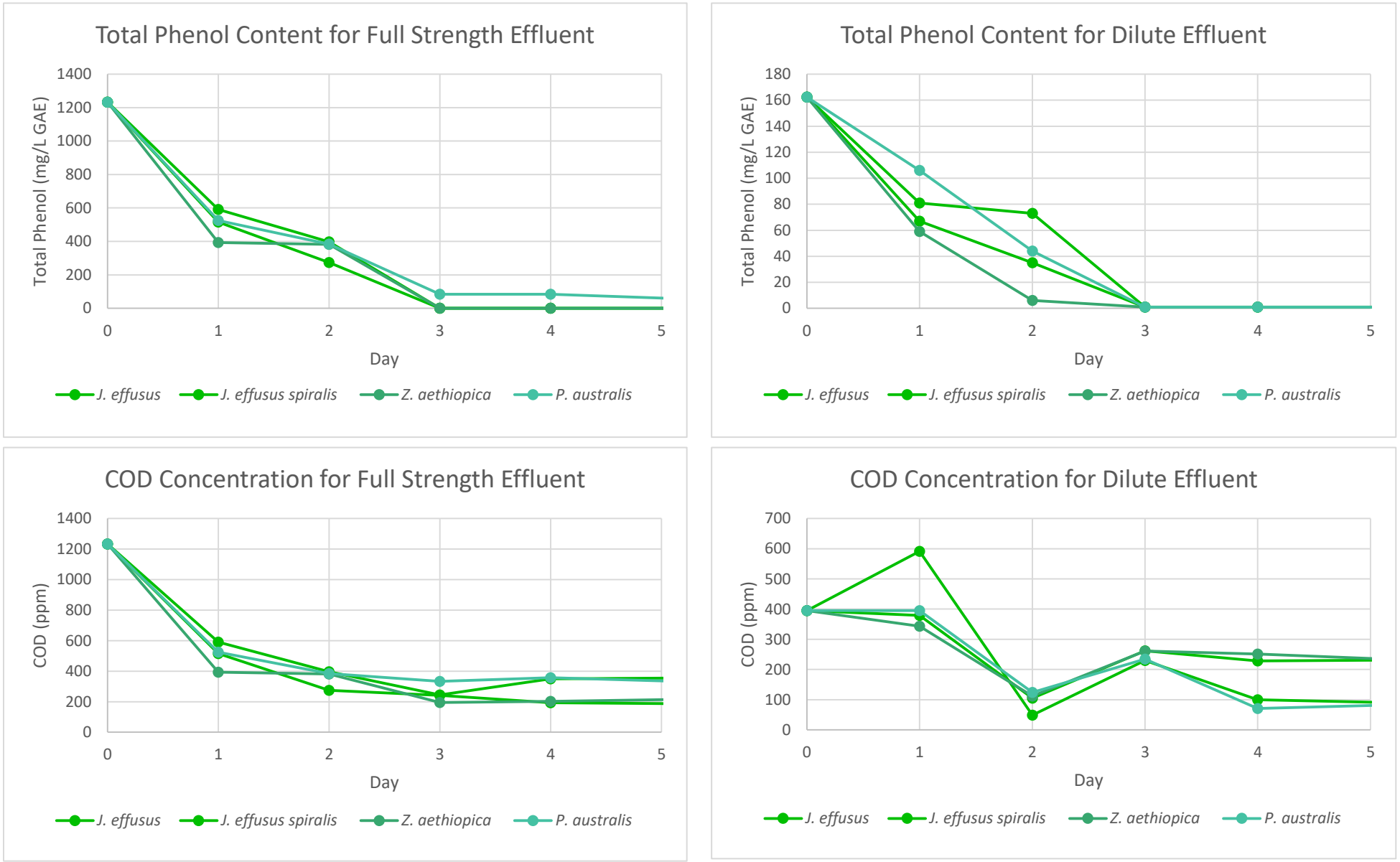


Figure 4: Phenol and COD Remediation Percentage Profiles for Each Macrophyte Species with Corresponding Effluent Strength

4.3 CW macrophyte configurations and phenol concentration gradients along the length and depth of the CW bed

Laboratory-scale CWs were set up based on the batch operation data (Section 4.2). By statistics and inspection (Appendix F), three CWs were set up as follows:

- CW A – *Zantedeschia aethiopica*
- CW B – *Juncus effusus*
- CW C – *Zantedeschia aethiopica* and *Juncus effusus*

4.3.1 The most effective CW configuration

Experiment 3 was conducted to determine the TPC gradients along the length and depth of the CW bed. Phenol solution was made to the same concentration of phenol as the pulp and paper mill effluent and used to determine the most effective CW macrophyte configuration as well as the TPC and COD gradients along the length and depth of the CW.

Table 3 is a summary of the phenol remediation % and COD removal % after six days of operation with a phenol solution equivalent to undiluted effluent fed to the three CW configurations (absorbance readings of TPC and COD for all three CWs can be found in Appendix G):

CW Configuration	Phenol Remediation %	COD Removal %
A – Z. aethiopica	75	74
B – J. effusus	73	78
C – Z. aethiopica& J. effusus	78	74

Table 3: Phenol Remediation % and COD Removal % over the Three Different CW Macrophyte Configurations:

CW C is the most effective at phenol remediation reaching 78%, followed by CW A at 75%. CW B had the highest COD % removal at 78% followed by both CW A and C. Statistically, both the TPC and COD values are not different (by the paired t-test – Appendix H). Due to this the trend in phenol remediation by day, over the six days was investigated to determine the most effective CW configuration. Figure 5 shows the phenol remediation % and the COD removal

across six days of operation. Figure 5 shows that CW C had a sharp increase in phenol remediation % within the first day that CW A and B do not display.

The difference may be due to CW C having two types of macrophytes, *Z. aethiopica* and *J. effusus,* that have two different environments around the root structures (Ojuederie and Babalola, 2017), allowing for a greater variety of microorganism communities within the CW. This variability may have resulted in a fitter, more biodiverse microbial community which was able to more rapidly respond to the phenol. This hypothesis was not, however, tested.

Due to the final TPC and COD concentrations not being statistically different, the initial sharp increase in CW C lead to CW C being chosen as the most effective CW configuration



Figure 5: Phenol Remediation and COD Profiles for the Three CWs for Phenol Solution

4.3.2 TPC and COD gradients along the length and depth of the CW bed – pure phenol solution

The gradients of TPC and COD along the length and depth of the CW bed were investigated using pure phenol solution in the three laboratory-scale CWs (Experiment 3) and then using pulp and paper mill effluent (Experiment 4) in the most effective CW configuration, CW C.

Figure 6 shows the TPC and COD gradients along the length and depth of CW C bed using pure phenol solution as the effluent to be treated (Experiment 3). Absorbance reading for TPC and COD can be found in Appendix I. For gradients along the length of the CW, samples were taken at the inlet (2 cm from the inlet), middle of the CW (50 cm from the inlet) and outlet (2 cm from the outlet); for gradients along the depth of the CW, samples were taken in the middle of the CW at surface water level, middle and bottom (1 cm below the water surface, 6 cm from the water surface and 1 cm above the bottom of the CW).

Along the length of the CW, the highest TPC and COD concentration is at the inlet. From Figure 5, steady state is assumed after three days of operation. Before this stage, the TPC concentration is higher in the outlet than the middle of the CW. After steady state the TPC concentration in the middle and outlet are not statistically different. This indicates that most remediation is occurring within the first half of the CW and the general trend is TPC decreases along the length of the CW bed. COD removal shows the same trend and decreases along the length of the CW bed.

Along the depth of the CW in the first few days of operation, the highest TPC and COD concentrations was at the bottom of the CW bed. After six days of operation, TPC was the lowest in the middle depths of the CW – indicating that phenol remediation occurs maximally in the middle depths of the CW, which Is predominantly where the roots and microorganism

environments are. COD increases as depth is increased. Therefore, COD removal occurs in the middle to top area of the CW bed.

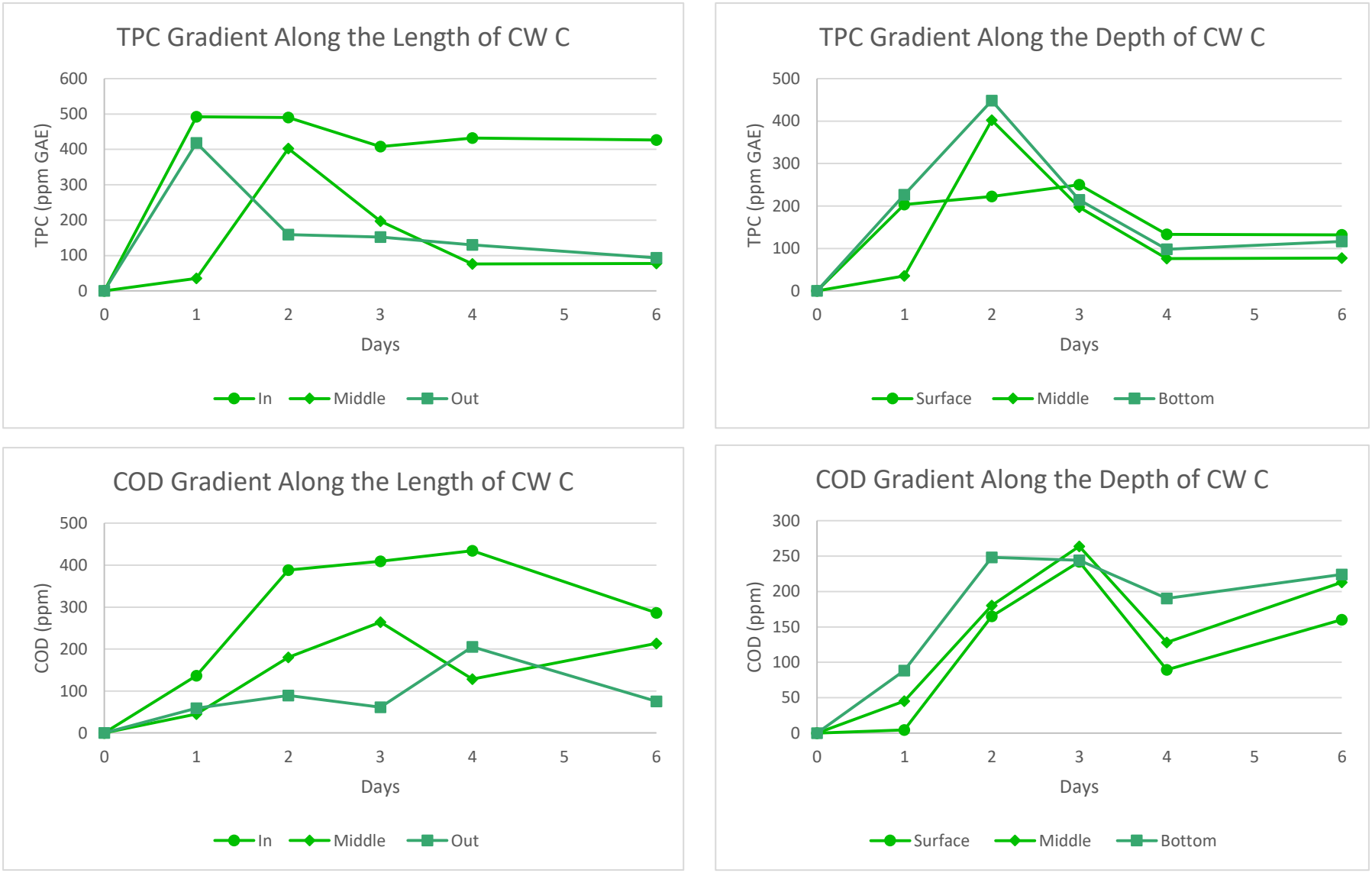


Figure 6: TPC and COD Gradients Along the Length and Depth of CW C Using Pure Phenol Solution

4.3.3 TPC and COD gradients along the length and depth of the CW bed – pulp and paper mill effluent

Experiment 4 was conducted in the same way as Experiment 3, except using pulp and paper mill effluent in CW C. Figure 7 shows the TPC gradients along the length and the depth of the CW C bed. Absorbance readings for TPC and COD can be found in Appendix J.

Along the length of the CW, the highest TPC and COD concentration, is as expected from the previous experiment, at the inlet. After six days of operation both the TPC and COD followed the same trend and both decreased in concentration along the length of the CW C bed. This indicates that phenol remediation is at its highest in the first half, length-wise of the CW. However, remediation is still occurring in the second half.

Along the depth of the CW, TPC and COD concentration are highest at the surface and bottom of the CW, with the middle level of the CW having the lowest TPC and COD concentrations. This indicates that phenol remediation occurs at its highest in the middle depth of the CW, where the root and microorganism community is most numerous.

TPC and COD gradients along the length and depth of the CW for pulp and paper mill effluent showed the same general trends as the pure phenol solution trends. The fact that the artificial effluent displays similar behavior to the real effluent is useful since it demonstrates that phenol can be used for lab-scale experiments.

A CW design for optimum effluent remediation results would therefore preferably be a shallow CW bed that allows for the root system of the macrophytes (and microorganism community) to occupy most of effluent flow path through the CW.

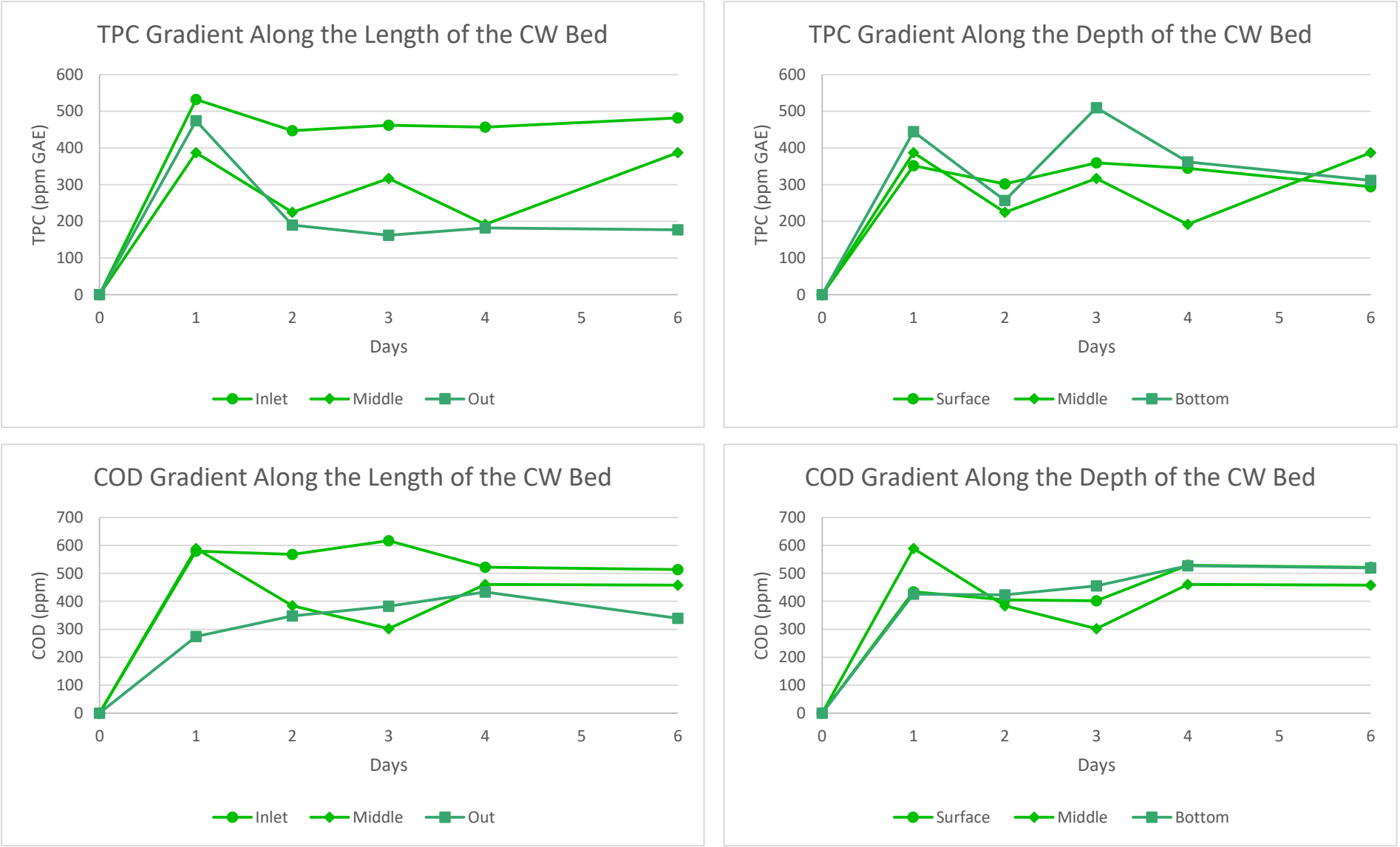


Figure 7: TPC and COD Gradients Along the Length and Depth of CW C Using Pulp and Paper Mill Effluent

4.4 Outlet total phenolic content and carbon oxygen demand for discharge purposes

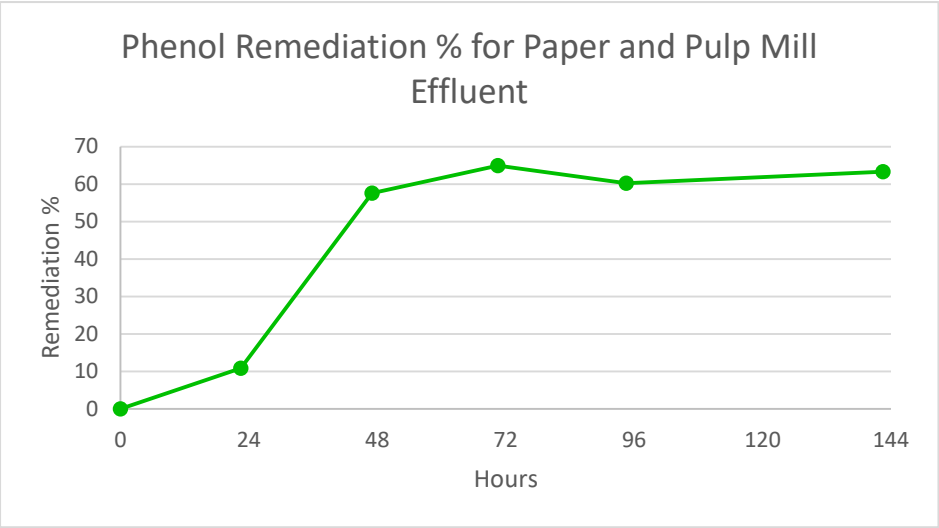
In order for the treated effluent to be discharged into the environment it has to have a total phenolic content value of less than 1ppm (Hussain et al., 2015) and a carbon oxygen demand value of less than 75pmm ("National Water Act No. 36 of 1998," 1999). Experiment 5 used the outlet TPC and COD results from Experiment 4 to determine if the laboratory-scale CW treatment system allows for the effluent to be discharged into the environment after six days' operation. The TPC and COD absorbance readings can be found in Appendix J. A calibration curve was constructed in order to convert the absorbance readings into concentration readings. A summary of Figure 8 - the outlet TPC and COD values per day of operation are shown in Table 4:

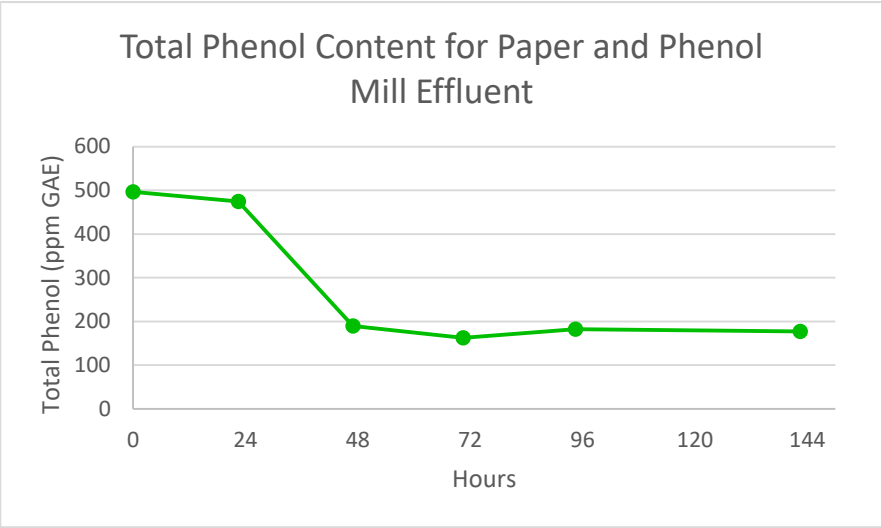
Day	TPC (ppm)	TPC Remediation %	COD (ppm)	COD Removal %
0	496	0	580	0
1	475	11	274	53
2	190	58	348	39
3	162	65	382	38
4	182	60	433	17
6	177	63	339	34

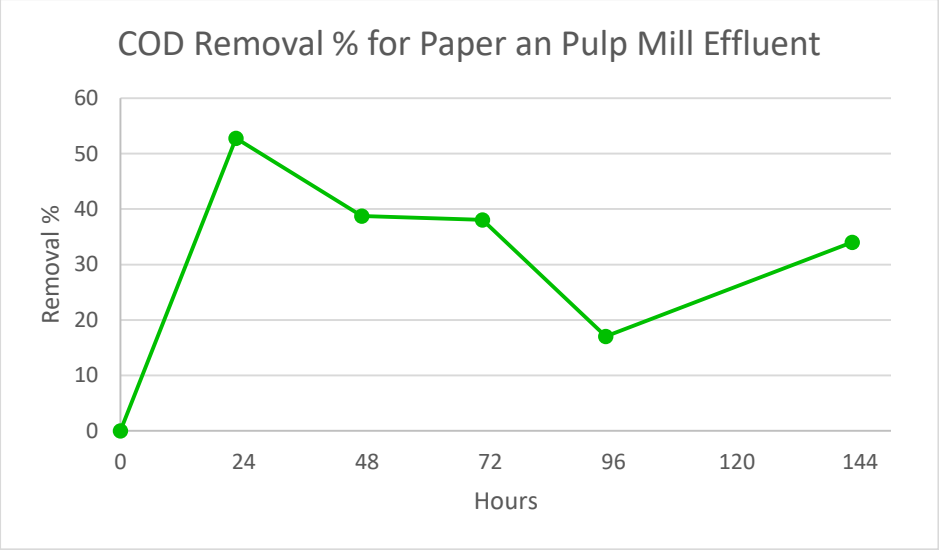
Table 4: TPC and COD Outlet Values for Pulp and Paper Mill Effluent per Day

Figure 8 shows a fast rate of phenol remediation within the first three days, thereafter it started to stabilize between 60-65% phenol remediation. COD removal fluctuated throughout the operation period. This may be due to the macrophytes and the microbial systems adjusting to the changing environments (Ojuederie and Babalola, 2017) and/or natural growth cycles (Panikov, 1995). After six days of CW operation on CW C (*Z. aethiopica* and *J. effusus)* the TPC value reached 177ppm GAE at 63% effluent remediation with a 34% COD removal at 339ppm, showing a decrease in TPC and COD. This is statistically different to the discharge limits of a TPC value of 1ppm and COD of 75ppm. A residence time for the CWs used in this study to reach TPC discharge limits would be approximately 65 days.

However, due to the CW being 1.00m in length, it is likely that increasing the size (and consequent residence time) of the CW (as seen in a similar study by Stefanakis and Thullner (2016) which had larger CWs and reached 100% phenol removal) would create a larger treatment area and therefore increase TPC remediation and COD removal. By doing this, it is potentially possible that the CW treatment system could remediate the pulp and paper effluent to a water quality that could be discharged to the environment.







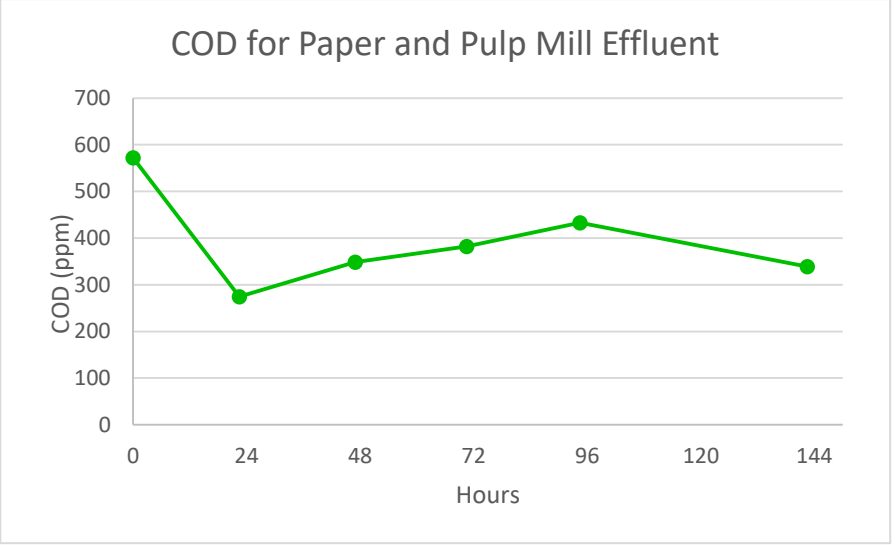


Figure 8: TPC and COD Outlet Values for Pulp and Paper Mill Effluent

4.5 Phenol remediation kinetics for batch and continuous operation

Reaction kinetics were investigated for both batch and continuous operation. The temperature was recorded and reported when samples were taken as kinetics are known to be temperature dependent.

4.5.1 Batch phenol remediation kinetics

Experiment 6 conducted batch kinetics of phenol remediation on the different effluent strengths, namely full-strength effluent and 2/3 diluted effluent and the four different macrophyte species (*P. australis, J. effusus spiralis, J. effusus* and *Z. aethiopica)*. The temperature ranged between 20-23˚C during the sampling period of 4 days. Four days was used as the time period as results in Section 4.2 showed that phenol remediation % stayed constant after 4 days. The data was fitted to a zero-order graph, first order graph and second order graph, with the first order graph yielding more of a straight line than zero and second order calculation, therefore first order reaction was assumed. Equation 2.6 was used to determine approximate batch phenol remediation kinetics.

$$\frac{dC}{dt} = -kC \qquad \text{Equation 2.6}$$

Where *C* is the concentration, *t* is the time and *k* is the rate constant. Table 5 shows the different rate constants, *k* (1/h), for the different macrophyte species at a temperature range of 20-23˚C by using the slope of the graph (-*k*) of ln*C* vs *t*. Figure 9 and 10 are the approximate batch kinetic graph for the macrophyte species - *J. effusus* and the different pulp and paper effluent strengths. All other species and effluent strength graphs can be found in Appendix K.

The slope in Figure 9 of -0.1259 and -1.7424 in Figure 10 are the rate constant, -*k* (1/s), was converted to *k* (1/h) as seen in Table 5 below. The same conversion was done for all other species and effluent strength rate constant values.

	Rate Constant, *k*(1/h)	
Effluent Type	**Full Strength**	**Dilute**
J. effuses	0.0726	0.0053
J. effusus spiralis	0.0674	0.0036
Z. aethiopica	0.0587	0.0042
P. australis	0.0741	0.0056

Table 5: Approximate Batch Kinetic Rate Constants for Full Strength and Dilute Pulp and Paper Effluent Strengths Across all Macrophyte Species with units 1/h at 20-23˚C.

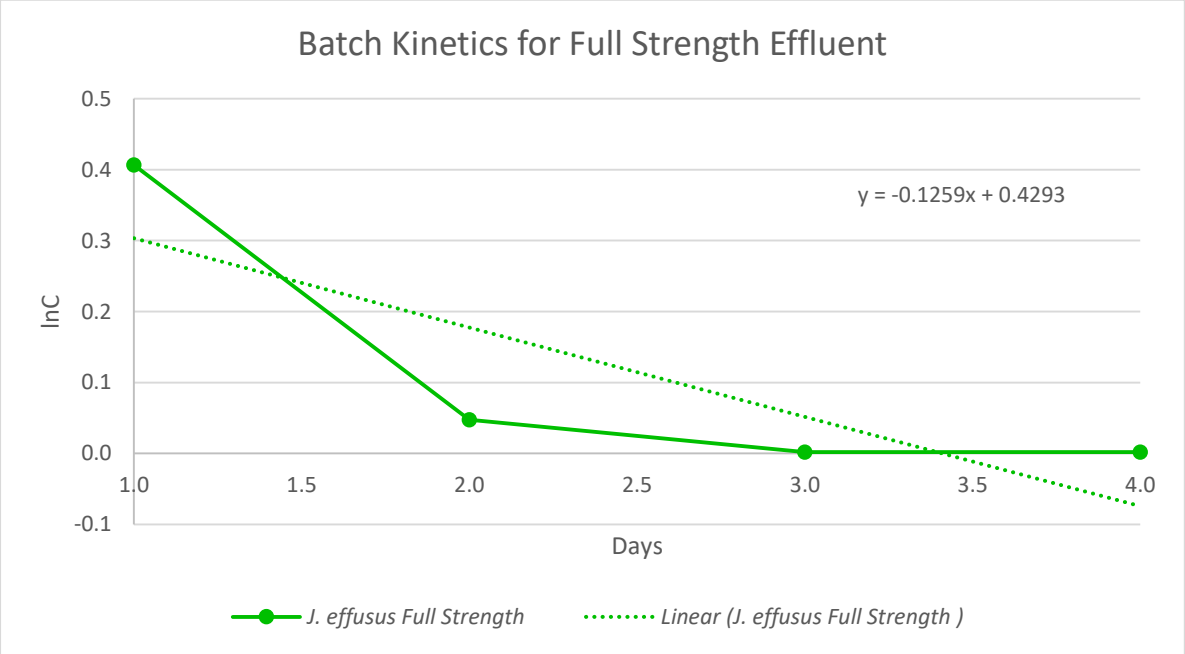


Figure 9: Batch Kinetics for Full Strength Effluent with J. effusus as the Macrophyte Species at 20-23˚C

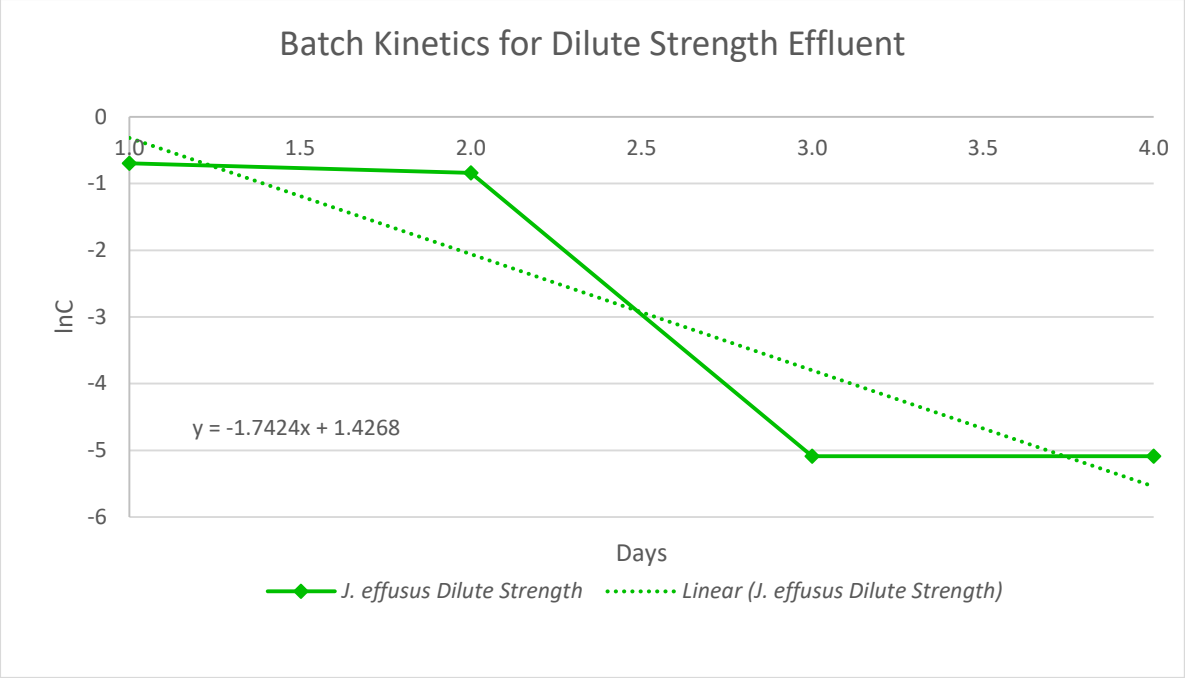


Figure 10: Batch Kinetics for Dilute Strength Effluent with J. effusus as the Macrophyte Species at 20-23˚C

Effluent (phenol) remediation took place at a faster rate for full-strength pulp and paper mill effluent than dilute pulp and paper mill effluent across all macrophyte species. *P. australis* had the greatest rate constant for the full-strength effluent and the dilute effluent, and therefore, a greater remediation rate than all the other macrophyte species of 0.0741/h and 0.0056/h respectively. By the paired t-test, both effluent strength types are not statistically different and within the effluent strength type, the macrophyte species are also not statistically different. These results correspond to the results found in Section 4.2 where the full-strength effluent had a higher phenol remediation % (and here, a greater remediation rate) than the phenol remediation % in the dilute strength effluent.

4.5.2 Continuous flow phenol remediation kinetics

Experiment 7 was conducted to determine continuous kinetics of phenol remediation on CW C, (*Zantedeschia aethiopica* and *Juncus effusus* CW configuration) for pulp and paper mill effluent. The temperature ranged between 15-18˚C.

The continuous rate of remediation was found using the approximate number of tanks the CWs represented to determine the reactor model it most represented (Fogler, 2006; Levenspiel, 1972). Data from previous studies (Appendix L) was used to determine the approximate number of tanks-in-series equation (Equations 2.8 to 2.15) in Section 2.

The approximate number of tanks was found to be 71.6 tanks. Therefore, CW C was modeled as a plug-flow reactor based on: If the value of $N \rightarrow \infty$ then the CW is best described as the plug-flow equation and if $N \rightarrow 0$ the CW is best described as the perfectly mixed reactor (Fogler, 2006). Equation 2.6 (see Section 4.5.1) was used to determine the approximate phenol remediation kinetics of pulp and paper mill effluent.

Samples were taken at different residence times once steady state was achieved (TPC values remained constant). Absorbance readings for batch and continuous kinetics can be found in Appendix M.

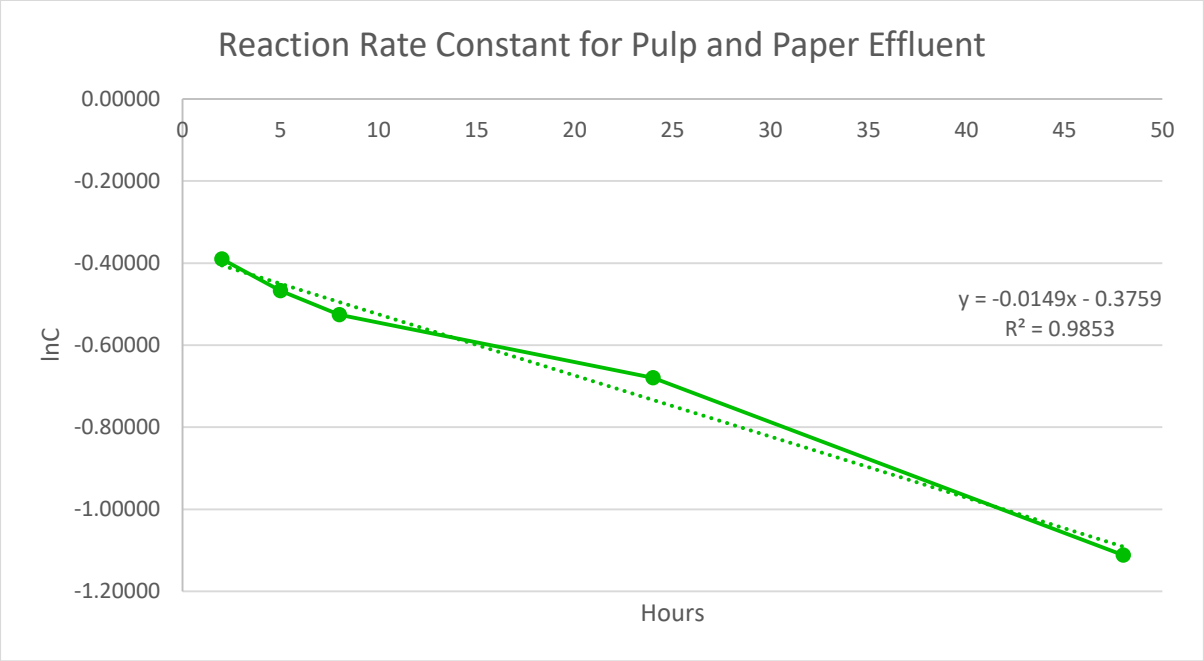


Figure 11: Reaction Rate Constant for Phenol Remediation in CW C at a Temperature Range of 15-18˚C

Similarly, to the batch kinetics, the slope of Figure 11 of -0.0149, -*k* (/s), was converted to *k*(/h). The rate constant for pulp and for paper (phenol-rich) mill effluent remediation was found to be 0.015/h.

4.5.3 Batch vs continuous phenol remediation kinetics

The rate constant for the continuous operation (0.015/h) is significantly different to the batch operation rate constants for full strength effluent (ranging between 0.06-0.07/h), by the paired t-test. The differences in the rate constant values may be explained by the following reasons:

- The mode of operation, batch and continuous, the nature of the remediation reaction and the temperature in which the experiments were conducted.
- Phenol remediation is an endothermic reaction (Maskow et al., 2010; Park et al., 2015; Y. Wu et al., 2014) and therefore, the rate constants should be greater in higher temperatures.
- Experiment 6 (batch operation) was conducted in temperatures of 20-23˚C whereas Experiment 7 (continuous operation) was conducted in temperatures of 15-18˚C.

Pulp and paper mill phenol-rich effluent can be remediated in batch or continuous operation; however, batch operation has shown to have a faster reaction rate than the continuous operation.

5. Factors influencing pulp and paper mill effluent remediation using constructed wetlands

In this chapter, the results are presented as a paper which has been submitted to Science of the Total Environment for peer review and possible publication. Since the paper has described some literature and materials and methods, the reader's indulgence is requested for any potential repetition.

The first author conducted the experimental work, the co-authors contributed through supervision.

Factors Influencing Pulp and Paper Mill Effluent Remediation Using Constructed Wetlands

Samantha Pearson[1], Craig Sheridan[2, 4,*], Uwe Kappelmeyer[3]

[1] University of the Witwatersrand, School of Chemical and Metallurgical Engineering, 1 Jan Smuts Avenue, Braamfontein, 2194, South Africa, spearson626@gmail.com

[2] Centre in Water Research and Development, 1 Jan Smuts Avenue, Braamfontein, 2194, Gauteng, South Africa, craig.sheridan@wits.ac.za*

[3] Helmholtz Zentrum für Umweltforschung UFZ, Leipzig-Halle, Dept. of Environmental Biotechnology, Permoserstraße 15, D-04318 Leipzig, Germany, uwe.kappelmeyer@ufz.de

[4]School of Geography, Archaeology and Environmental Studies, 1 Jan Smuts Avenue, Braamfontein, 2194, South Africa

---

Abstract

Pulp and paper industry produces the third largest volume of wastewater in the industrial sector. The pulp and paper mill effluent is high in phenolic compounds that are highly toxic to the environment. In this study, constructed wetlands, as a sustainable alternative, were tested at laboratory-scale for phenol remediation from pulp and paper mill effluent from a pulp and paper mill plant on the east coast of South Africa, with a total phenol content of approximately 500ppm. Constructed wetlands were operated in batch mode to determine the most effective emergent macrophyte species in phenol remediation and the most effective effluent concentration and in continuous mode using *Zantedeschia aethiopica* and *Juncus effusus* and a combination thereof to determine phenol remediation. Kinetic studies on both batch and continuous operation were investigated. Results showed phenol remediation does occur in laboratory-scale constructed wetlands with complete phenol remediation in batch operation with an average rate constant of 0.058-0.074/h at 22˚C and 65% phenol remediation in continuous operation with an average rate constant of 0.015/h at 15˚C.

Keywords: Constructed Wetlands; Phenol Remediation; Pulp and Paper Mill Effluent; Phenol-Rich Effluent; Industrial Wastewater Treatment

1. Introduction

The pulp and paper industry is one of the world's largest industries; producing the third largest volume of wastewater in the industrial sector (Ashrafi et al., 2015). Approximately two billion m^3 per annum of paper and pulp industrial wastewater is generated worldwide (Purkarová et al., 2018). The Kraft process is the most commonly used process in the pulp and paper industry. The process results in pulp (as the product) and black liquor as the major by-product. One tone of pulp produced, generates seven tones of black liquor (Oliveira et al., 2018), creating a phenol-rich effluent which requires treatment.

Several components of pulp and paper mill effluent have been identified as carcinogenic, mutagenic, clastogenic or endocrine disrupting (Singh and Chandra, 2019). Phenol is a major component in the effluent as a priority pollutant. It has a limit of 1mg/L in wastewater for discharge (WHO Chronicle, 2011). A characteristic odor is produced at a low level of 5mg/L and aquatic life is threatened at 9-25mg/L (Rani and Shanker, 2018).

Currently, black liquor is treated using a variety of techniques. Worldwide, the most common method used is the Timlinson recovery process. The process involves concentrating the black liquor in an evaporator and burning it in a recovery furnace. This allows the inorganic chemicals to be recovered as well as providing an energy and heat source. However, during the burning process, air pollutants such as NO_x and SO_x are released (Oliveira et al., 2018). Physicochemical treatment methods include adsorption, filtration, reverse osmosis, membrane technology and wet oxidation. These methods have high operating costs due to their complexity (Kamali and Khodaparast, 2015).

Biological treatment is an alternative option that utilizes bacteria that can break down the toxic and hazardous substances (Vashi et al., 2018). These treatment options require large amount of mechanical energy (for aeration), are costly and lead to environmental degradation through improper disposal and the by-products thereof, such as sludge and bio-solids (Sundaravadivel and Vigneswaran, 2001; Vymazal, 2014; Wu et al., 2015). For this reason, treatment methods that are cost-effective and sustainable reduce the operating problems such as corrosion and environmental problems such as water and air pollution (Pola et al., 2019) are actively being sought.

An alternative is to use constructed wetlands (CWs). CWs are considered a "green" option for wastewater treatment (Stefanakis et al., 2016), are natural and environmentally friendly systems (Saeed and Sun, 2012). A green option or green technology is an option that is environmentally friendly, one that does not release harmful substances into the environment and does not, itself, use fossil fuels to carry out the intended process. CWs are used to treat a variety of effluents from a wide range of industries, to treat industrial and agricultural wastewaters, as well as acid mine drainage and landfill leachates (Scholz, 2011; Sundaravadivel and Vigneswaran, 2001), treatments of aquaculture ponds (Li et al., 2007), removal of preservatives (Chen, 2011), heavy metal removal (Carapeto and Purchase, 2000; Xu et al., 2019), diesel and petroleum wastewaters (Al-Isawi et al., 2016; Mustapha et al., 2018) and pharmaceutical wastewaters (Vo et al., 2018; Vystavna et al., 2017). They are a good alternative wastewater treatment method as they have lower costs once established, as well as less operational and maintenance requirements (Wu et al., 2015).

Phenolic compounds, the primary chemical of concern are thought to be removed in CWs by means of microbial transformation and adsorption to particles of clay or organic

matter (Rossmann et al., 2012). Few studies have been done on phenol treatment by CWs and the kinetics thereof. However, the studies done previously, on similar organic compounds, such as benzene and MTBE, show promising removal efficiency results (A et al., 2017; Stefanakis et al., 2016).

The present study focuses on the remediation of pulp and paper mill effluent (obtained from a pulp and paper mill on the east coast of South Africa) by constructed wetlands by better understanding the effects of system parameters (e.g., batch and continuous operation, macrophyte species) and investigating the kinetic response of the systems. The specific objectives of the study are to evaluate: (i) the effect of system parameters such as macrophyte species and concentration, (ii) the efficiency of CWs in the remediation of pulp and paper mill (phenol-rich) effluent, and (iii) the approximate remediation kinetics in batch and continuous CW operation.

2. Materials and Methods

2.1 Project Description

The project consisted of two phases. Phase 1, the batch phase (as CWs can be operated in batch and continuously (Fan et al., 2013)), consisted of five different species of emergent macrophytes: *Phragmites australis* (Common Reed), *Chondropetalum tectorum* (Cape Thatching Reed), *Juncus effusus spiralis* (Common Spiral Rush), *Juncus effusus* (Common Rush) and *Zantedeschia aethiopica* (Arum Lily)., prepared in triplicate, that were each dosed with paper and pulp mill (phenolic-rich) effluent in different concentrations to determine the species that was most effective in the remediation of phenolic compounds, COD and color. Phase 2, the continuous phase, used the results in Phase 1 to carry out the same remediation

experiments in a continuous flow laboratory-scale constructed wetlands. Both phases were exposed to the natural environment.

2.1.2 *Description of Laboratory-Scale Units*

Phase 1 – 15 identical x 20L (length x width x depth = 0.50m x 0.20m x 0.20m) pot plant trays were used (Figure 1). Each tray held three plants of the same species (triplicated for each species), topped with water to a pre-determined height of 0.16m to keep the water balance consistent. The five species investigated in this phase were: *Phragmites australis, Chondropetalum tectorum, Juncus effusus spiralis*, *Juncus effusus* and *Zantedeschia aethiopica*.

Phase 2 – Three laboratory-scale, horizontal flow constructed wetlands were used with a volume of 37L (length x width x depth = 1.00m x 0.185m x 0.20m), referred to as A-C (Figure 1). All beds were filled with commercial gravel (obtained from a nursery with a diameter of approximately 12mm) to a height of 0.16m with the water height set to 0.15m. The macrophytes used in this phase were based on results from Phase 1 (A – *Zantedeschia aethiopica,* B – *Juncus effusus* and C - *Zantedeschia aethiopica and Juncus effusus)*. All three beds were in operation for three weeks before the start of Phase 2 in this study, allowing for plant growth and biofilm development and stabilization to occur.



Figure 1: Laboratory-Scale Constructed Wetlands A-C for phase 2

2.2 Wastewater Classification and Other Solutions

Phenol-rich effluent was obtained from a paper and pulp company in South Africa and used in both phases of this study. The effluent had a total phenolic content (TPC) of approximately 500ppm Gallic acid equivalent determined by the Folin-Ciocalteu Method, with carbon oxygen demand (COD) of 925ppm and total organic carbon (TOC) of 1010ppm. A 450ppm phenol solution was made up for a stage in Phase 2 using distilled water and pure phenol obtained from Merck.

2.3 Methods and Sampling Procedures

Phenol was considered the most important component to monitor in the pulp and paper mill effluent as phenol is the most immediately toxic component in the effluent to the environment.

Phase 1: Experiment 1 was performed to determine which plant species would be most effective to use in a continuous flow laboratory-scale CW for the treatment of paper mill effluent as well as to determine the effect of effluent concentration on the degradation

process. Effluent strength was classified as full strength (pure effluent) or diluted by 2/3 and distilled water was used as a zero reference. Each tray was marked identically to a set volume of water to ensure the water balance could be closed. Full strength effluent was placed into the first tray of each species, similarity for diluted and distilled water. All trays were topped up to the predetermined mark every two days with distilled water for three weeks.

Experiment 2 was performed identically as Experiment 1 but over the period of one week to determine phenol remediation, the effects of effluent concentration on phenol remediation and the kinetic rates of degradation. Water samples were taken weekly from each of the trays in-between the individual plants and analyzed for TPC and COD. Samples were taken daily for 7 days and analyzed for total phenolic content (TPC) and carbon oxygen demand (COD). Batch kinetic data for phenol remediation was achieved by taking samples from the triplicate trays, 24 hours apart and analyzed for TPC.

Phase 2: Experiment 3 was performed in laboratory scale CWs with pure phenol solution of the same phenolic concentration as the pulp and paper effluent to determine the most effective CW configuration as determined by Phase 1 (based on the assumption that phenol was a representative amount of pulp and paper mill effluent). The approximate reaction kinetics for phenol remediation were also investigated. Continuous kinetic data was achieved by taking samples at different theoretical resident times by changing the inlet flowrate while keeping the load constant. This was done by adjusting the irrigation taps attached to the inlet pipe.

Experiment 4 was performed, in the same way as Experiment 3, on the laboratory-scale CW that showed the most promising results from Experiment 3. Pulp and paper mill effluent was used to determine effectiveness and approximate reaction kinetics. Kinetics for phenol

remediation were calculated as previously done in Experiment 3. Water samples were taken daily and were analyzed for TPC and COD. Water for Phase 2 was pumped in the CWs using a submersible centrifugal pump, with the flowrates controlled by irrigation taps.

2.4 Analysis

All samples were analyzed for TPC and COD. Evapotranspiration and rainfall was considered throughout the study. TPC was analyzed by the Folin-Ciocalteu colorimetric method following the procedure set out by Faustino et al., (2010) and reported in mg/L or ppm Gallic acid equivalent (GAE). A calibration curve from 0ppm GAE to 500ppm GAE was constructed using a spectrophotometer (SQ-2800 UV-Vis spectrophotometer, Merck, Germany) at a wavelength of 765nm. COD was determined by a colorimetric method (Gaudy and Ramanathan, 1964). All calibration curves used had a Pearson's product-moment correlation coefficient of at least 0.99 (R^2=0.99) to be considered usable.

2.5 Statistical tests

To investigate the level of significant differences between species effectiveness of pulp and paper mill wastewater treatment, wetlands and the combination, paired t-tests were used with a confidence level of 95%. T-tests were carried out at the same time as sampling to ensure results were not influenced by the variability of measurement versus time.

3 Results and Discussion

3.1 Phase 1 Experiments - Batch Operation

Batch experiments were conducted using all five species of macrophytes at different concentration doses (Experiment 1), namely full strength, diluted (by 2/3) for two weeks. The most widely used expressions of data presentation is in percent concentration reduction and mass removal (Stefanakis et al., 2016) and therefore, presented in this way. The endpoint phenol remediation and COD removal measurements are shown as percentages in Table 1:

	Phenol Remediation %		**COD Removal %**	
Effluent Type	**Full Strength**	**2/3 strength**	**Full Strength**	**2/3 strength**
Juncus effuses	100	58	73	17
Juncus effusus spiralis	100	96	78	36
Zantedeschia aethiopica	100	81	70	27
Phragmites australis	96	90	76	45
Chondropetalum tectorum	79	79	76	34

Table 1: Total Phenol Remediation and COD Removal Percentages for the Different Macrophyte Species after two weeks

Phenol remediation occurs within the CWs systems as batch operation. The full strength effluent showed greater phenol remediation than the dilute effluent. This may be due to a higher concentration of phenolic compounds resulting in a faster remediation process than a lower concentration of phenolic compounds. Similar results were seen for the COD removal over the two-week period. The setups with all five macrophyte species showed promising results with three of the five species (*J. effusus, J. effusus spiralis* and *Z. aethiopica)* reaching 100% phenol remediation determined by the Folin-Ciocalteu method and a COD removal of above 70%. Complete phenol remediation was found possible in previous studies by Stefanakis et al., (2016) and A et al., (2017). Statistically (by the t-test), the setup with *Chondropetalum tectorum* was the least efficient at phenol remediation by the Folin-Ciocalteu method and therefore removed from all further experiments. The experiment was

re-conducted over the period of one week (Experiment 2) to determine phenol remediation, the effects of effluent concentration on phenol remediation and the kinetic rates of degradation.

3.1.1 Factors influencing the remediation of pulp and paper effluent

The results from Experiment 2 are shown in Table 2, Figure 2 and 3. Table 2 is a summary of Figure 4 and the effect of effluent concentration and the effect macrophyte species have on the phenol remediation and COD removal. In general, *J. effusus* was most effective followed by *Z. aethiopica.* Similarly, to Experiment 1, and as expected the full strength effluent showed greater phenol remediation than the dilute effluent.

	Phenol Remediation %		**COD Removal %**	
Effluent Type	**Full Strength**	**Dilute**	**Full Strength**	**Dilute**
Juncus effuses	100	99	86	40
Juncus effusus spiralis	100	99	71	81
Zantedeschia aethiopica	100	99	81	48
Phragmites australis	97	99	76	75

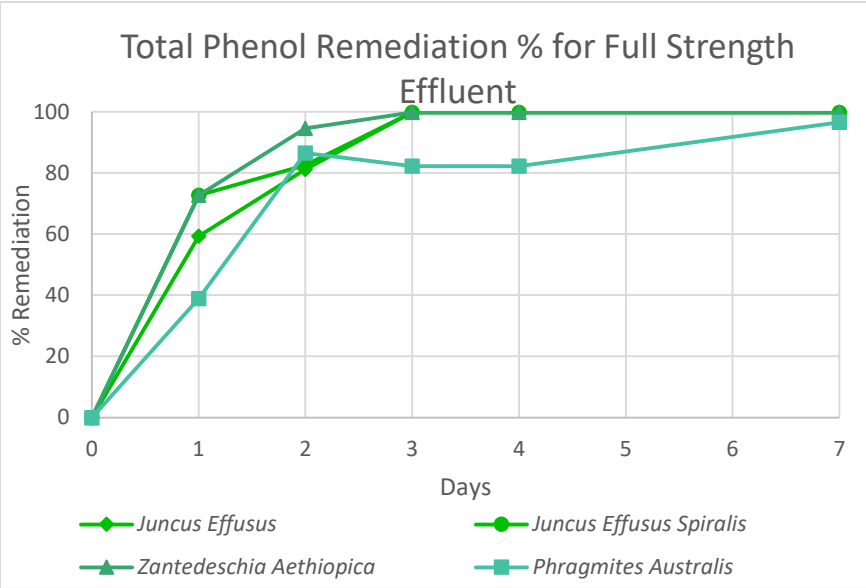
Table 2: Total Phenol Remediation and COD Removal Percentages for the Different Macrophyte Species after a week

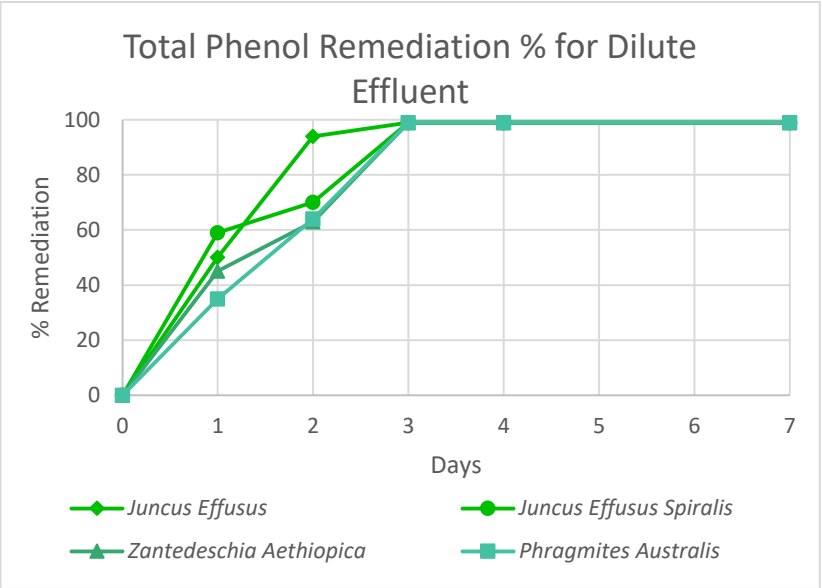
The phenol remediation and COD removal profiles for full strength effluent and dilute effluent over seven days are shown in Figure 2 and the concentration profiles for full strength effluent and dilute effluent over seven days are shown in Figure 3. Phenol remediation and COD removal have the same general trend as seen in both Figure 2 and 3. If phenol remediation occurs, COD is removed.

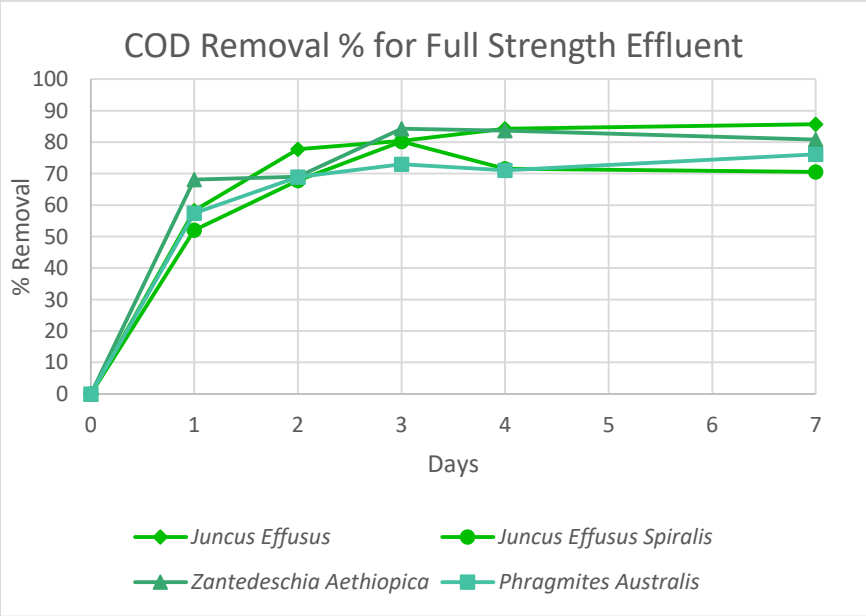
In general, for the full strength effluent, phenol remediation occurs exponentially in the first couple of days and then slowly starts to stabilize. All investigated setups result in 100% remediation, except for the *P. australis* which gradually tends towards 100% at 7 days, as all phenolic compounds have been removed. Likewise, the COD removal reaches stabilization in

three days between 70-85% removal, leaving the COD concentration between approximately 177-365ppm across all macrophyte species with *J. effusus* being the most effective species (COD at 177ppm), followed by *Z. aethiopica* (COD at 236ppm).

Comparatively, the dilute (by 2/3) effluent, phenol remediation also occurs at a greater rate, however, over the first three days where 99% phenol remediation is achieved. The dilute effluent shows fluctuations in the COD removal as phenol remediation is occurring. COD removal is relatively low (compared to the full strength effluent) with a high of 81% (75ppm) by the *J. effusus spiralis* followed by *P. australis* at 75% (100ppm).







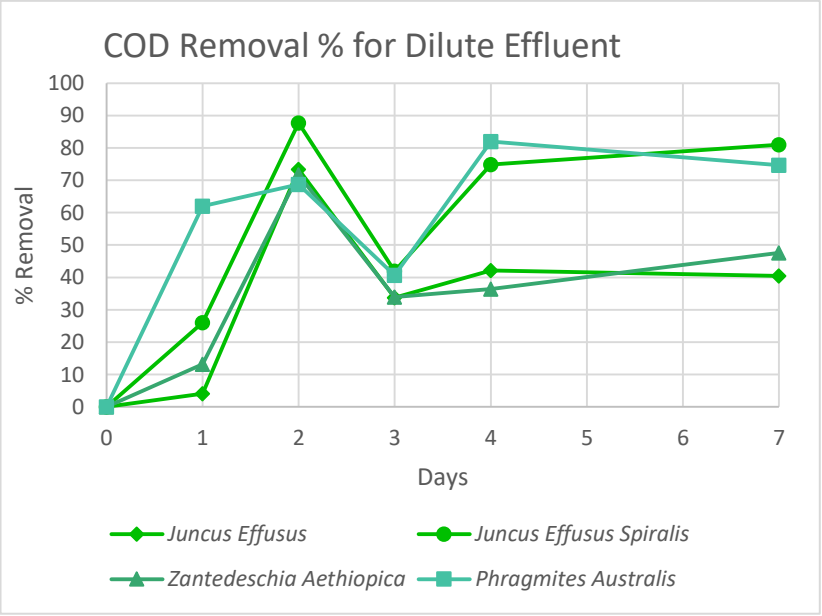
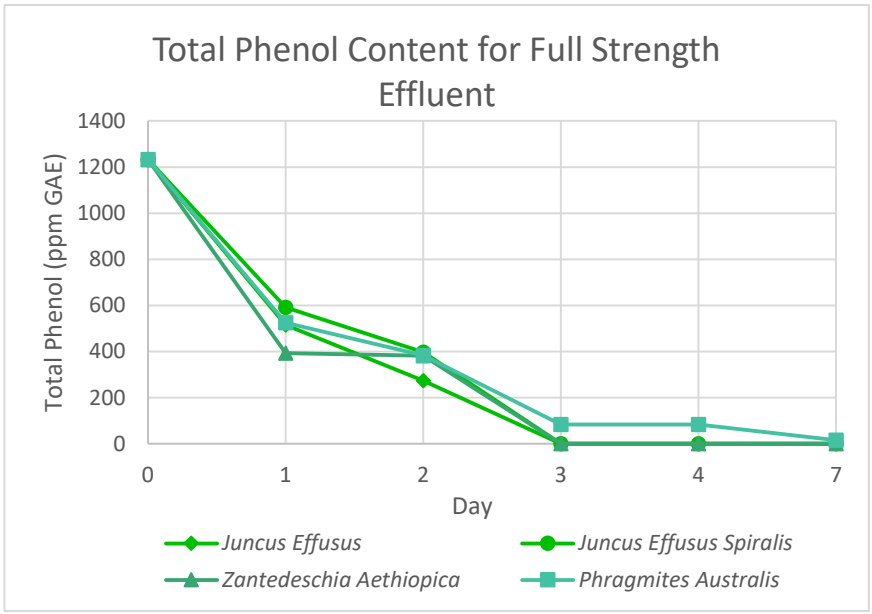
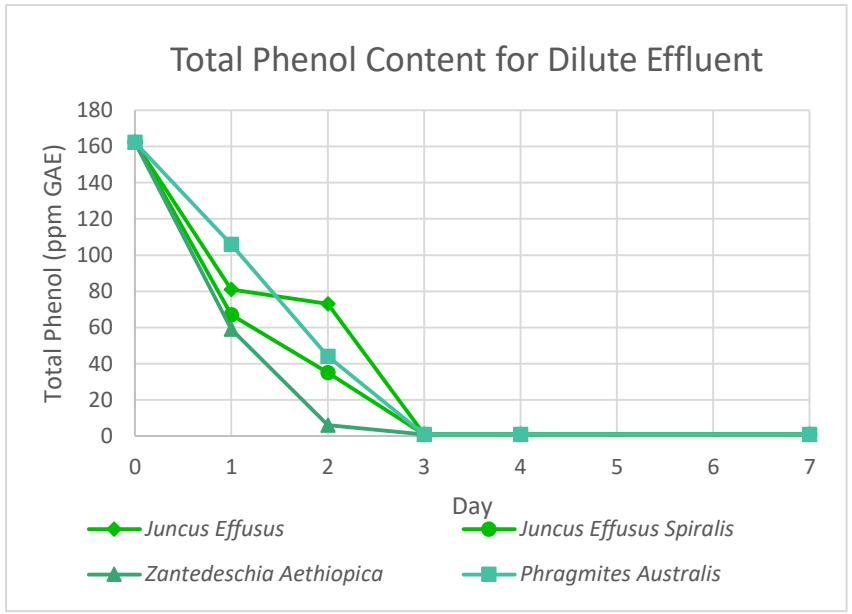
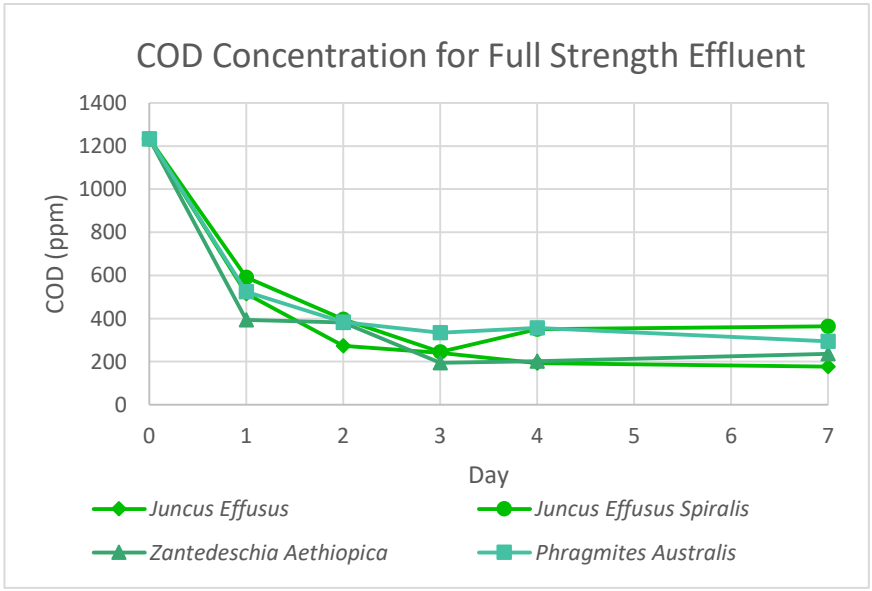


Figure 2: Phenol and COD Remediation Percentage Profiles for Each Macrophyte Species with Corresponding Effluent Strength







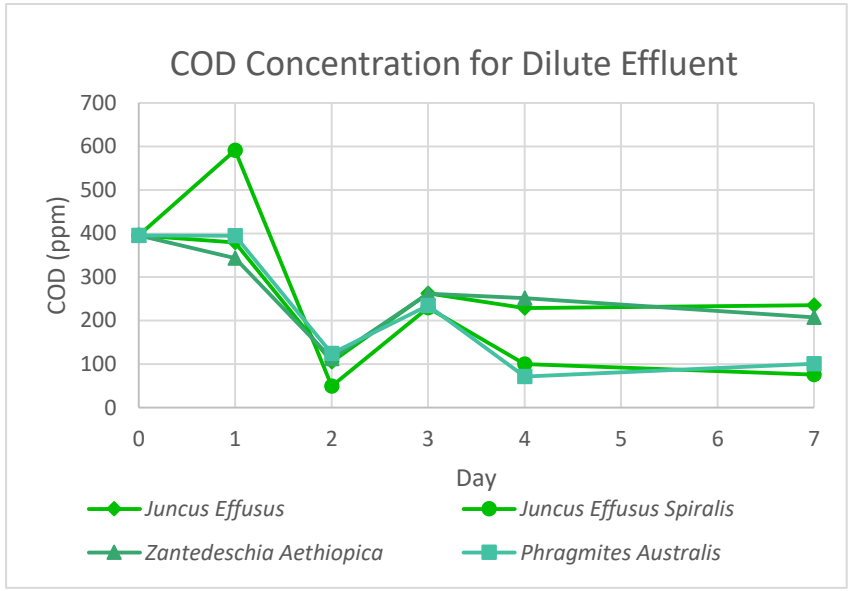


Figure 3: TPC and COD Concentration Profiles for Each Macrophyte Species with Corresponding Effluent Strength

3.1.2 Batch Kinetics

The equation used for the batch kinetics for phenol remediation was the first-order kinetic equation, after analyzing the data and choosing the order that yields a straight line graph (Equation 1):

$$\frac{dC}{dt} = -kC \qquad \text{Equation [1]}$$

Where *C* is the concentration, *t* is the time and *k* is the rate constant. Table 3 show the different rate constants, k, for the different macrophyte species at a temperature range of 20-23˚C by using the slope of the graph of ln*C* vs *t* (graphs not shown):

	Rate Constant, k(1/h)	
Effluent Type	**Full Strength**	**Dilute**
Juncus effusus	0.0726	0.0053
Juncus effusus spiralis	0.0674	0.0036
Zantedeschia aethiopica	0.0587	0.0042
Phragmites australis	0.0741	0.0056

Table 3: Batch Kinetic Rate Constants for Full Strength and Dilute Effluent across all Macrophyte Species with units 1/h at 20-23˚C.

In general, the full strength effluent has a greater rate constant than the dilute effluent. The macrophyte species with the least efficient kinetics for the full strength effluent was *Z. aethiopica* and *J. effusus spiralis* for the dilute strength effluent. However, all species' rate constants were not statistically different (by the t-test). Overall, across both effluent types, *P. australis* has the greatest rate constant and therefore the faster reaction rate and the most efficient kinetics for pulp and paper remediation.

Phase 1 was conducted to determine the best macrophytes to use in the laboratory-scale CWs in term of transformation effectiveness, kinetics and the effluent strength needed to get promising results in the continuous phase (Phase 2). By inspection and statistics, it was determined that effluent at full strength would be used and three identical laboratory-scale CWs that would each have a different macrophyte configuration, namely A – *Z. aethiopica,* B – *J. effusus* and C – a combination of *Z. aethiopica* and *J. effusus.*

3.2 Phase 2 Experiments – Continuous Operation

A phenol stock solution was used in place of the pulp and paper mill effluent to mimic the phenol concentrations in pulp and paper mill effluent. Due to the time limitations and distance, we were unable to get more effluent and therefore a phenol stock solution was used. The stock solution was a representative amount of pulp and paper effluent. The stock solution had the same phenol concentration as full strength effluent was used in all three CWs to determine the most effective CW macrophyte configuration in phenol remediation (Experiment 3). The macrophyte species had an influence in phenol remediation as seen in the batch phase experiment and therefore the optimum configuration in the continuous phase was investigated before using the phenol-rich pulp and paper mill effluent.

3.2.1 Optimum Wetland Configuration

The results from Experiment 3 are shown in Table 4 and Figure 4. Table 4 is a summary of Figure 4 and the effect macrophytes and respective configurations have on the pulp and paper mill effluent remediation and COD removal.

CW Configuration	Phenol Remediation %	COD Removal %
A – Z. aethiopica	75	74
B – J. effuses	73	78
C – Z. aethiopica& J. effusus	78	74

Table 4: Phenol Remediation % and COD Removal % over the Three Different CW Macrophyte Configurations

Phenol remediation and COD removal occurs within CW systems, as seen in Figure 4. All three CW configurations show phenol can be remediated to approximately 70% in 144 hours of continuous flow. CW C (*Z. aethiopica & J. effusus*) showed a greater phenol remediation in the first day than CW A and B. This may be due to the macrophyte variation that creates a variation in the micro-organism community or different oxygen release rates and root surfaces of the two species, and therefore different methods of phenol remediation occurring simultaneously allowing for faster phenol remediation (Grace Liu et al., 2011; Haberl et al., 2003). CW C shows a gradual increase in phenol remediation effectivity after the first 24 hours, reaching steady state after 96 hours. Steady state was concluded when two consecutive samples from CW C had TPC results that were not statistically different proven by the t-test. CW A has higher phenol remediation efficiency, at the end of the experiment, than CW B and C of 78%.



Figure 4: Phenol Remediation and COD Profiles for the Three CWs for Phenol Solution

However, the phenol remediation efficiency across all three CWs (Table 4) are not statistically different (by the t-test) from one another. This can also be seen in the phenol concentration profile in Figure 4 where the end concentration for all three CWs is approximately 100ppm GAE by the Folin-Ciocalteu method.

COD removal efficiency follows the same trend as phenol remediation efficiency for the first 72 hours, thereafter; CW C continues the upward trend and CW A and B decrease at 96 hours. The decrease seen in CW A and B may be due to adaptation of the microbial community within the system that resulted in oxygen consumption and then reached steady state after the environment stabilised (Asina et al., 2017; Panikov, 1995). CW C may not have had the same decrease in COD removal % at 96 hours due to the macrophyte variation of *Z. aethiopica & J. effusus* and therefore the microbial community which may not have needed to adjust to environmental conditions as much as CW A and B may have needed to (Grace Liu et al., 2011; Haberl et al., 2003). CW B had the highest COD removal efficiency with 78%, however, the final COD removal efficiency for all three CWs (Table 4), are not statistically different. This can also be seen in the COD concentration profile in Figure 4 where the end concentrations for all three CWs are approximately 80ppm.

CW C was taken to be the optimum CW (Experiment 3) as the initial phenol remediation efficiency is the greatest and the phenol remediation process begins at the start-up phase in CW A, compared to later in CW B, resulting in a more efficient system in the beginning stages and a lower toxin concentration (phenolic compounds) being exposed to the surrounding environment.

3.2.2 Pulp and Paper Effluent Remediation by Continuous Flow CW

Phenol-rich effluent was used as an influent to the optimum CW, with the *Z. aethiopica & J. effusus* configuration (Experiment 4). The total phenol remediation efficiency and COD removal is shown in Figure 5. The phenol remediation efficiency and COD removal efficiency are relative to the influent sample, taken at the same time as the effluent sample.

Pulp and paper effluent can be remediated by constructed wetlands. Effluent remediation occurs rapidly within the first 48 hours after start-up of operation reaching 58% remediation efficiency with a TPC of 190ppm GAE outflow concentration. Remediation efficiency gradually starts to stabilize and reaches steady state at approximately 96 hours of operation. Steady state was concluded when two consecutive samples had TPC results that were not statistically different determined by the t-test. In this CW configuration, phenol remediation efficiency stabilizes at 63% and a TPC of approximately 177ppm GAE outflow concentration at 144 hours of operation, as seen in Figure 6.

COD removal occurs rapidly in the first 24 hours reaching 53% and approximately outflow concentration of 274ppm, thereafter COD removal decreases, until the effluent remediation reaches steady state (96 hours). COD outflow concentration at 96 hours is approximately 433ppm at 17% COD removal. This decrease in COD removal efficiency, may be due to the CW microbial community adjusting to the change in the environment caused by the different organic components in the effluent (Stefanakis and Thullner, 2016). Once steady state in effluent remediation is achieved, COD removal efficiency increases to 34%

at approximately 34ppm in the following 48 hours. The increase in COD removal efficiency may be due to the microbial community stabilizing adequately adapting to the change in environmental conditions to start the COD removal process again (Asina et al., 2017).

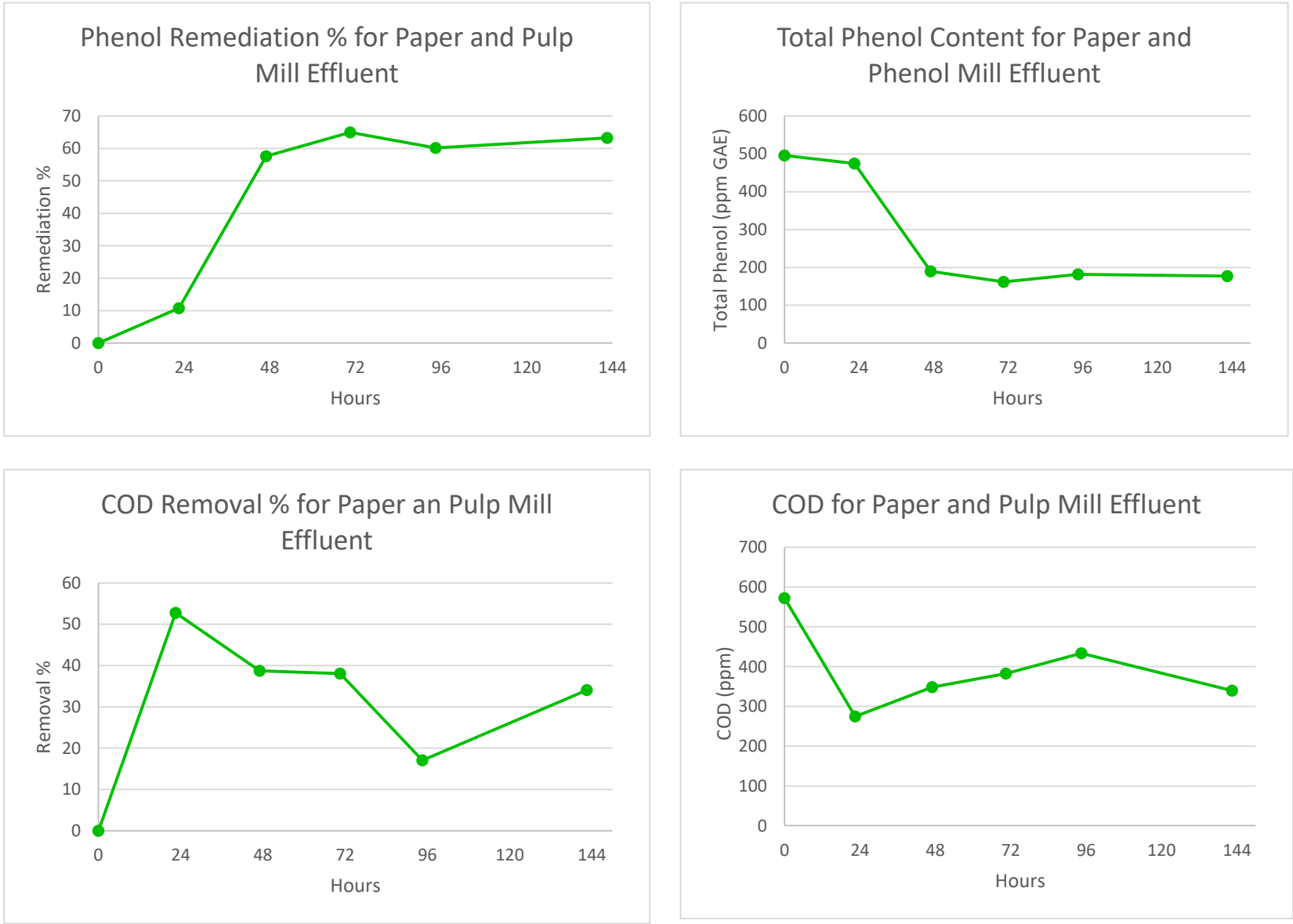


Figure 5: Phenol and COD Removal as Percentages and Concentration Profiles for Paper and Pulp Mill Effluent

A phenol remediation efficiency of 63% and COD removal efficiency of 34% resulted in a substantial color change. A photo of vials with feed and outlet (with no filtration) is shown in Figure 6.

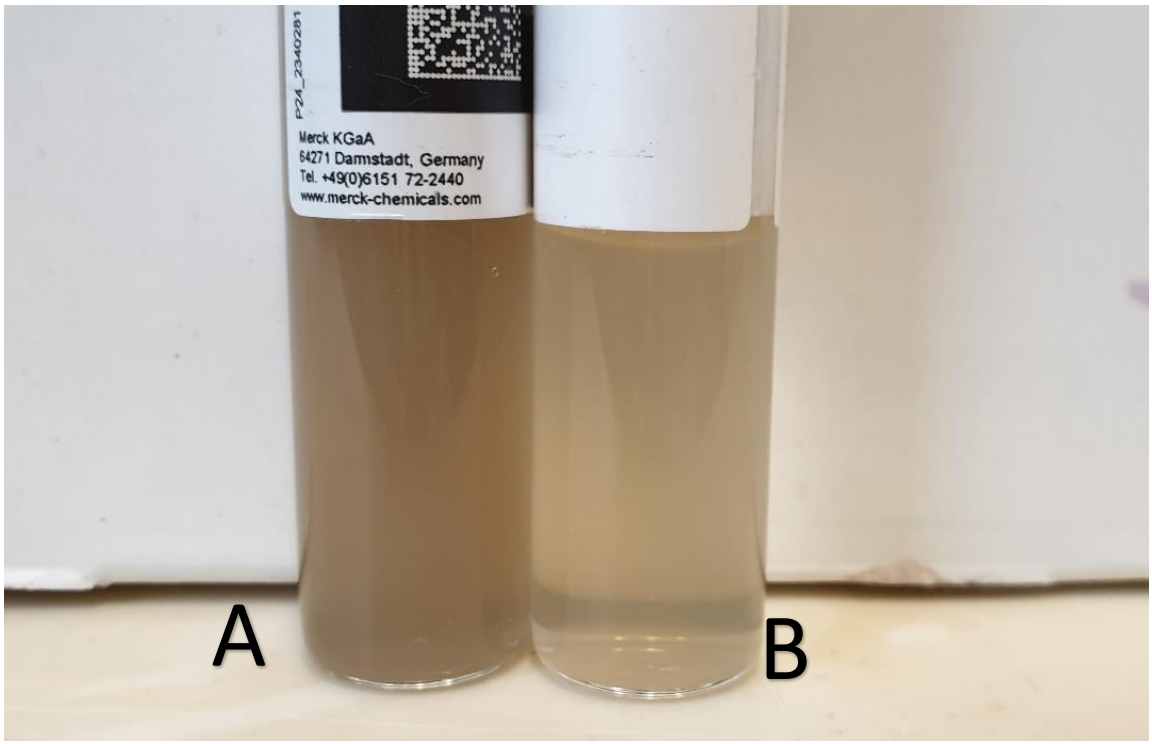


Figure 6: Inlet (A) and Outlet (B) Color Comparisons

The phenol solution has a higher phenol remediation and COD removal than pulp and phenol-rich paper mill effluent. This may be due to the pulp and paper effluent has other organic components, as seen by the TOC test which resulted in a TOC value of 1010ppm. The bigger the mixture of components in the paper mill effluent was, the more components the micro-organisms have available to them which may be the reason the phenol remediation process (which is the main component of testing in this study) is not as effective as it is when using the phenol solution.

3.2.3 Continuous Kinetics

Kinetics were investigated for both continuous experiments for phenol remediation. The continuous rate of remediation was found using the approximate number tanks the CWs represented to determine the reactor model it most represented (Fogler, 2006; Levenspiel, 1972). This reactor was found in previous studies (data not shown) to behave as a plug-flow reactor and therefore Equation 1 can be used (Fogler, 2006). Table 5 is a summary of the rate constants found for each CW (temperatures ranges between 9 and 11˚C) and the phenol remediation efficiency.

CW Configuration	***k*(/h)**
A – Z. aethiopica	0.0064
B – J. effuses	0.0095
C – Z. aethiopica& J. effusus	0.0067

Table 5: Continuous Kinetic Rate Constants for each CW Configuration at a Temperature Range of 9-11˚C.

The rate constant, *k*, was calculated using the slope of the graph of ln*C* vs *t* (graphs not shown). CW B has the greatest rate constant of 9.5×10^{-3}/h, however had the lowest phenol remediation % (as seen in Table 4 and Figure 5), followed by CW C with a rate constant of 6.7×10^{-3}/h and the highest phenol remediation.

The same procedure was followed for pulp and paper mill effluent kinetics for phenol remediation. Figure 7 shows that graph from which the rate constant was found for Experiment 4, the temperature during this experiment ranged between 15-18˚C:

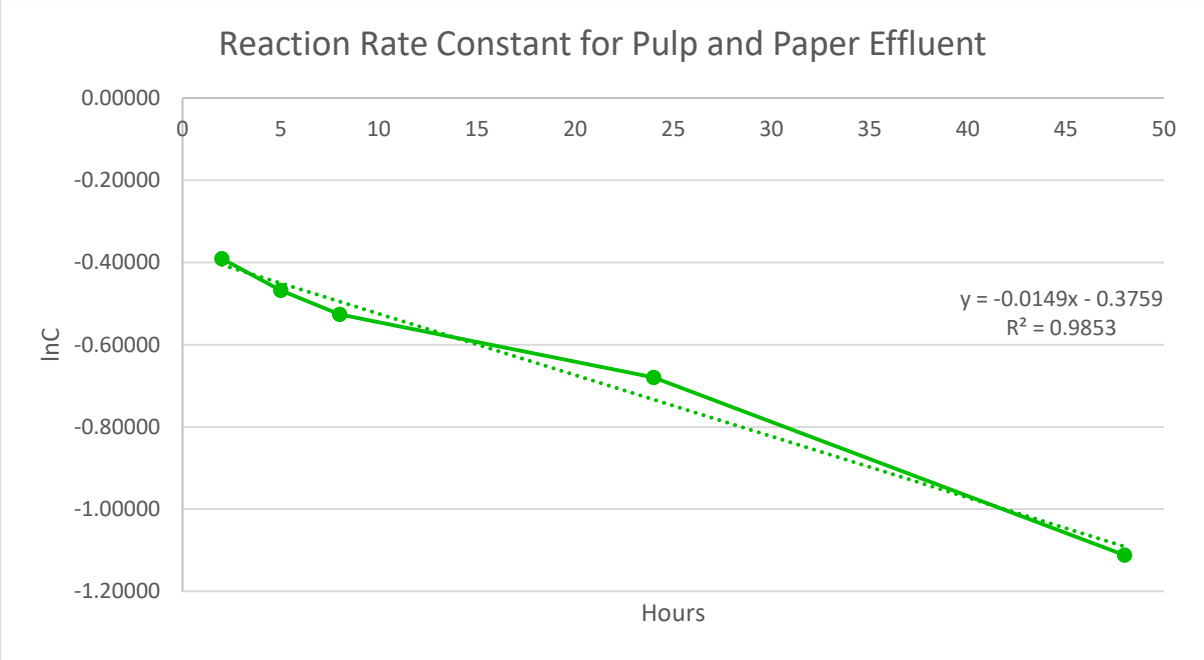


Figure 7: Reaction Rate Constant for Phenol Remediation in CW C at a Temperature Range of 15-18˚C

The rate constant for pulp and for paper and pulp mill effluent was found to be $15x10^{-3}$/h. This is significantly different to the rate constants found for the phenol solution in Table 5. However, the temperature difference at the time of conducting the experiments must be noted as rate constants are influenced by temperatures and this may be the reason for the difference in results. In general phenol remediation is an endothermic reaction (Maskow et al., 2010; Park et al., 2015; Y. Wu et al., 2014) and therefore the rate constants will be greater in higher temperatures. Another reason may be the different organic composition of the pulp and paper effluent to the phenol solution.

Summary and Conclusion

Remediation of phenol-rich paper and pulp mill effluent can be achieved using constructed wetlands in both batch and continuous operation modelled as a plug-flow reactor. Macrophytes used in the CW has an effect on phenol remediation, with the configuration of *J. effusus* and *Z. aethiopica* being the most effective during the startup phase of CW

configuration for phenol remediation and COD removal. Batch operation can reach a phenol remediation of 97-100% with a COD removal of 80-86% with an average rate constant of 0.058-0.074/h. Continuous operation can reach phenol remediation of 60-65% with a COD removal of 30-35% with an average rate constant of 0.015/h. Temperature has a large influence on rate constants and therefore the reaction rates. Phenol-rich pulp and paper mill effluent remediation is more efficient at higher temperatures.

This result confirms the few similar previous studies of phenolic like compounds done by Stefanakis et al., a 2014, b 2016; Stefanakis and Thullner, 2016. Further studies can be conducted on a CW that is longer in length which is expected to achieve the same or improved results, which can further be investigated by the use of phenol solution and/or effluent over an extended time period. Upscaling the constructed wetlands and effluent volumes may also be an option for increasing the total removal of both phenolic compounds and COD. Another opportunity would be to use different types of phenol-rich effluents such as effluents form the petroleum, plastic and pharmaceutical industries and investigate the constructed wetland remediation efficiencies for each effluent type.

Acknowledgments

The authors would like to thank the Centre in Water Research and Development (CIWaRD) and the School of Chemical and Metallurgical Engineering at the University of the Witwatersrand for financial support to conduct this study and the technical support given.

6. Conclusions

Industrial effluent from the pulp and paper industry is phenol-rich with a high chemical oxygen demand. Current treatment methods are costly and/or release harmful gases and substances to the environment. This study has shown that laboratory-scale constructed wetlands can be used to remediate the phenol-rich industrial effluent in a way that does not harm the surrounding.

This study found that CWs can remediate full strength phenol-rich effluent in both laboratory-scale batch and continuous operation. Batch operation reached 100% phenol remediation with low COD values with macrophyte species *J. effusus spiralis*, *J. effusus* and *Z. aethiopica* with an average rate constant of 0.06/h. Continuous operation reached highs of 78% phenol remediation with a combination of *J. effusus* and *Z. aethiopica* macrophytes with a rate constant of 0.015/h.

Based on the results obtained from this study, optimum horizontal subsurface-flow constructed wetland design specifications can be made. CWs should be shallow in depth as phenol and COD concentration gradients for both pulp and paper mill effluent and artificial effluent underwent a decrease in concentration in the middle depths of the CW bed. Concentrations decreased along the length of the CW bed, therefore longer CW beds may prove to be more beneficial at phenol-rich effluent remediation in terms of percentage remediation. These design specifications may allow for the system to be sufficient enough to discharge the treated water into the environment, with TPC value of less than 1ppm and a COD value of less than 75ppm.

Temperature plays a role on the phenol remediation kinetics. This study showed that experiments done in warmer temperatures had greater rate constants and therefore, faster

remediation rates. This is therefore a factor that needs to be considered when designing CWs for remediating pulp-and-paper effluent, especially in colder climates.

This study can be expanded upon in a number of ways. The same experiments can be conducted on a CW that has been made longer and shallower to meet the design specifications stated earlier in order to determine by how much the results are improved. This study was mainly conducted in the Johannesburg winter months and could be redone in the summer months as the temperature would be warmer, aiding remediation reaction rates and to determine by how much the reaction rate increases. The result may be different if conducted in a coastal area where the climate might favor different emergent macrophyte species.

Other industrial phenol-rich effluents could be tested in this treatment system to determine if the same phenol remediation trend is followed. Other industrial phenol-rich effluents can be sourced from the textile industry, the oil, gas and coal industry, the pharmaceutical industry and the agricultural industry in pesticides and insecticides (Anku et al., 2017). The current CW system could also potentially be used to treat other kinds of industrial effluents that do not contain phenol, such as acid mine drainage wastewaters, diesel and petroleum wastewaters and heavy metal removal.

References

A, D., Fujii, D., Soda, S., Machimura, T., Ike, M., 2017. Removal of phenol, bisphenol A, and 4-tert-butylphenol from synthetic landfill leachate by vertical flow constructed wetlands. Sci. Total Environ. 578, 566–576. https://doi.org/10.1016/j.scitotenv.2016.10.232

Aghdam, M.A., Kariminia, H.-R., Safari, S., 2016. Removal of lignin, COD, and color from pulp and paper wastewater using electrocoagulation. Desalination Water Treat. 57, 9698–9704. https://doi.org/10.1080/19443994.2015.1040461

Akratos, C.S., Tsihrintzis, V.A., 2007. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecol. Eng. 29, 173–191. https://doi.org/10.1016/j.ecoleng.2006.06.013

Al-Isawi, R.H.K., Scholz, M., Al-Faraj, F.A.M., 2016. Assessment of diesel-contaminated domestic wastewater treated by constructed wetlands for irrigation of chillies grown in a greenhouse. Environ. Sci. Pollut. Res. 23, 25003–25023. https://doi.org/10.1007/s11356-016-7706-x

Almuktar, S.A.A.A.N., Abed, S.N., Scholz, M., 2018. Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. Environ. Sci. Pollut. Res. 25, 23595–23623. https://doi.org/10.1007/s11356-018-2629-3

Anku, W.W., Mamo, M.A., Govender, P.P., 2017. Phenolic Compounds in Water: Sources, Reactivity, Toxicity and Treatment Methods. Phenolic Compd. - Nat. Sources Importance Appl. https://doi.org/10.5772/66927

Arivoli, A., Mohanraj, R., Seenivasan, R., 2015. Application of vertical flow constructed wetland in treatment of heavy metals from pulp and paper industry wastewater. Environ. Sci. Pollut. Res. 22, 13336–13343. https://doi.org/10.1007/s11356-015-4594-4

Ashrafi, O., Yerushalmi, L., Haghighat, F., 2015. Wastewater treatment in the pulp-and-paper industry: A review of treatment processes and the associated greenhouse gas emission. J. Environ. Manage. 158, 146–157. https://doi.org/10.1016/j.jenvman.2015.05.010

Asina, F., Brzonova, I., Kozliak, E., Kubátová, A., Ji, Y., 2017. Microbial treatment of industrial lignin: Successes, problems and challenges. Renew. Sustain. Energy Rev. 77, 1179–1205. https://doi.org/10.1016/j.rser.2017.03.098

Aysu, T., Esim, N., 2016. Supercritical liquefaction of common reed (Phragmites australis) with alkali catalysts. Energy Sources Part Recovery Util. Environ. Eff. 38, 1336–1344. https://doi.org/10.1080/15567036.2014.919369

Belmont, M.A., Metcalfe, C.D., 2003. Feasibility of using ornamental plants (Zantedeschia aethiopica) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants—a laboratory-scale study. Ecol. Eng. 21, 233–247. https://doi.org/10.1016/j.ecoleng.2003.10.003

Blainski, A., Lopes, G.C., de Mello, J.C.P., 2013. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. Mol. Basel Switz. 18, 6852–6865. https://doi.org/10.3390/molecules18066852

Bond, W.J., Honig, M., Maze, K.E., 1999. Seed size and seedling emergence: an allometric relationship and some ecological implications. Oecologia 120, 132–136. https://doi.org/10.1007/s004420050841

Brandão, Y.B., Benachour, J.G.C. de O. and M., 2017. Phenolic Wastewaters: Definition, Sources and Treatment Processes. Phenolic Compd. - Nat. Sources Importance Appl. https://doi.org/10.5772/66366

Burton, F.L., Tchobanoglous, G., Tsuchihashi, R., Stensel, H.D., Inc, M.& E., 2013. Wastewater Engineering: Treatment and Resource Recovery. McGraw-Hill Education.

Cao, C., Guo, L., Chen, Y., Guo, S., Lu, Y., 2011. Hydrogen production from supercritical water gasification of alkaline wheat straw pulping black liquor in continuous flow system. Int. J. Hydrog. Energy, 2010 Asian/APEC BioH2 36, 13528–13535. https://doi.org/10.1016/j.ijhydene.2011.07.101

Carapeto, C., Purchase, D., 2000. Distribution and Removal of Cadmium and Lead in a Constructed Wetland Receiving Urban Runoff. Bull. Environ. Contam. Toxicol. 65, 322–329. https://doi.org/10.1007/s0012800131

Cardoso, M., de Oliveira, É.D., Passos, M.L., 2009. Chemical composition and physical properties of black liquors and their effects on liquor recovery operation in Brazilian pulp mills. Fuel 88, 756–763. https://doi.org/10.1016/j.fuel.2008.10.016

Chandra, R., Singh, S., Raj, A., 2006. Seasonal bacteriological analysis of Gola river water contaminated with pulp paper mill waste in Uttaranchal, India. Environ. Monit. Assess. 118, 393–406. https://doi.org/10.1007/s10661-006-1508-4

Chen, H., 2011. Surface-Flow Constructed Treatment Wetlands for Pollutant Removal: Applications and Perspectives. Wetlands 31, 805–814. https://doi.org/10.1007/s13157-011-0186-3

Chen, L.-Y., Cheng, C.-W., Liang, J.-Y., 2015. Effect of esterification condensation on the Folin–Ciocalteu method for the quantitative measurement of total phenols. Food Chem. 170, 10–15. https://doi.org/10.1016/j.foodchem.2014.08.038

Cheung, L.M., Cheung, P.C.K., Ooi, V.E.C., 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem.

Coulson, J.M., Sinnott, R.K., Richardson, J.F., 1999. Coulson & Richardson's Chemical Engineering. Butterworth-Heinemann.

Cowling, R.M., Richardson, D.M., Pierce, S.M., 2004. Vegetation of Southern Africa. Cambridge University Press.

Dan, A., Fujii, D., Soda, S., Machimura, T., Ike, M., 2017. Removal of phenol, bisphenol A, and 4-tert-butylphenol from synthetic landfill leachate by vertical flow constructed wetlands. Sci. Total Environ. 578, 566–576. https://doi.org/10.1016/j.scitotenv.2016.10.232

Economopoulou, M.A., Tsihrintzis, V.A., 2003. Design Methodology and Area Sensitivity Analysis of Horizontal Subsurface Flow Constructed Wetlands. Water Resour. Manag. 17, 147–174. https://doi.org/10.1023/A:1023615324178

Fan, J., Liang, S., Zhang, B., Zhang, J., 2013. Enhanced organics and nitrogen removal in batch-operated vertical flow constructed wetlands by combination of intermittent aeration and step feeding strategy. Environ. Sci. Pollut. Res. 20, 2448–2455. https://doi.org/10.1007/s11356-012-1130-7

Faustino, H., Gil, N., Baptista, C., Duarte, A.P., 2010. Antioxidant Activity of Lignin Phenolic Compounds Extracted from Kraft and Sulphite Black Liquors. Molecules 15, 9308–9322. https://doi.org/10.3390/molecules15129308

Fogler, H.S., 2006. Elements of Chemical Reaction Engineering. Prentice Hall PTR.

Gaudy, A.F., Ramanathan, M., 1964. A Colorimetric Method for Determining Chemical Oxygen Demand. J. Water Pollut. Control Fed. 36, 1479–1487.

Ghassemzadeh, F., Yousefzadeh, H., Arbab-Zavar, M.H., 2008. Arsenic phytoremediation by Phragmites australis: green technology. Int. J. Environ. Stud. 65, 587–594. https://doi.org/10.1080/00207230802273387

Gosling, S.N., Arnell, N.W., 2016. A global assessment of the impact of climate change on water scarcity. Clim. Change 134, 371–385. https://doi.org/10.1007/s10584-013-0853-x

Grace Liu, P.-W., Chang, T.C., Whang, L.-M., Kao, C.-H., Pan, P.-T., Cheng, S.-S., 2011. Bioremediation of petroleum hydrocarbon contaminated soil: Effects of strategies and microbial community shift. Int. Biodeterior. Biodegrad. 65, 1119–1127. https://doi.org/10.1016/j.ibiod.2011.09.002

Greenway, M., 2007. The Role of Macrophytes in Nutrient Removal using Constructed Wetlands, in: Singh, S.N., Tripathi, R.D. (Eds.), Environmental Bioremediation Technologies. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 331–351. https://doi.org/10.1007/978-3-540-34793-4_15

Haberl, R., Grego, S., Langergraber, G., Kadlec, R.H., Cicalini, A.-R., Dias, S.M., Novais, J.M., Aubert, S., Gerth, A., Thomas, H., Hebner, A., 2003. Constructed wetlands for the treatment of organic pollutants. J. Soils Sediments 3, 109. https://doi.org/10.1007/BF02991077

Headley, T.R., Tanner, C.C., 2012. Constructed Wetlands With Floating Emergent Macrophytes: An Innovative Stormwater Treatment Technology. Crit. Rev. Environ. Sci. Technol. 42, 2261–2310. https://doi.org/10.1080/10643389.2011.574108

Hussain, A., Dubey, S.K., Kumar, V., 2015. Kinetic study for aerobic treatment of phenolic wastewater. Water Resour. Ind. 11, 81–90. https://doi.org/10.1016/j.wri.2015.05.002

Ilyas, H., Masih, I., 2017. Intensification of constructed wetlands for land area reduction: a review. Environ. Sci. Pollut. Res. 24, 12081–12091. https://doi.org/10.1007/s11356-017-8740-z

Jørgensen, S.E., Löffler, H., Rast, W., Straškraba, M. (Eds.), 2005. Chapter 3 - Evaluating Lake and Reservoir Water Quality, in: Developments in Water Science, Lake and Reservoir Management. Elsevier, pp. 107–168. https://doi.org/10.1016/S0167-5648(05)80024-3

Kadlec, R.H., Knight, R., Vymazal, J., Brix, H., Cooper, P., Haberl, R., 2000. Constructed Wetlands for Pollution Control. IWA Publishing.

Kamali, M., Khodaparast, Z., 2015. Review on recent developments on pulp and paper mill wastewater treatment. Ecotoxicol. Environ. Saf. 114, 326–342. https://doi.org/10.1016/j.ecoenv.2014.05.005

Keddy, P.A., 2010. Wetland Ecology: Principles and Conservation. Cambridge University Press.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., Veneklaas, E.J., 2006. Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. Ann. Bot. 98, 693–713. https://doi.org/10.1093/aob/mcl114

Levenspiel, O., 1972. Chemical reaction engineering. Wiley.

Li, G., Wu, Z., Cheng, S., Liang, W., He, F., Fu, G., Zhong, F., 2007. Application of constructed wetlands on wastewater treatment for aquaculture ponds. Wuhan Univ. J. Nat. Sci. 12, 1131–1135. https://doi.org/10.1007/s11859-007-0116-7

Maskow, T., Kemp, R., Buchholz, F., Schubert, T., Kiesel, B., Harms, H., 2010. What heat is telling us about microbial conversions in nature and technology: from chip- to megacalorimetry. Microb. Biotechnol. 3, 269–284. https://doi.org/10.1111/j.1751-7915.2009.00121.x

Matthews, D.J., Moran, B.M., Otte, M.L., 2004. Zinc tolerance, uptake, and accumulation in the wetland plants <Emphasis Type="Italic">Eriophorum angustifolium, Juncus effusus</Emphasis>, and <Emphasis Type="Italic">Juncus articulatus</Emphasis>. Wetlands 24, 859. https://doi.org/10.1672/0277-5212(2004)024[0859:ZTUAAI]2.0.CO;2

Mekonnen, M.M., Hoekstra, A.Y., 2016. Four billion people facing severe water scarcity. Sci. Adv. 2, e1500323. https://doi.org/10.1126/sciadv.1500323

Mustapha, H.I., Bruggen, J.J.A. van, Lens, P.N.L., 2018. Optimization of Petroleum Refinery Wastewater Treatment by Vertical Flow Constructed Wetlands Under Tropical Conditions: Plant Species Selection and Polishing by a Horizontal Flow Constructed Wetland. Water. Air. Soil Pollut. 229, 137. https://doi.org/10.1007/s11270-018-3776-3

National Water Act No. 39 of 1999 [WWW Document], 1999. . Cent. Environ. Rights. URL https://cer.org.za/virtual-library/legislation/national/water/national-water-act-1998 (accessed 8.15.19).

Novák, V., 2012. Methods of Evapotranspiration Estimation. Evapotranspiration Soil-Plant-Atmosphere Syst. 165–215. https://doi.org/10.1007/978-94-007-3840-9_9

Ojuederie, O.B., Babalola, O.O., 2017. Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. Int. J. Environ. Res. Public. Health 14. https://doi.org/10.3390/ijerph14121504

Oki, T., Kanae, S., 2006. Global Hydrological Cycles and World Water Resources. Science 313, 1068–1072. https://doi.org/10.1126/science.1128845

Oliveira, R.C.P., Mateus, M., Santos, D.M.F., 2018. Chronoamperometric and chronopotentiometric investigation of Kraft black liquor. Int. J. Hydrog. Energy 16817–16823. https://doi.org/10.1016/j.ijhydene.2018.01.046

Panikov, N.S., 1995. Microbial Growth Kinetics. Springer Science & Business Media.

Park, H., Kwon, O., Ryu, K., 2015. Thermal stability and degradation kinetics of polyphenols and polyphenylenediamines enzymatically synthesized by horseradish peroxidase. Korean J. Chem. Eng. 32, 1847–1852. https://doi.org/10.1007/s11814-015-0011-4

Plessis, A. du, 2017. Water Scarcity and Other Significant Challenges for South Africa. Freshw. Chall. South Afr. Its Up. Vaal River 119–125. https://doi.org/10.1007/978-3-319-49502-6_7

Poerschmann, J., Schultze-Nobre, L., 2014. Sorption determination of phenols and polycyclic aromatic hydrocarbons in a multiphase constructed wetland system by solid phase microextraction. Sci. Total Environ. 482–483, 234–240. https://doi.org/10.1016/j.scitotenv.2014.03.004

Pola, L., Collado, S., Oulego, P., Díaz, M., 2019. Production of carboxylic acids from the non-lignin residue of black liquor by hydrothermal treatments. Bioresour. Technol. 284, 105–114. https://doi.org/10.1016/j.biortech.2019.03.066

Ramanjaneyulu, P.S., Raman, V.A., Sayi, Y.S., Ramakumar, K.L., Venugopal, V., 2005. Determination of Boron in U3O8 by Spectrophotometry. Trans. Indian Ceram. Soc. 64, 163–165. https://doi.org/10.1080/0371750X.2005.11012207

Rani, M., Shanker, U., 2018. Photocatalytic degradation of toxic phenols from water using bimetallic metal oxide nanostructures. Colloids Surf. Physicochem. Eng. Asp. 553, 546–561. https://doi.org/10.1016/j.colsurfa.2018.05.071

Rocha, A.C.S., Almeida, C.M.R., Basto, M.C.P., Vasconcelos, M.T.S.D., 2015. Influence of season and salinity on the exudation of aliphatic low molecular weight organic acids (ALMWOAs) by Phragmites australis and Halimione portulacoides roots. J. Sea Res. 95, 180–187. https://doi.org/10.1016/j.seares.2014.07.001

Rossmann, M., de Matos, A.T., Abreu, E.C., e Silva, F.F., Borges, A.C., 2012. Performance of constructed wetlands in the treatment of aerated coffee processing wastewater: Removal of nutrients and phenolic compounds. Ecol. Eng. 49, 264–269. https://doi.org/10.1016/j.ecoleng.2012.08.017

Saeed, T., Sun, G., 2012. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. J. Environ. Manage. 112, 429–448. https://doi.org/10.1016/j.jenvman.2012.08.011

Samuelsson, L.N., Babler, M.U., Brännvall, E., Moriana, R., 2016. Pyrolysis of kraft pulp and black liquor precipitates derived from spruce: Thermal and kinetic analysis. Fuel Process. Technol. 149, 275–284. https://doi.org/10.1016/j.fuproc.2016.04.029

Schmitt, D., Beiser, N., Regenbrecht, C., Zirbes, M., Waldvogel, S.R., 2017. Adsorption and separation of black liquor-derived phenol derivatives using anion exchange resins. Sep. Purif. Technol. 181, 8–17. https://doi.org/10.1016/j.seppur.2017.03.004

Scholz, M., 2011. Wetland Case Studies. Wetl. Syst. 19–126. https://doi.org/10.1007/978-1-84996-459-3_2

Scholz, M., Lee, B., 2005. Constructed wetlands: a review. Int. J. Environ. Stud. 62, 421–447. https://doi.org/10.1080/00207230500119783

Schultze-Nobre Luciana, Wiessner Arndt, Bartsch Cindy, Paschke Heidrun, Stefanakis Alexandros I., Aylward Lara A., Kuschk Peter, 2017. Removal of dimethylphenols and ammonium in laboratory-scale horizontal subsurface flow constructed wetlands. Eng. Life Sci. 17, 1224–1233. https://doi.org/10.1002/elsc.201600213

Sheridan, C., 2012. Chemical engineering modelling of a vegetated submerged reedbed for winery effluent treatment. University of the Witwatersrand, Johannesburg, Gauteng.

Singh, A.K., Chandra, R., 2019. Pollutants released from the pulp paper industry: Aquatic toxicity and their health hazards. Aquat. Toxicol. 211, 202–216. https://doi.org/10.1016/j.aquatox.2019.04.007

Sokkanathan, G., Sharmila, V.G., Kaliappan, S., Banu, J.R., Yeom, I.T., Rani, R.U., 2018. Combinative treatment of phenol-rich retting-pond wastewater by a hybrid upflow anaerobic sludge

blanket reactor and solar photofenton process. J. Environ. Manage. 206, 999–1006. https://doi.org/10.1016/j.jenvman.2017.11.083
Stefanakis, A., Akratos, C.S., Tsihrintzis, V.A., 2014. Vertical Flow Constructed Wetlands: Eco-engineering Systems for Wastewater and Sludge Treatment. Newnes.
Stefanakis, A.I., Seeger, E., Dorer, C., Sinke, A., Thullner, M., 2016. Performance of pilot-scale horizontal subsurface flow constructed wetlands treating groundwater contaminated with phenols and petroleum derivatives. Ecol. Eng. 95, 514–526. https://doi.org/10.1016/j.ecoleng.2016.06.105
Stefanakis, A.I., Thullner, M., 2016. Fate of Phenolic Compounds in Constructed Wetlands Treating Contaminated Water, in: Phytoremediation. Springer, Cham, pp. 311–325. https://doi.org/10.1007/978-3-319-41811-7_16
Struchkov, P.A., Beloborodov, V.L., Il'yasov, I.R., Savvatee, A.M., Kolkhir, V.K., Voskoboinikova, I.V., 2016. Assessment of Total Polyphenol Compounds in the Complex Phytopreparations Angionorm and Prostanorm Using the Folin-Ciocalteu Method. Pharm. Chem. J. 50, 486–490. https://doi.org/10.1007/s11094-016-1474-2
Sundaravadivel, M., Vigneswaran, S., 2001. Constructed Wetlands for Wastewater Treatment. Crit. Rev. Environ. Sci. Technol. 31, 351–409. https://doi.org/10.1080/20016491089253
Tee, H.C., Seng, C.E., Noor, A.Md., Lim, P.E., 2009. Performance comparison of constructed wetlands with gravel- and rice husk-based media for phenol and nitrogen removal. Sci. Total Environ. 407, 3563–3571. https://doi.org/10.1016/j.scitotenv.2009.02.017
US EPA, 2000. Constructed Wetland Treatment of Municipal Wastewaters [WWW Document]. URL https://nepis.epa.gov/Exe/ZyNET.exe/30004TBD.TXT?ZyActionD=ZyDocument&Client=EPA&Index=1995+Thru+1999&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C95thru99%5CTxt%5C00000016%5C30004TBD.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL (accessed 8.13.19).
Vashi, H., Iorhemen, O.T., Tay, J.H., 2018. Aerobic granulation: A recent development on the biological treatment of pulp and paper wastewater. Environ. Technol. Innov. 9, 265–274. https://doi.org/10.1016/j.eti.2017.12.006
Vo, H.-N.-P., Bui, X.-T., Nguyen, T.-M.-H., Koottatep, T., Bandyopadhyay, A., 2018. Insights of the Removal Mechanisms of Pharmaceutical and Personal Care Products in Constructed Wetlands. Curr. Pollut. Rep. 4, 93–103. https://doi.org/10.1007/s40726-018-0086-8
Vymazal, J., 2014. Constructed wetlands for treatment of industrial wastewaters: A review. Ecol. Eng. 73, 724–751. https://doi.org/10.1016/j.ecoleng.2014.09.034
Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ., Contaminants in Natural and Constructed Wetlands: Pollutant Dynamics and Control 380, 48–65. https://doi.org/10.1016/j.scitotenv.2006.09.014
Vystavna, Y., Frkova, Z., Marchand, L., Vergeles, Y., Stolberg, F., 2017. Removal efficiency of pharmaceuticals in a full scale constructed wetland in East Ukraine. Ecol. Eng. 108, 50–58. https://doi.org/10.1016/j.ecoleng.2017.08.009
Wolverton, B.C., Barlow, R.M., Mcdonald, R.C., 1975. Application of vascular aquatic plants for pollution removal, energy, and food production in a biological system (No. N-75-22938; NASA-TM-X-72726). National Aeronautics and Space Administration, Bay St. Louis, Miss. (USA). National Space Technology Labs.
Woodard & Curran, Inc., 2006. 5 - Waste Characterization, in: Woodard & Curran, Inc. (Ed.), Industrial Waste Treatment Handbook (Second Edition). Butterworth-Heinemann, Burlington, pp. 83–126. https://doi.org/10.1016/B978-075067963-3/50007-2

Wu, H., Zhang, J., Ngo, H.H., Guo, W., Hu, Z., Liang, S., Fan, J., Liu, H., 2015. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. Bioresour. Technol. 175, 594–601. https://doi.org/10.1016/j.biortech.2014.10.068

Wu, S., Kuschk, P., Brix, H., Vymazal, J., Dong, R., 2014. Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. Water Res. 57, 40–55. https://doi.org/10.1016/j.watres.2014.03.020

Wu, Y., Luo, H., Wang, H., Zhang, L., Liu, P., Feng, L., 2014. Fast adsorption of nickel ions by porous graphene oxide/sawdust composite and reuse for phenol degradation from aqueous solutions. J. Colloid Interface Sci. 436, 90–98. https://doi.org/10.1016/j.jcis.2014.08.068

Xu, X., Mills, G., Lindell, A., Peck, E., Korotasz, A., Burgess, E., 2019. The performance of a free surface and metal-removing constructed wetland: How a young wetland becomes mature. Ecol. Eng. 133, 32–38. https://doi.org/10.1016/j.ecoleng.2019.04.020

Yadav, S.K., 2006. Human Health Implications Due to Water Toxicity by Pulp and Paper Mill. J. Hum. Ecol. 20, 91–96. https://doi.org/10.1080/09709274.2006.11905909

Yalcuk, A., Ugurlu, A., 2009. Comparison of horizontal and vertical constructed wetland systems for landfill leachate treatment. Bioresour. Technol. 100, 2521–2526. https://doi.org/10.1016/j.biortech.2008.11.029

Zika, P.F., 2003. The native subspecies of Juncus effusus (Juncaceae) in western North America. Brittonia 55, 150–156. https://doi.org/10.1663/0007-196X(2003)055[0150:TNSOJE]2.0.CO;2

Appendices

Appendix A Pulp and Paper Effluent …… 65

Appendix B Emergent Macrophyte Species ……..66

Appendix C Experiment 1 Absorbance Readings for TPC and COD ……68

Appendix D Experiment 1 Paired t-test Results ……..69

Appendix E Experiment 2 Absorbance Readings for TPC and COD ……70

Appendix F Statistics and Inspection for CW Set-up Based on Batch Data……73

Appendix G Experiment 3 Absorbance Readings for TPC and COD……74

Appendix H Experiment 3 Paired t-test Results……75

Appendix I Experiment 3 Absorbance Readings for TPC and COD for Gradient Profiles

……76

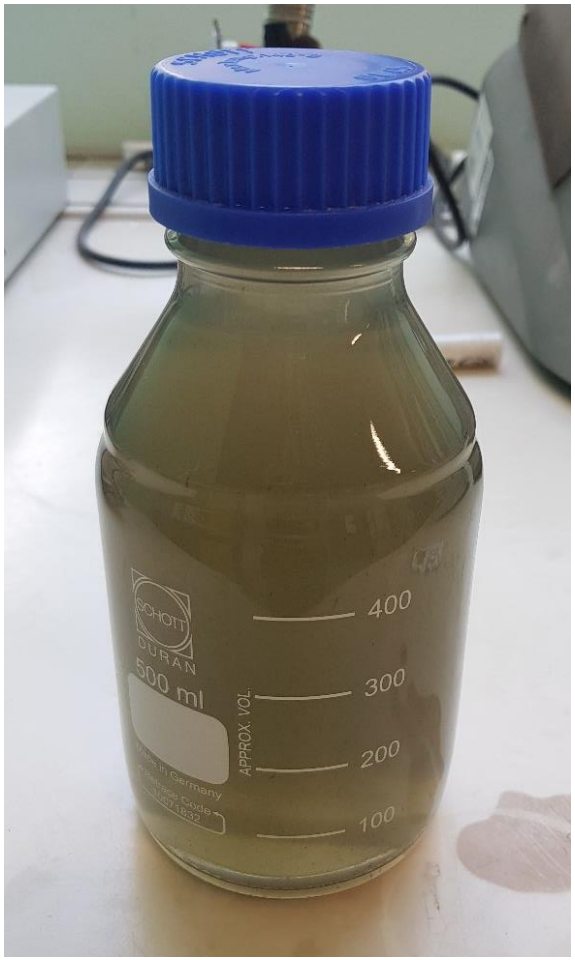
Appendix J Experiment 4 Absorbance Readings for TOC and COD……79

Appendix K Approximate Batch Reaction Kinetics for Different Macrophyte Species and Different Effluent Strengths……80

Appendix L Tracer Studies on the Laboratory-Scale CWs Done Previously……..83

Appendix M Experiment 6 and 7 Absorbance Readings for TPC for Batch and Continuous Kinetics…… 85

Appendix A: Pulp and paper effluent





Appendix B: Emergent macrophyte species



Phragmites australis (Common Reed)



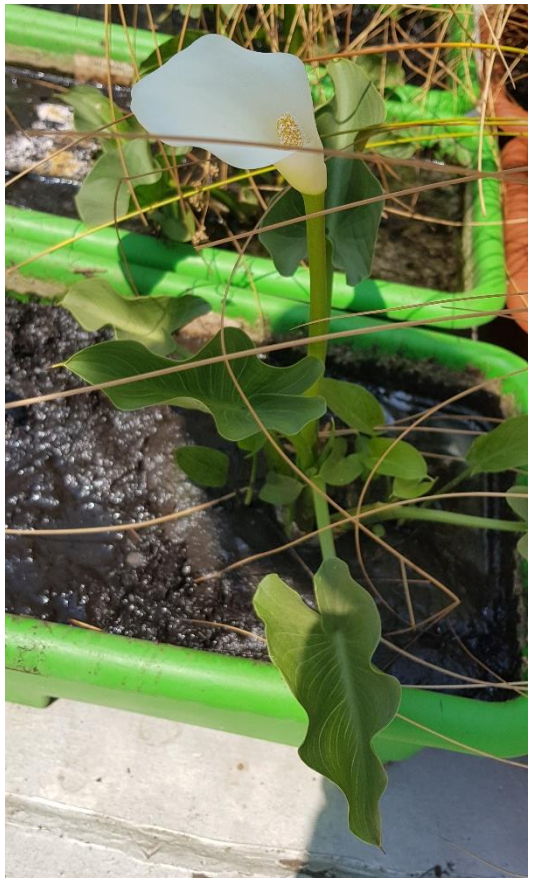
Chondropetalum tectorum (Cape Thatching Reed)



Juncus effusus spiralis (Common Spiral Rush)



Juncus effusus (Common Rush)



Zantedeschia aethiopica (Arum Lily)

Appendix C: Experiment 1 absorbance readings for TPC and COD

Phase 1, Experiment 1, 04032019, TPC					Phase 1, Experiment 1, 11042019, COD					Phase 1, Experiment 1, 11032019, TPC					Phase 1, Experiment 1, 11032019, TPC			
TRAY #	ABS	ppm	av ppm		TRAY #	ABS	ppm	av ppm		TRAY #	ABS	ppm	av ppm		TRAY #	ABS	ppm	av ppm
A1L	0.225	69	96		A1L	0.423	414	423		A1L	0.2	42	49		A1L	0.404	390	398
A1R	0.273	122			A1R	0.909	2034			A1R	0.212	56			A1R	0.412	402	
A2L	0.26	108	87		A2L	0.46	537	422		A2L	0.209	52	45		A2L	0.537	580	577
A2R	0.222	66			A2R	0.385	287			A2R	0.197	39			A2R	0.516	550	
A3L	0.2	41	48		A3L	0.406	357	340		A3L	0.215	59	52		A3L	0.442	445	428
A3R	0.212	54			A3R	0.391	307			A3R	0.203	45			A3R	0.407	395	
B1L	0.227	71	91		B1L	0.628	1097	974		B1L	0.22	65	67		B1L	0.506	536	486
B1R	0.263	111			B1R	0.541	807			B1R	0.225	70			B1R	0.422	416	
B2L	0.224	68	61		B2L	0.404	351	307		B2L	0.211	54	54		B2L	0.473	489	488
B2R	0.212	54			B2R	0.374	251			B2R	0.21	53			B2R	0.459	469	
B3L	0.222	66	74		B3L	0.887	1961	226		B3L	0.22	65	62		B3L	0.437	438	513
B3R	0.238	83			B3R	0.365	221			B3R	0.216	60			B3R	0.483	503	
C1L	0.212	54	52		C1L	0.364	217	447		C1L	0.218	62	59		C1L	0.394	376	386
C1R	0.208	50			C1R	0.496	657			C1R	0.212	56			C1R	0.397	380	
C2L	0.227	71	65		C2L	0.441	474	355		C2L	0.198	40	41		C2L	0.475	492	476
C2R	0.216	59			C2R	0.365	221			C2R	0.2	42			C2R	0.44	442	
C3L	0.223	67	63		C3L	0.315	54	273		C3L	0.22	65	67		C3L	0.433	432	455
C3R	0.216	59			C3R	0.379	267			C3R	0.225	70			C3R	0.443	446	
D1L	0.197	38	53		D1L	0.407	361	389		D1L	0.183	23	39		D1L	0.437	438	456
D1R	0.224	68			D1R	0.419	401			D1R	0.211	54			D1R	0.45	456	
D2L	0.239	84	66		D2L	0.527	761	457		D2L	0.2	42	52		D2L	0.449	455	518
D2R	0.205	47			D2R	0.433	447			D2R	0.218	62			D2R	0.486	508	
D3L	0.209	51	51		D3L	0.429	434	229		D3L	0.207	50	50		D3L	0.492	516	475
D3R	0.189	29			D3R	0.366	224			D3R	0.203	45			D3R	0.457	466	
E1L	0.195	36	44		E1L	0.443	481	491		E1L	0.2	42	39		E1L	0.423	418	426
E1R	0.203	44			E1R	0.925	2087			E1R	0.197	39			E1R	0.556	608	
E2L	0.145	-20	82		E2L	0.55	837	488		E2L	0.223	68	69		E2L	0.514	548	563
E2R	0.237	82			E2R	0.334	117			E2R	0.224	69			E2R	0.521	558	
E3L	0.206	48	48		E3L	0.384	284	376		E3L	0.186	26	39		E3L	0.45	456	509
E3R	0.207	49			E3R	0.409	367			E3R	0.208	51			E3R	0.48	499	

Appendix D: Experiment 1 paired t-test results

Paired t-test on TPC Experiment 1					
	J. effusus	***C. tectorum***	***J. effusus spiralis***	***Z. aethiopica***	***P. australis***
J. effusus		0.00649	0.75474	0.78077	0.38147
C. tectorum	0.00649		0.06423	0.31992	0.78550
J. effusus spiralis	0.75474	0.06423		0.69693	0.36221
Z. aethiopica	0.78077	0.31992	0.69693		0.48095
P. australis	0.38147	0.78550	0.36221	0.48095	

Paired t-test on COD Experiment 1					
	J. effusus	***C. tectorum***	***J. effusus spiralis***	***Z. aethiopica***	***P. australis***
J. effusus		0.84338	0.89383	0.68916	0.67494
C. tectorum	0.84338		0.95222	0.85447	0.81026
J. effusus spiralis	0.89383	0.95222		0.80651	0.76996
Z. aethiopica	0.68916	0.85447	0.80651		0.93324
P. australis	0.67494	0.81026	0.76996	0.93324	

To be statistically different the t value for the paired test must be less than 0.05 at a confidence level of 95%., numbers in red indicated that they were not statistically different.

Appendix E: Experiment 2 absorbance readings for TPC and COD

Experiment 2, D1, 08042019, TPC				Experiment 2, D1, 08042019, COD				Experiment 2, D2, 08052019, TPC				Experiment 2, D2, 08052019, COD			
Tray #	ABS	ppm	ave ppm	Tray #	ABS	ppm	ave ppm	Tray #	ABS	ppm	ave ppm	Tray #	ABS	ppm	ave ppm
A1L	0.283	35.3	29.3	A1L	0.42	329.2	329.2	A1L	0.267	19.3011	23.80135	A1L	0.264	123.6737	123.6737
A1R	0.271	23.3		A1R	0.42	329.2		A1R	0.276	28.30161		A1R	0.264	123.6737	
A2L	0.284	36.3	80.8	A2L	0.445	379.2	379.2	A2L	0.312	64.30365	73.30416	A2L	0.253	105.3393	105.3393
A2R	0.373	125.3		A2R	0.445	379.2		A2R	0.33	82.30468		A2R	0.253	105.3393	
A3L	0.408	160.3	190.8	A3L	0.513	515.2	515.2	A3L	0.267	19.3011	22.30127	A3L	0.354	273.6822	273.6822
A3R	0.469	221.3		A3R	0.513	515.2		A3R	0.273	25.30144		A3R	0.354	273.6822	
B1L	0.296	48.3	53.3	B1L	0.571	631.2	517.2	B1L	0.273	25.30144	30.30172	B1L	0.265	125.3405	125.3405
B1R	0.306	58.3		B1R	0.571	403.2		B1R	0.283	35.30201		B1R	0.265	125.3405	
B2L	0.317	69.3	67.3	B2L	0.457	591.2	497.2	B2L	0.283	35.30201	34.80198	B2L	0.219	48.66943	48.66943
B2R	0.313	65.3		B2R	0.457	403.2		B2R	0.282	34.30195		B2R	0.219	48.66943	
B3L	0.379	131.3	128.3	B3L	0.551	591.2	591.2	B3L	0.269	21.30121	22.8013	B3L	0.428	397.0226	397.0226
B3R	0.373	125.3		B3R	0.551	591.2		B3R	0.272	24.30138		B3R	0.428	397.0226	
C1L	0.296	48.3	50.8	C1L	0.513	515.2	515.2	C1L	0.271	23.30132	22.8013	C1L	0.289	165.3427	165.3427
C1R	0.301	53.3		C1R	0.513	515.2		C1R	0.27	22.30127		C1R	0.289	165.3427	
C2L	0.313	65.3	59.3	C2L	0.427	343.2	343.2	C2L	0.254	6.300358	6.300358	C2L	0.257	112.0064	112.0064
C2R	0.301	53.3		C2R	0.427	343.2		C2R	0.254	6.300358		C2R	0.257	112.0064	
C3L	0.377	129.3	128.3	C3L	0.452	393.2	393.2	C3L	0.333	85.30485	95.80544	C3L	0.419	382.0217	382.0217
C3R	0.375	127.3		C3R	0.452	393.2		C3R	0.354	106.306		C3R	0.419	382.0217	
E1L	0.296	48.3	41.8	E1L	0.526	541.2	541.2	E1L	0.253	5.300301	3.300188	E1L	0.243	88.6717	88.6717
E1R	0.283	35.3		E1R	0.526	541.2		E1R	0.249	1.300074		E1R	0.243	88.6717	
E2L	0.346	98.3	105.8	E2L	0.576	641.2	641.2	E2L	0.289	41.30235	44.30252	E2L	0.264	123.6737	123.6737
E2R	0.361	113.3		E2R	0.576	641.2		E2R	0.295	47.30269		E2R	0.264	123.6737	
E3L	0.538	290.3	286.8	E3L	0.518	525.2	525.2	E3L	0.361	113.3064	105.806	E3L	0.42	383.6885	383.6885
E3R	0.531	283.3		E3R	0.518	525.2		E3R	0.346	98.30559		E3R	0.42	383.6885	

Experiment 2, D3, 08062019, TPC					Experiment 2, D3, 08062019, COD					Experiment 2, D4, 08072019, TPC					Experiment 2, D4, 08072019, COD			
Tray #	ABS	ppm	ave ppm		Tray #	ABS	ppm	ave ppm		Tray #	ABS	ppm	ave ppm		Tray #	ABS	ppm	ave ppm
A1L	0.088	-105.762	-102.637		A1L	0.3	183.6875	182.8541		A1L	0.101	-89.5	-85.125		A1L	0.177	-21.3333	-14.6667
A1R	0.093	-99.5113			A1R	0.299	182.0207			A1R	0.108	-80.75			A1R	0.185	-8	
A2L	0.102	-88.26	-78.2589		A2L	0.302	187.0213	262.0298		A2L	0.127	-57	-58.875		A2L	0.326	227	228.6667
A2R	0.118	-68.2578			A2R	0.392	337.0383			A2R	0.124	-60.75			A2R	0.328	230.3333	
A3L	0.142	-38.2543	-34.5039		A3L	0.354	273.6978	241.1941		A3L	0.145	-34.5	-39.5		A3L	0.307	195.3333	193.6667
A3R	0.148	-30.7535			A3R	0.315	208.6904			A3R	0.137	-44.5			A3R	0.305	192	
B1L	0.099	-92.0105	-92.0105		B1L	0.354	273.6978	378.7097		B1L	0.121	-64.5	-52		B1L	0.264	123.6667	120.3333
B1R	0.099	-92.0105			B1R	0.48	483.7216			B1R	0.141	-39.5			B1R	0.26	117	
B2L	0.054	-148.267	-138.266		B2L	0.306	193.6887	229.5261		B2L	0.08	-115.75	-102.625		B2L	0.246	93.66667	99.5
B2R	0.07	-128.265			B2R	0.349	265.3635			B2R	0.101	-89.5			B2R	0.253	105.3333	
B3L	0.198	31.75361	39.25446		B3L	0.355	275.3646	244.5278		B3L	0.162	-13.25	-5.75		B3L	0.397	345.3333	350.3333
B3R	0.21	46.75531			B3R	0.318	213.6909			B3R	0.174	1.75			B3R	0.403	355.3333	
C1L	0.084	-110.763	-98.2612		C1L	0.312	203.6898	187.0213		C1L	0.153	-24.5	-11.375		C1L	0.304	190.3333	192.8333
C1R	0.104	-85.7597			C1R	0.292	170.3527			C1R	0.174	1.75			C1R	0.307	195.3333	
C2L	0.11	-78.2589	-84.5096		C2L	0.353	272.0309	261.1963		C2L	0.11	-78.25	-44.5		C2L	0.35	267	251.1667
C2R	0.1	-90.7603			C2R	0.34	250.3618			C2R	0.164	-10.75			C2R	0.331	235.3333	
C3L	0.13	-53.2561	-41.3797		C3L	0.323	222.0252	194.5221		C3L	0.111	-77	-62.625		C3L	0.318	213.6667	202
C3R	0.149	-29.5034			C3R	0.29	167.019			C3R	0.134	-48.25			C3R	0.304	190.3333	
E1L	0.087	-107.012	-98.8862		E1L	0.311	202.023	217.0247		E1L	0.111	-77	-76.375		E1L	0.236	77	78.66667
E1R	0.1	-90.7603			E1R	0.329	232.0264			E1R	0.112	-75.75			E1R	0.238	80.33333	
E2L	0.115	-72.0082	-62.6321		E2L	0.334	240.3606	234.5266		E2L	0.114	-73.25	-64.5		E2L	0.233	72	71.16667
E2R	0.13	-53.2561			E2R	0.327	228.6927			E2R	0.128	-55.75			E2R	0.232	70.33333	
E3L	0.25	96.76099	83.6345		E3L	0.401	352.04	333.7046		E3L	0.223	63	83.625		E3L	0.397	345.3333	357
E3R	0.229	70.50801			E3R	0.379	315.3692			E3R	0.256	104.25			E3R	0.411	368.6667	

Experiment 2, D6, 14042019, TPC					Experiment 2, D6, 14042019, COD			
Tray #	ABS	ppm	ave ppm		Tray #	ABS	ppm	ave ppm
A1L	0.104	-85.75	-75.75		A1L	0.186	-6.33333	2.833333
A1R	0.12	-65.75			A1R	0.197	12	
A2L	0.114	-73.25	-78.25		A2L	0.347	262	235.3333
A2R	0.106	-83.25			A2R	0.315	208.6667	
A3L	0.143	-37	-40.75		A3L	0.287	162	177
A3R	0.137	-44.5			A3R	0.305	192	
B1L	0.132	-50.75	-46.375		B1L	0.274	140.3333	117
B1R	0.139	-42			B1R	0.246	93.66667	
B2L	0.102	-88.25	-84.5		B2L	0.217	45.33333	75.33333
B2R	0.108	-80.75			B2R	0.253	105.3333	
B3L	0.155	-22	-16.375		B3L	0.417	378.6667	363.6667
B3R	0.164	-10.75			B3R	0.399	348.6667	
C1L	0.164	-10.75	-18.25		C1L	0.285	158.6667	185.3333
C1R	0.152	-25.75			C1R	0.317	212	
C2L	0.156	-20.75	-15.75		C2L	0.321	218.6667	207
C2R	0.164	-10.75			C2R	0.307	195.3333	
C3L	0.109	-79.5	-58.875		C3L	0.318	213.6667	236.1667
C3R	0.142	-38.25			C3R	0.345	258.6667	
E1L	0.104	-85.75	-79.5		E1L	0.216	43.66667	52.83333
E1R	0.114	-73.25			E1R	0.227	62	
E2L	0.124	-60.75	-58.875		E2L	0.246	93.66667	100.3333
E2R	0.127	-57			E2R	0.254	107	
E3L	0.187	18	16.125		E3L	0.386	327	294.5
E3R	0.184	14.25			E3R	0.347	262	

Appendix F: Statistics and inspection for CW set-up based on batch data

	COD % Removal					TPC % Remediation				
% Removed	Day 1	Day 2	Day 3	Day 4	Day 7	Day 1	Day 2	Day 3	Day 4	Day 7
J. effusus Full Strength	58	78	80	84	86	59	95	107	108	109
J. effusus spiralis Full Strength	52	68	80	72	71	73	95	92	101	103
Z. aethiopica Full Strength	68	69	84	84	81	73	80	109	113	113
P. australis Full Strength	57	69	73	71	76	39	77	82	82	97
J. effusus Dilute Strength	4	73	34	42	40	50	55	148	136	148
J. effusus spiralis Dilute Strength	-26	88	42	75	81	59	79	185	163	152
Z. aethiopica Dilute Strength	13	72	34	36	48	63	96	152	127	110
P. australis Dilute Strength	-62	69	41	82	75	35	73	139	140	136

Red – Lowest % and Statistically Different

Orange – Middle % and Statistically Different

Green – Highest % and Statistically Different

Optimal Wetland System Looking at all data

1. ***Zantedeschia aethiopica***
2. ***Juncus effusus***
3. ***Zantedeschia aethiopica***
4. ***Juncus effusus***

Appendix G: Experiment 3 absorbance readings for TPC and COD

Experiment 3, D1, 23072019, TPC		
CW #	**ABS**	**ppm**
CW A in	0.345	411
CW A out	0.159	158
CW B in	0.283	404
CW B out	0.053	327
CW C in	0.288	492
CW C out	0.111	418

Experiment 3, D1, 23072019, COD		
CW #	**ABS**	**ppm**
CW A in	0.356	350
CW A out	0.279	117
CW B in	0.435	215
CW B out	0.288	68
CW C in	0.57	136
CW C out	0.337	59

Experiment 3, D2, 24072019, TPC		
CW #	**ABS**	**ppm**
CW A in	0.71	390
CW A out	0.379	154
CW B in	0.653	433
CW B out	0.363	143
CW C in	0.61	490
CW C out	0.374	159

Experiment 3, D2, 24072019, COD		
CW #	**ABS**	**ppm**
CW A in	0.608	475
CW A out	0.309	194
CW B in	0.599	379
CW B out	0.362	142
CW C in	0.695	409
CW C out	0.414	61

Experiment 3, D3, 25072019, TPC		
CW #	**ABS**	**ppm**
CW A in	0.63	357
CW A out	0.372	98
CW B in	0.531	311
CW B out	0.362	142
CW C in	0.58	408
CW C out	0.318	152

Experiment 3, D3, 25072019, COD		
CW #	**ABS**	**ppm**
CW A in	0.629	479
CW A out	0.281	129
CW B in	0.551	331
CW B out	0.355	135
CW C in	0.699	409
CW C out	0.349	61

Experiment 3, D4, 26072019, TPC		
CW #	**ABS**	**ppm**
CW A in	0.68	422
CW A out	0.378	73
CW B in	0.645	397
CW B out	0.322	74
CW C in	0.67	432
CW C out	0.321	130

Experiment 3, D4, 26072019, COD		
CW #	**ABS**	**ppm**
CW A in	0.654	502
CW A out	0.425	54
CW B in	0.624	404
CW B out	0.413	193
CW C in	0.722	434
CW C out	0.274	205

Experiment 3, D6, 28072019, TPC		
CW #	**ABS**	**ppm**
CW A in	0.674	434
CW A out	0.341	110
CW B in	0.632	384
CW B out	0.35	102
CW C in	0.682	426
CW C out	0.358	93

Experiment 3, D6, 28072019, COD		
CW #	**ABS**	**ppm**
CW A in	0.506	306
CW A out	0.295	79
CW B in	0.596	376
CW B out	0.301	81
CW C in	0.526	286
CW C out	0.299	75

Appendix H: Experiment 3 paired t-test results

COD Paired T-Tests			
	CW A	CW B	CW C
CW A		0.720864	0.740922
CW B	0.720864		0.176881
CW C	0.740922	0.176881	
TPC Paired T-Tests			
	CW A	CW B	CW C
CW A		0.462479	0.284593
CW B	0.462479		0.264652
CW C	0.284593	0.264652	

Appendix I: Experiment 3 absorbance readings for TPC and COD for gradient profiles

Experiment 3, D1, 23072019, TPC				Experiment 3, D1, 23072019, COD				Experiment 3, D2, 24072019, TPC				Experiment 3, D2, 24072019, COD		
CW #	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**
CW A In	0.288	492		CW A In	0.57	136		CW A In	0.653	433		CW A In	0.695	409
CW A Out	0.111	418		CW A Out	0.337	59		CW A Out	0.363	143		CW A Out	0.414	61
CW A Surf	0.043	61		CW A Surf	0.224	44		CW A Surf	0.507	287		CW A Surf	0.385	238
CW A Mid	0.016	22		CW A Mid	0.265	95		CW A Mid	0.468	248		CW A Mid	0.4	122
CW A Bot	0.129	184		CW A Bot	0.308	99		CW A Bot	0.498	278		CW A Bot	0.468	224
CW B In	0.283	404		CW B In	0.435	215		CW B In	0.71	390		CW B In	0.599	379
CW B Out	0.053	327		CW B Out	0.288	68		CW B Out	0.379	154		CW B Out	0.362	142
CW B Surf	0.229	117		CW B Surf	0.255	35		CW B Surf	0.554	334		CW B Surf	0.35	130
CW B Mid	0.082	41		CW B Mid	0.22	0		CW B Mid	0.529	309		CW B Mid	0.371	151
CW B Bot	0.029	75		CW B Bot	0.225	5		CW B Bot	0.478	258		CW B Bot	0.434	214
CW C In	0.345	411		CW C In	0.356	350		CW C In	0.61	490		CW C In	0.608	475
CW C Out	0.159	158		CW C Out	0.279	117		CW C Out	0.374	159		CW C Out	0.309	194
CW C Surf	0.293	204		CW C Surf	0.224	4		CW C Surf	0.442	222		CW C Surf	0.385	165
CW C Mid	0.143	35		CW C Mid	0.265	45		CW C Mid	0.622	402		CW C Mid	0.4	180
CW C Bot	0.025	227		CW C Bot	0.308	88		CW C Bot	0.668	448		CW C Bot	0.468	248

Experiment 3, D3, 25072019, TPC				Experiment 3, D3, 25072019, COD				Experiment 3, D4, 26072019, TPC				Experiment 3, D4, 26072019, COD		
CW #	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**
CW A In	0.58	408		CW A In	0.699	409		CW A In	0.67	432		CW A In	0.722	434
CW A Out	0.318	152		CW A Out	0.349	61		CW A Out	0.321	130		CW A Out	0.274	205
CW A Surf	0.359	139		CW A Surf	0.391	171		CW A Surf	0.312	64		CW A Surf	0.311	91
CW A Mid	0.311	91		CW A Mid	0.46	240		CW A Mid	0.321	73		CW A Mid	0.273	53
CW A Bot	0.352	132		CW A Bot	0.469	249		CW A Bot	0.324	76		CW A Bot	0.231	11
CW B In	0.531	311		CW B In	0.551	331		CW B In	0.645	397		CW B In	0.624	404
CW B Out	0.362	142		CW B Out	0.355	135		CW B Out	0.322	74		CW B Out	0.413	193
CW B Surf	0.376	156		CW B Surf	0.371	151		CW B Surf	0.365	117		CW B Surf	0.461	241
CW B Mid	0.327	107		CW B Mid	0.467	247		CW B Mid	0.326	78		CW B Mid	0.493	273
CW B Bot	0.459	239		CW B Bot	0.524	304		CW B Bot	0.357	109		CW B Bot	0.491	271
CW C In	0.63	357		CW C In	0.629	479		CW C In	0.68	422		CW C In	0.654	502
CW C Out	0.372	98		CW C Out	0.281	129		CW C Out	0.378	73		CW C Out	0.425	54
CW C Surf	0.470	250		CW C Surf	0.462	242		CW C Surf	0.381	133		CW C Surf	0.309	89
CW C Mid	0.417	197		CW C Mid	0.584	264		CW C Mid	0.324	76		CW C Mid	0.348	128
CW C Bot	0.434	214		CW C Bot	0.464	244		CW C Bot	0.346	98		CW C Bot	0.41	190

Experiment 3, D6, 28072019, TPC				Experiment 3, D6, 28072019, COD		
CW #	**ABS**	**ppm**		**CW #**	**ABS**	**ppm**
CW A In	0.674	434		CW A In	0.506	306
CW A Out	0.341	110		CW A Out	0.295	79
CW A Surf	0.285	37		CW A Surf	0.516	296
CW A Mid	0.294	46		CW A Mid	0.498	278
CW A Bot	0.407	159		CW A Bot	0.593	373
CW B In	0.632	384		CW B In	0.596	376
CW B Out	0.35	102		CW B Out	0.301	81
CW B Surf	0.363	115		CW B Surf	0.552	332
CW B Mid	0.435	187		CW B Mid	0.431	211
CW B Bot	0.418	170		CW B Bot	0.574	354
CW C In	0.682	426		CW C In	0.526	286
CW C Out	0.358	93		CW C Out	0.299	75
CW C Surf	0.38	132		CW C Surf	0.38	160
CW C Mid	0.325	77		CW C Mid	0.433	213
CW C Bot	0.364	116		CW C Bot	0.444	224

Appendix J: Experiment 4 absorbance readings for TOC and COD

Experiment 4, D1, 31072019, TPC		
CW Area	**ABS**	**ppm**
In	0.442	532
Out	0.419	475
Surface	0.37	352
Middle	0.384	387
Bottom	0.407	445

Experiment 4, D1, 31072019, COD		
CW Area	**ABS**	**ppm**
In	0.8	580
Out	0.494	274
Surface	0.654	434
Middle	0.809	589
Bottom	0.646	426

Experiment 4, D2, 01082019, TPC		
CW Area	**ABS**	**ppm**
In	0.408	447
Out	0.305	190
Surface	0.35	302
Middle	0.319	225
Bottom	0.332	257

Experiment 4, D2, 01082019, COD		
CW Area	**ABS**	**ppm**
In	0.788	568
Out	0.568	348
Surface	0.625	405
Middle	0.604	384
Bottom	0.643	423

Experiment 4, D3, 02082019, TPC		
CW Area	**ABS**	**ppm**
In	0.414	462
Out	0.294	162
Surface	0.373	360
Middle	0.356	317
Bottom	0.433	510

Experiment 4, D3, 02082019, COD		
CW Area	**ABS**	**ppm**
In	0.837	617
Out	0.602	382
Surface	0.622	402
Middle	0.522	302
Bottom	0.675	455

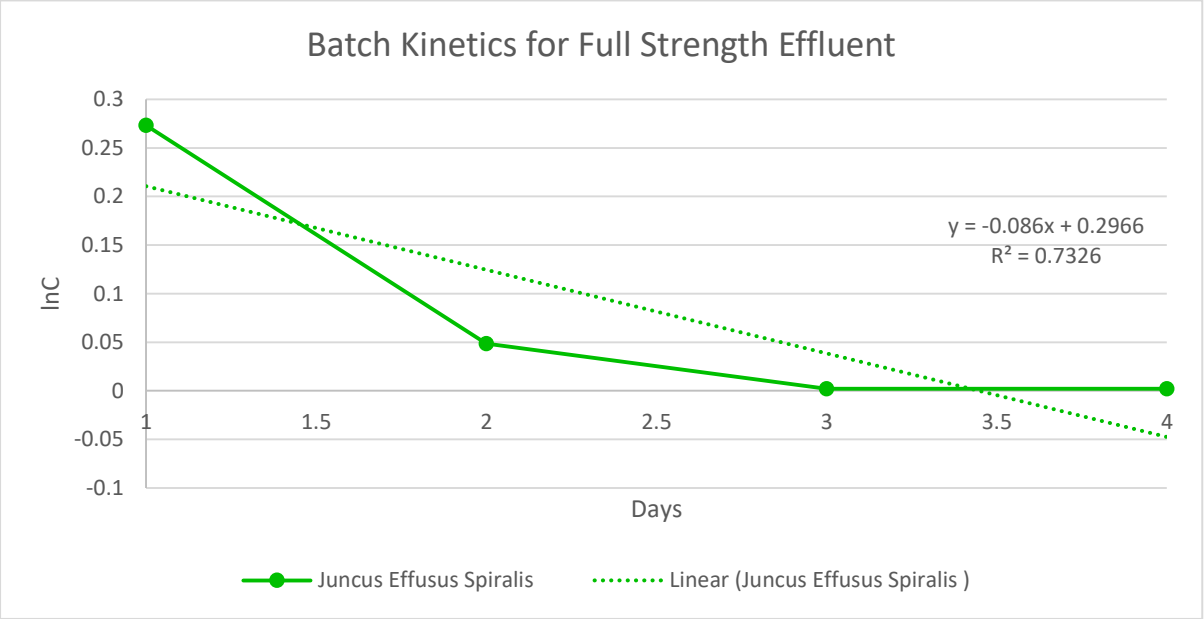
Experiment 4, D4, 03082019, TPC		
CW Area	**ABS**	**ppm**
In	0.412	457
Out	0.302	182
Surface	0.367	345
Middle	0.306	192
Bottom	0.374	362

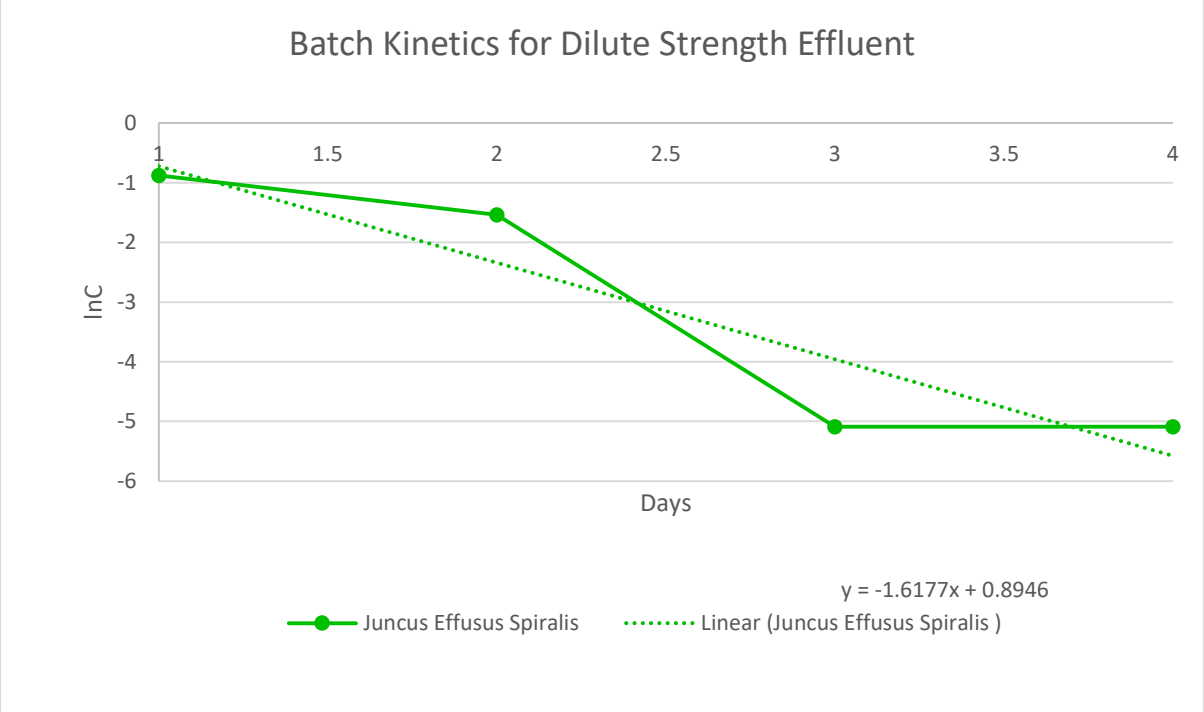
Experiment 4, D4, 03082019, COD		
CW Area	**ABS**	**ppm**
In	0.742	522
Out	0.653	433
Surface	0.749	529
Middle	0.68	460
Bottom	0.747	527

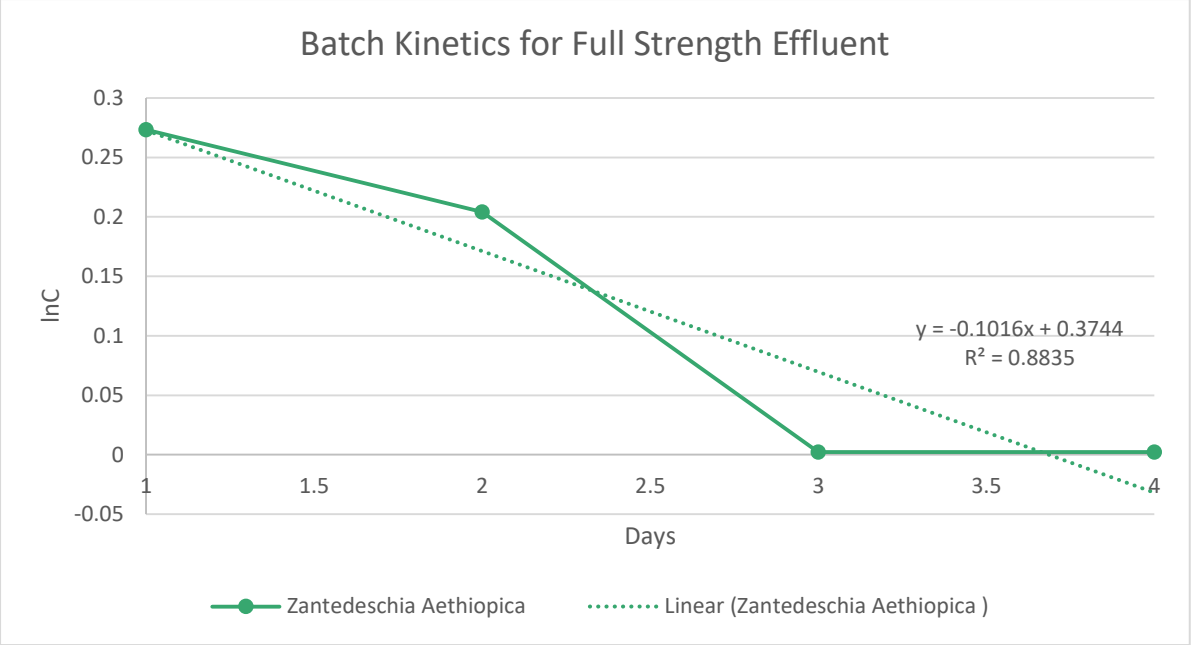
Experiment 4, D6, 05082019, TPC		
CW Area	**ABS**	**ppm**
In	0.422	482
Out	0.3	177
Surface	0.347	295
Middle	0.384	387
Bottom	0.354	312

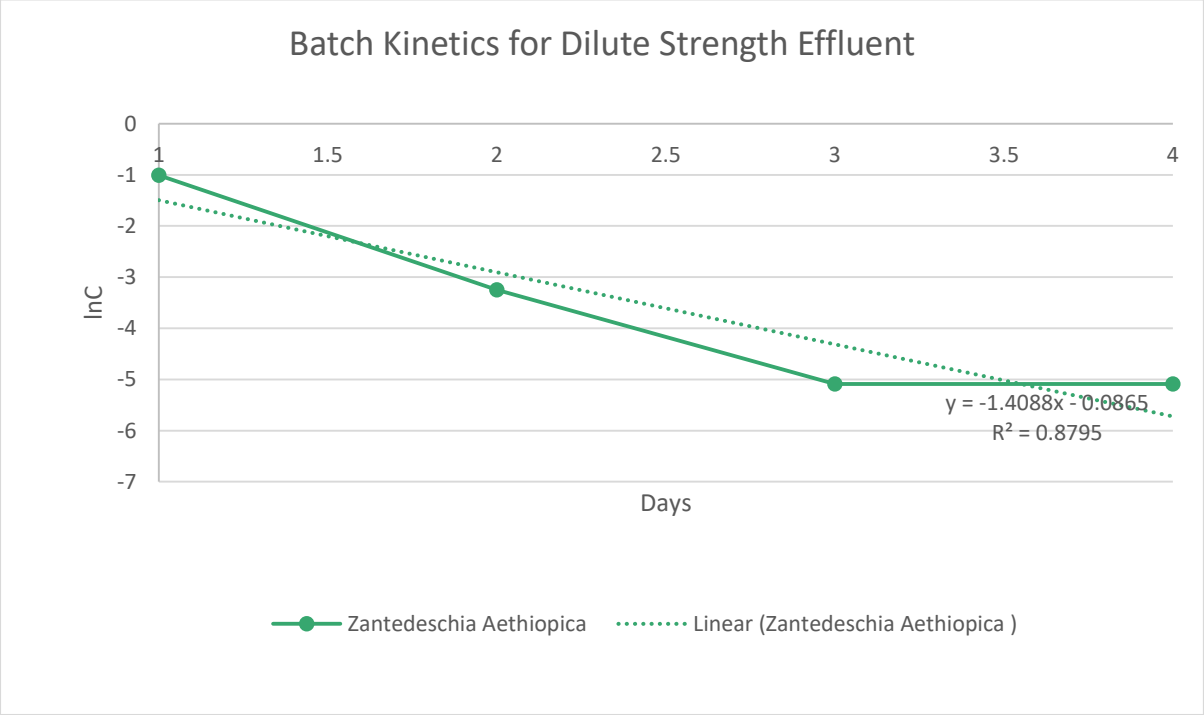
Experiment 4, D6, 05082019, COD		
CW Area	**ABS**	**ppm**
In	0.734	514
Out	0.559	339
Surface	0.741	521
Middle	0.678	458
Bottom	0.74	520

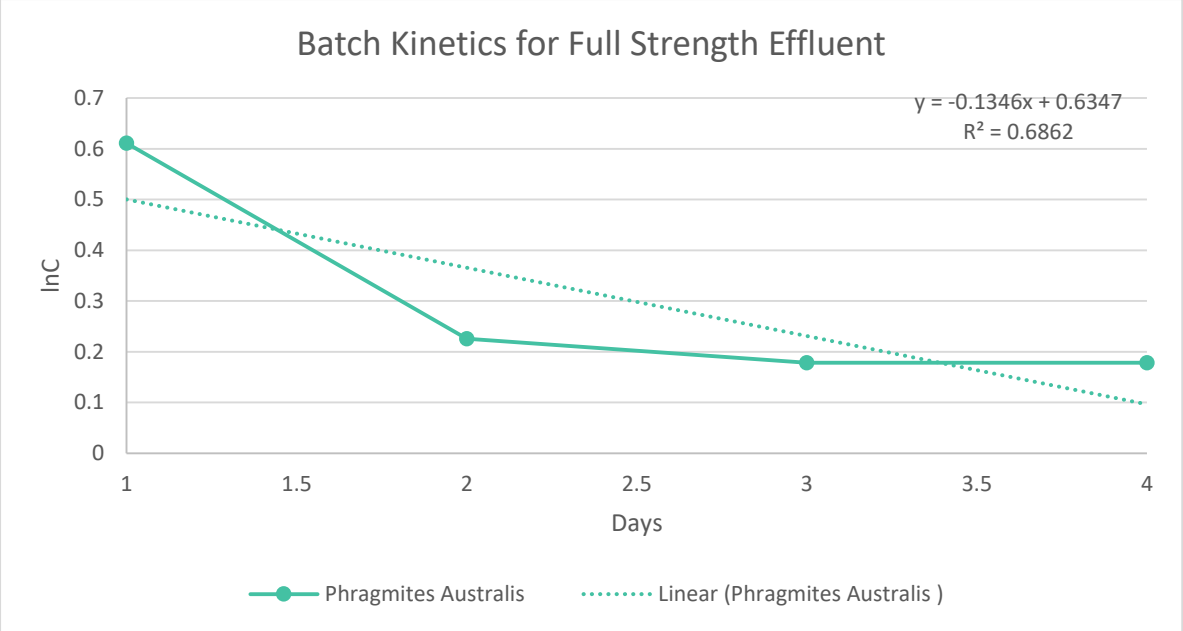
Appendix K: Approximate batch reaction kinetics for different macrophyte species and different effluent strengths

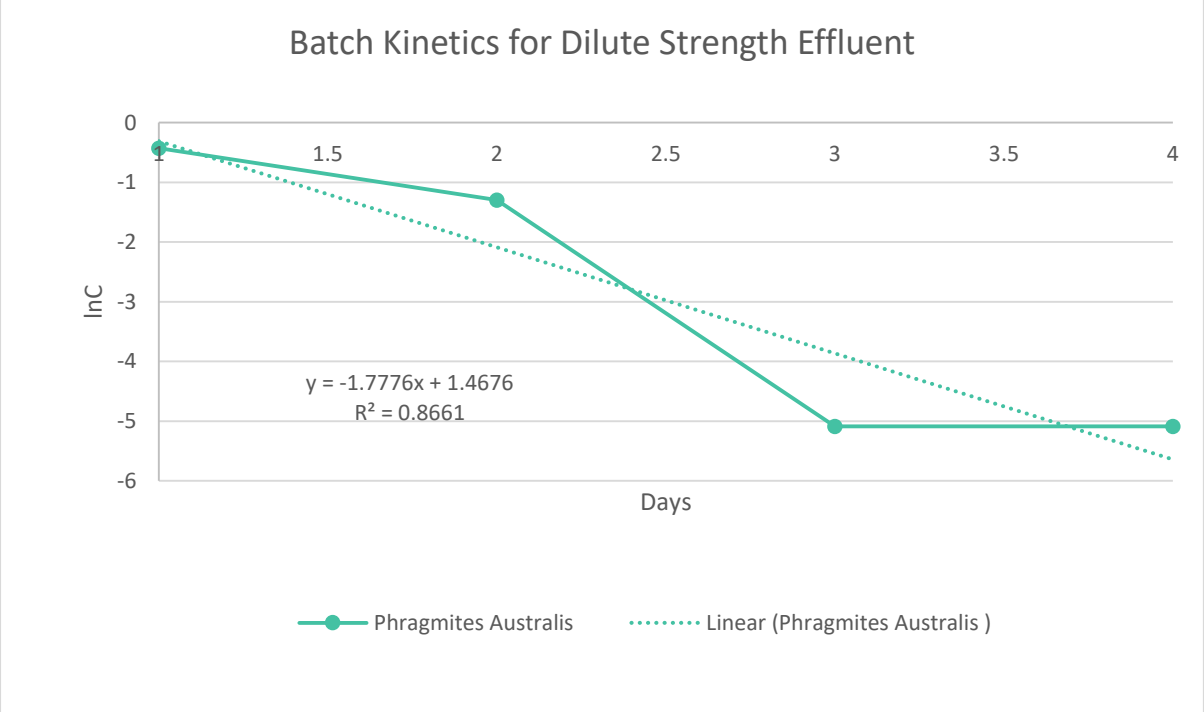





Batch Kinetics for Full Strength Effluent
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
lnC
1
1.5
2
2.5
3
3.5
4
Days
y = -0.1016x + 0.3744
R² = 0.8835
Zantedeschia Aethiopica
Linear (Zantedeschia Aethiopica)


Batch Kinetics for Dilute Strength Effluent
0
-1
-2
-3
-4
-5
-6
-7
lnC
1
1.5
2
2.5
3
3.5
4
Days
y = -1.4088x - 0.0865
R² = 0.8795
Zantedeschia Aethiopica
Linear (Zantedeschia Aethiopica)


Batch Kinetics for Full Strength Effluent
y = -0.1346x + 0.6347
R² = 0.6862
lnC
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
1.5
2
2.5
3
3.5
4
Days
Phragmites Australis
Linear (Phragmites Australis)


Batch Kinetics for Dilute Strength Effluent
lnC
0
-1
-2
-3
-4
-5
-6
1
1.5
2
2.5
3
3.5
4
y = -1.7776x + 1.4676
R² = 0.8661
Days
Phragmites Australis
Linear (Phragmites Australis)

Appendix L: Tracer studies on the laboratory-scale CWs done previously

Calculating mean residence time and variance

Sub totals	Total	t	C(t)	Integration co-effs	Co-effs* C(t)	E(t)	t*E(t)	Co-effs*t*E(t)	Co-effs*E(t)	(t-tm)^2	t^2*E(t)	Co-effs*t^2*E(t)	(t-tm)^2*E(t)
0	0	0.5	0.244007	1	0.244006654	4.52696E-05	2.26348E-05	2.26348E-05	4.52696E-05	62.57668	1.13174E-05	1.13174E-05	0.002832823
1	1	1	0.17152	4	0.686079875	3.18214E-05	3.18214E-05	0.000127286	0.000127286	54.91614	3.18214E-05	0.000127286	0.001747511
2	2	1.5	2.495075	2	4.990149761	0.000462902	0.000694353	0.001388705	0.000925804	47.75559	0.001041529	0.002083058	0.022106148
3	3	1.833333	11.6516	4	46.606419	0.002161678	0.003963076	0.015852305	0.008646712	43.25968	0.00726564	0.029062559	0.093513489
4	4	2	25.48449	2	50.96898401	0.004728041	0.009456082	0.018912163	0.009456082	41.09505	0.018912163	0.037824327	0.194299087
5	5	2.166667	37.93299	4	151.7319532	0.007037563	0.015248053	0.060992213	0.028150252	38.98598	0.033037449	0.132149795	0.27436631
6	6	2.333333	55.72239	2	111.4447839	0.010337963	0.024121915	0.048243829	0.020675927	36.93247	0.056284468	0.112568935	0.381806509
7	7	2.5	88.81875	4	355.2750018	0.016478205	0.041195512	0.164782049	0.06591282	34.93451	0.102988781	0.411955123	0.575658016
8	8	2.666667	124.9042	2	249.8083692	0.023172998	0.061794662	0.123589324	0.046345997	32.99211	0.164785765	0.329571531	0.76452604
9	9	2.833333	149.7293	4	598.9173468	0.027778714	0.078706357	0.314825429	0.111114857	31.10526	0.223001345	0.892005382	0.864064121
10	10	3	173.2677	2	346.535442	0.032145701	0.096437104	0.192874207	0.064291402	29.27397	0.289311311	0.578622622	0.941032221
11	11	3.166667	212.715	4	850.8600919	0.039464209	0.124969995	0.499879982	0.157856836	27.49823	0.395738319	1.582953276	1.085195956
12	12	3.333333	246.9258	2	493.8516718	0.045811211	0.152704037	0.305408074	0.091622422	25.77805	0.509013457	1.018026913	1.180923721
13	13	3.5	292.5924	4	1170.36951	0.054283551	0.189992428	0.759969712	0.217134203	24.11343	0.664973498	2.659893992	1.308962359
14	14	3.666667	333.4853	2	666.9706596	0.061870265	0.226857637	0.453715275	0.123740529	22.50436	0.831811337	1.663622674	1.392350453
15	15	3.833333	357.3939	4	1429.575628	0.066305932	0.25417274	1.016690959	0.265223729	20.95084	0.974328836	3.897315344	1.389165091
16	16	4	371.9664	2	743.9327584	0.069009507	0.27603803	0.55207606	0.138019015	19.45288	1.104152119	2.208304239	1.342433889
17	17	4.083333	363.8984	4	1455.593746	0.067512693	0.275676829	1.102707315	0.270050771	18.72474	1.125680384	4.502721536	1.264157434
18	18	4.166667	364.4673	2	728.9346848	0.06761824	0.281742665	0.563485331	0.135236479	18.01048	1.173927773	2.347855545	1.217836969
19	19	4.25	356.2839	4	1425.135682	0.066100001	0.280925002	1.123700009	0.264400002	17.31011	1.19393126	4.77572504	1.144198417
20	20	4.333333	341.2286	2	682.4572121	0.063306845	0.274329663	0.548659326	0.126613691	16.62363	1.188761873	2.377523747	1.05238975
21	21	4.416667	327.2195	4	1308.87793	0.060707786	0.268126055	1.072504218	0.242831144	15.95104	1.184223408	4.736893631	0.968352468
22	22	4.5	314.3366	2	628.673288	0.058317682	0.262429569	0.524859137	0.116635364	15.29234	1.180933059	2.361866117	0.891813874
23	23	4.583333	299.9822	4	1199.928891	0.055654561	0.255083403	1.020333613	0.222618243	14.64753	1.169132265	4.67652906	0.815201754
24	24	4.666667	297.9216	2	595.8432064	0.055272262	0.257937222	0.515874444	0.110544524	14.0166	1.203707035	2.407414071	0.77472944
25	25	4.75	285.7979	4	1143.191735	0.053023004	0.251859267	1.007437067	0.212092014	13.39957	1.196331517	4.78532607	0.710485436
26	26	4.833333	277.4915	2	554.9830147	0.051481944	0.248829395	0.497658791	0.102963888	12.79642	1.202675411	2.405350821	0.658784774
27	27	4.916667	271.5179	4	1086.071764	0.050373691	0.247670646	0.990682586	0.201494763	12.20717	1.217714012	4.870856047	0.614920046
28	28	5	264.1787	2	528.3573163	0.049012062	0.245060308	0.490120616	0.098024123	11.6318	1.22530154	2.450603079	0.570098431
29	29	5.083333	260.5599	4	1042.239788	0.048340696	0.245731871	0.982927483	0.193362784	11.07032	1.24913701	4.996548039	0.535146942
30	30	5.166667	261.6421	2	523.2841677	0.048541461	0.250797546	0.501595093	0.097082921	10.52273	1.295787323	2.591574646	0.510788635
31	31	5.25	259.1217	4	1036.486699	0.048073859	0.252387758	1.009551032	0.192295435	9.989028	1.32503573	5.300142919	0.480211096
32	32	5.333333	251.3023	2	502.6045553	0.046623156	0.24865683	0.497313661	0.093246311	9.469215	1.326169762	2.652339523	0.441484681
33	33	5.416667	244.6684	4	978.6737474	0.045392404	0.245875524	0.983502096	0.181569618	8.963291	1.331825755	5.327303021	0.406865339
34	34	5.5	249.079	2	498.1580288	0.046210682	0.254158752	0.508317503	0.092421364	8.471256	1.397873134	2.795746267	0.391462535
35	35	5.583333	238.5945	4	954.3778128	0.044265521	0.247149161	0.988596644	0.177062085	7.99311	1.379916148	5.519664593	0.3538192
36	36	5.666667	238.7651	2	477.5302917	0.044297189	0.251017406	0.502034812	0.088594379	7.528853	1.422431968	2.844863936	0.333507043
37	37	5.75	235.9858	4	943.9433147	0.043781553	0.251743931	1.006975723	0.175126213	7.078485	1.447527602	5.790110406	0.309907077
38	38	5.833333	230.4257	2	460.851409	0.042750004	0.249375026	0.498750052	0.085500009	6.642006	1.454687652	2.909375305	0.283945784
39	39	5.916667	209.7782	4	839.1127511	0.038919349	0.230272816	0.921091265	0.155677397	6.219416	1.362447497	5.449789986	0.242055607

$$\int_0^{\infty} C(t)\,dt = \int_0^{4} C(t)\,dt + \int_4^{12} C(t)\,dt$$

$\int_0^4 C(t)\,dt$	h1 0.1171875
	Area under C(t) curve 1 1486.2334
$\int_4^{12} C(t)\,dt$	h2 3.285714286
	Area under C(t) curve 2 3903.840994
	Total Area under C(t) curve 5390.074395

$$t_m = \int_0^{\infty} tE(t)\,dt = \int_0^{4} tE(t)\,dt + \int_4^{\infty} tE(t)dt$$

$\int_0^4 tE(t)\,dt$	h1 0.1171875
	Area under t*E(t) curve 1 1.430422979
$\int_4^{\infty} tE(t)\,dt$	h2 3.285714286
	Area under t*E(t) curve 2 6.980119301
	tm 8.41054228

$$\int_0^{\infty} t^2E(t)dt = \int_0^{4} t^2E(t)\,dt + \int_4^{100} t^2E(t)\,dt =$$

$\int_0^{10} t^2E(t)\,dt$	h 0.1171875
	Area under t^2E(t) curve 1 7.920389765
$\int_4^{100} t^2E(t)dt$	h 3.285714286
	Area under t^2E(t) curve 2 71.1719161
	Total area under t^2*E(t) cur 79.09230586
	variance 8.355084419
	standard deviation 2.890516289
	h1 0.1171875
	Area under E(t) curve 1 0.275735229
	h2 3.285714286
	Area under E(t) curve 2 0.724264771
	Total area under E(t) curve 1
normalised std	
	0.118114399
	71.67939132 tanks

40	40	6	205.857	2	411.713929	0.03819186	0.229151158	0.458302315	0.076383719	5.810714	1.374906945	2.749813891	0.221921976
41	41	6.083333	207.5484	4	830.1937268	0.038505671	0.234242832	0.936971329	0.154022684	5.415901	1.42497723	5.699908921	0.208542921
42	42	6.166667	205.3297	2	410.6593592	0.038094034	0.234913212	0.469826425	0.076188069	5.034978	1.448631476	2.897262953	0.191802616
43	43	6.25	199.6737	4	798.6949102	0.037044707	0.23152942	0.926117679	0.148178829	4.667943	1.447058874	5.788235495	0.172922579
44	44	6.333333	193.7784	2	387.5568953	0.035950978	0.227689529	0.455379059	0.071901957	4.314797	1.442033686	2.884067371	0.155121174
45	45	6.416667	188.5158	4	754.0631181	0.034974616	0.22442045	0.897681798	0.139898462	3.97554	1.440031218	5.760124871	0.139042982
46	46	6.5	178.0148	2	356.0295506	0.033026404	0.214671627	0.429343254	0.066052808	3.650172	1.395365575	2.79073115	0.120552049
47	47	6.583333	172.4319	4	689.7277682	0.031990642	0.210605062	0.842420248	0.127962569	3.338693	1.386483325	5.545933299	0.106806919
48	48	6.666667	169.3471	2	338.6942604	0.031418329	0.209455526	0.418911052	0.062836658	3.041102	1.396370174	2.792740348	0.095546348
49	49	6.75	162.898	4	651.5918959	0.030221841	0.20399743	0.81598972	0.120887366	2.757401	1.376982653	5.507930612	0.083333726
50	50	6.833333	158.5335	2	317.0669061	0.029412109	0.200982742	0.401965483	0.058824217	2.487588	1.373382067	2.746764135	0.07316521
51	51	6.916667	153.4027	4	613.6106051	0.02846021	0.196849789	0.787399154	0.113840842	2.231664	1.361544371	5.446177482	0.063513637
52	52	7	146.7351	2	293.4701584	0.027223201	0.190562408	0.381124815	0.054446402	1.98963	1.333936854	2.667873708	0.054164085
53	53	7.083333	141.26	4	565.0399027	0.026207426	0.185635934	0.742543736	0.104829704	1.761484	1.314921199	5.259684796	0.046163951
54	54	7.166667	135.4315	2	270.8629497	0.025126086	0.180070286	0.360140571	0.050252173	1.547227	1.290503714	2.581007429	0.038875748
55	55	7.25	132.3827	4	529.5307234	0.024560455	0.1780633	0.712253202	0.098241821	1.346858	1.290958928	5.163835712	0.033079455
56	56	7.333333	132.2919	2	264.5838886	0.024543621	0.179986556	0.359973111	0.049087242	1.160379	1.319901408	2.639802816	0.028479905
57	57	7.416667	131.8385	4	527.3539864	0.024459495	0.18140792	0.725631679	0.097837979	0.987789	1.345442071	5.381768286	0.024160813
58	58	7.5	127.7749	2	255.5498917	0.0237056	0.177791998	0.355583995	0.047411199	0.829087	1.333439982	2.666879965	0.01965401
59	59	7.583333	123.3408	4	493.3630689	0.022882943	0.173528987	0.694115949	0.091531774	0.684275	1.315928154	5.263712616	0.015658218
60	60	7.666667	117.75	2	235.4999687	0.021845707	0.167483751	0.334967503	0.043691413	0.553351	1.284042094	2.568084189	0.012088342
61	61	7.75	112.7411	4	450.9645122	0.020916433	0.162102353	0.648409412	0.083665731	0.436316	1.256293236	5.025172944	0.009126176
62	62	7.833333	109.2969	2	218.5938828	0.020277446	0.158839992	0.317679984	0.040554892	0.33317	1.244246605	2.48849321	0.00675584
63	63	7.916667	103.762	4	415.0479551	0.019250567	0.152400323	0.609601291	0.077002268	0.243913	1.206502556	4.826010222	0.004695466
64	64	8	100.0313	1	100.0313399	0.018558434	0.148467472	0.148467472	0.018558434	0.168545	1.187739776	1.187739776	0.003127931
0	64	8	100.0313	1	100.0313399	0.018558434	0.148467472	0.148467472	0.018558434	0.168545	1.187739776	1.187739776	0.003127931
1	65	8.166667	93.82623	4	375.3049159	0.017407223	0.142158991	0.568635963	0.069628893	0.059475	1.160965092	4.643860368	0.0010353
2	66	8.333333	88.52557	2	177.0511324	0.016423812	0.136865096	0.273730193	0.032847623	0.005961	1.14054247	2.28108494	9.7906E-05
3	67	8.5	85.01907	4	340.0762948	0.015773265	0.134072756	0.536291022	0.063093061	0.008003	1.139618422	4.558473687	0.000126228
4	68	8.666667	82.07193	2	164.1438518	0.015226492	0.131962933	0.263925865	0.030452984	0.0656	1.14367875	2.2873575	0.000998853
5	69	8.833333	75.40334	4	301.6133742	0.013989296	0.12357211	0.494288441	0.055957182	0.178752	1.091553642	4.366214567	0.002500618
6	70	9	70.26905	2	140.5381065	0.01303675	0.117330752	0.234661503	0.0260735	0.34746	1.055976764	2.111953527	0.004529754
7	71	9.166667	65.62189	4	262.4875682	0.01217458	0.111600317	0.446401267	0.04869832	0.571724	1.023002905	4.092011619	0.006960501
8	72	9.333333	61.49043	2	122.9808598	0.011408086	0.106475465	0.212950931	0.022816171	0.851543	0.99377101	1.987542019	0.009714479
9	73	9.5	56.51469	4	226.0587605	0.010484955	0.099607077	0.398428309	0.041939822	1.186918	0.946267233	3.785068933	0.012444784
10	74	9.666667	53.94353	2	107.887052	0.010007937	0.096743393	0.193486786	0.020015874	1.577848	0.935186131	1.870372261	0.015791008
11	75	9.833333	52.3667	4	209.4668173	0.009715395	0.095534722	0.382138888	0.038861582	2.024334	0.939424767	3.757699068	0.019667209
12	76	10	51.14749	2	102.2949792	0.009489199	0.094891992	0.189783984	0.018978398	2.526376	0.948919919	1.897839839	0.023973284
13	77	10.25	46.02218	4	184.0887243	0.00853832	0.087517782	0.350071128	0.034153281	3.383605	0.897057266	3.588229063	0.0288903
14	78	10.5	41.40334	2	82.80668906	0.007681405	0.080654753	0.161309505	0.01536281	4.365834	0.846874904	1.693749808	0.033535736
15	79	10.75	41.44201	4	165.7680557	0.007688579	0.082652226	0.330608906	0.030754317	5.473062	0.888511435	3.554045739	0.042080074
16	80	11	38.27915	2	76.5582964	0.007101785	0.078119632	0.156239265	0.01420357	6.705291	0.859315956	1.718631912	0.047619535
17	81	11.25	34.52021	4	138.0808523	0.006404404	0.072049543	0.288198172	0.025617615	8.06252	0.810557359	3.242229436	0.051635635
18	82	11.5	32.80175	2	65.60350642	0.006085585	0.069984222	0.139968444	0.012171169	9.544749	0.804818551	1.609637101	0.058085377
19	83	11.75	30.97393	4	123.8957103	0.005746475	0.067521081	0.270084323	0.0229859	11.15198	0.7933727	3.173490801	0.064084562
20	84	12	28.35574	2	56.71147373	0.005260732	0.063128784	0.126257568	0.010521464	12.88421	0.757545408	1.515090817	0.067780359
21	85	24.41667	8.523881	4	34.09552477	0.001581403	0.038612596	0.154450385	0.006325613	256.196	0.942790889	3.771163556	0.405149217
22	86	36	2.129044	2	4.258088949	0.000394994	0.014219767	0.028439534	0.000789987	761.1782	0.511911606	1.023823212	0.30066045
23	87	48	0.535622	4	2.142487713	9.93719E-05	0.004769851	0.019079405	0.000397488	1567.325	0.228952855	0.915811421	0.155748078
24	88	60	0.134751	2	0.269501979	2.49998E-05	0.00149999	0.002999981	4.99997E-05	2661.472	0.089999419	0.179998838	0.066536374
25	89	72	0.0339	4	0.135601836	6.28942E-06	0.000452838	0.001811354	2.51577E-05	4043.619	0.03260437	0.13041748	0.025432032
26	90	84	0.008529	2	0.017057257	1.58228E-06	0.000132912	0.000265824	3.16457E-06	5713.766	0.011164596	0.022329192	0.009040801
27	91	96	0.002146	4	0.00858248	3.98069E-07	3.82146E-05	0.000152858	1.59227E-06	7671.913	0.003668601	0.014674405	0.003053949
28	92	100	0.001354	1	0.001354495	2.51294E-07	2.51294E-05	2.51294E-05	2.51294E-07	8388.629	0.002512943	0.002512943	0.002108014

Appendix M: Experiment 6 and 7 absorbance readings for TPC for batch and continuous kinetics

Batch Kinetics	Day 1		Day 2		Day 3		Day 4	
Species and Effluent	ABS	ppm	ABS	ppm	ABS	ppm	ABS	ppm
J. effusus Dilute	0.287	81	0.312	70	0.102	0	0.124	0
J. effusus Full	0.408	191	0.267	22	0.148	0	0.137	0
J. effusus spiralis Dilute	0.317	67	0.282	35	0.054	0	0.101	0
J. effusus spiralis Full	0.377	128	0.272	23	0.198	0	0.162	0
Z. aethiopica Dilute	0.313	59	0.254	6	0.11	0	0.164	0
Z. aethiopica Full	0.346	128	0.354	96	0.149	0	0.134	0
P. australis Dilute	0.326	106	0.295	44	0.13	0	0.114	0
P. australis Full	0.538	287	0.346	106	0.229	84	0.256	74

Continuous Kinetics			
τ		ABS	ppm
2	In	0.745	532
	Out	0.367	361
5	In	0.412	447
	Out	0.326	284
8	In	0.407	440
	Out	0.314	266
24	In	0.754	587
	Out	0.344	294
48	In	0.762	631
	Out	0.298	204