DESSERTATION IN PARTIAL FULFILLMENT OF THE DEGREE OF MASTER OF SCIENCE

# The Development and Operation of Plant Microbial Fuel Cells using Municipal Sludge


**Prepared by:**

**Mohamedjaffer Gulamhussein**
**GLMMOH003**

**Supervised by:**

**Dr Dyllon Randall**

Department of Civil Engineering
University of Cape Town, Private Bag Rondebosch, 7700
South Africa 7700

# Declaration

I know the meaning of plagiarism and declare that all the work in the document, save for that which is properly acknowledged, is my own. This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

Signed by candidate

Signed: M.G. Date: 30th April 2018

# Abstract

Wastewater treatment accounts for 3-5% of the total electricity demand in developed countries. However, wastewater is estimated to have 9.3 times more energy than which is required to treat it. A sediment microbial fuel cell (SMFC) can potentially be used to treat wastewater and produce electricity by utilising the organics found in the wastewater. The challenge associated with using SMFCs is efficiency and longevity. Literature has shown that the efficiency can be increased by growing plants in a SMFC. Plants release organics and oxygen into the rhizosphere which can increase microbial growth and increase oxygen at the cathode.

This research undertook to design a batch plant microbial fuel cell (PMFC) and operate it on three different municipal sludge streams namely, thickened waste activated sludge (WAS), liquid WAS and primary sludge (PS). In addition, three indigenous South African plant species, namely, *C. papyrus nanus, W. thyrsiflora* and *P. australis* were tested based on their power output potential and organic removal potential.

The highest PPD (1036 ± 59 $mW/m^3$) was obtained from the system using thickened WAS as substrate and planted with *W. thyrsiflora*. This was followed by liquid WAS as substrate planted with *W. thyrsiflora* (290 ± 21 $mW/m^3$) and the lowest in the unplanted system using PS (119 ± 31 $mW/m^3$).

It was also found that COD utilisation for power generation was most efficient when using WAS. Thickened WAS produced 1330 $mW/m^3$ per gram of COD consumed followed by liquid WAS with 508 $mW/(m^3 \cdot gCOD)$ and the lowest conversion in PS i.e. 124 $mW/(m^3 \cdot gCOD)$. Based on these factors WAS was chosen as the most suitable feed for a PMFC. Furthermore, it was found that utilising the PS in an anaerobic digestion would have over 500 times more power output making its use in a PMFC not viable.

The highest organic removal efficiencies were observed when systems were planted with *C. papyrus.* When using WAS, *C. papyrus* achieved 62.2 ± 12.8%, 62.8 ± 9.6%, 58.5 ± 14.0%, 75.4 ± 8.4%, 95.3 ± 2.8% and 94.4 ± 3.5% removal efficiencies of VSS, COD, TKN, TP, FSA and OP respectively. When using PS, *C. papyrus* achieved 59.4 ± 9.7% 45.7 ± 10.4%, 82.0 ± 3.3%, 65.6 ± 3.2%, 97.4 ± 2.4% and 78.5 ± 2.8% removal efficiencies of VSS, COD, TKN, TP FSA and OP respectively.

Therefore, it was noticed that *W. thyrsiflora* produced the highest power densities, but the *C. papyrus* produced the highest organic removal. The decision between the two plants was made based on the plant species ability to grow in sludge. It was noticed that the *W. thyrsiflora* died in thickened WAS. When using liquid WAS and PS, the old roots died, and new roots grew on the surface for *W. thyrsiflora*. Given the uncertainty of the plants ability to survive in the long term, *C. papyrus* was chosen as the most suitable plant species as it was able to grow in all three sludge types.

Using WAS and *C. papyrus*, three more optimisation experiments were conducted. In the first one, it was found that using a separator between the electrodes increased the power density by 35%. The power output increased from 141 ± 16 mW/m$^3$ to 191 ± 16 mW/m$^3$ when a separator was used. It was noticed that the separator system had more horizontal root growth along the top surface just under the cathode of the PMFC as the separator limited vertical root growth. This may be the reason for higher power densities since more roots meant more oxygen release that can be consumed by the cathode.

The second optimisation experiment focused on the use of multiple electrodes. It was found that using multiple electrodes was more efficient than single electrodes. Furthermore, it was noticed that connecting the multiple electrodes in parallel within a set-up was more efficient than connecting them in series. The peak power densities followed the order of: parallel connection 443 mW/m$^3$, series connection 296 ± 46 mW/m$^3$ and 156 ± 17 mW/m$^3$ for the control.

The third optimisation experiment was focused on varying electrode distance. It was noticed that the highest peak power density was achieved when the electrode distance was halved (664 ± 122 mW/m$^3$) followed by the system with 1.5 times electrode spacing which produced 453 ± 74 mW/m$^3$ and the lowest for the standard design (290 mW/m$^3$).

From the three optimisation experiments, it was noticed that some variables have a larger impact on the performance of the PMFC than others. Halving the electrode distance increased the PPD 2.3 times, while doubling the electrodes increased it 2.8 times. Adding a separator only increased it by 1.4 times. This indicates that more focus should be attributed to the electrode distance and number of electrodes.

In summary, this research found that, of the three plant species investigated, using *C. papyrus* with WAS substrate was the most practical and best performing combination for a PMFC. Furthermore, having a separator between the electrodes, having multiple electrodes connected in parallel within a set-up and decreasing the electrode distance to half all increased the power production.

# Acknowledgements

My passion of wastewater treatment and its use in developing a sustainable world drove me towards pursuing a master's degree. At the beginning of this research, I was uncertain about this topic as I was new to experimental work. However, the friendly nature, encouragement and understanding of this research topic that my supervisor, Dr Dyllon Randall, provided me with made me interested and excited about the potential of PMFC. I would like to offer my gratitude to Dr Dyllon Randall for always being approachable, providing me with guidance and support during the course of this research. I would not have been able to finish this thesis in 15 months without his excellent and timely feedback. The weekly meetings with Dr Dyllon were insightful, motivational and always helped to keep me on track.

I would also like to thank the Water Quality Lab technicians, Njabulo Thela and Hector Mafungwa who aided me with collecting feed, buying equipment and providing advice along the way. To the researchers working in the lab, I thank you a lot, the late nights and long hours in the lab would not be an enjoyable experience without you (Suzanne Lambert, Johan Du Toit Matthieu Quevauvilliers, Aaron Mwidu and Chris Gaszynski).

I would also like to thank the chemical engineering electronics workshop for constantly providing me with electrical equipment in particular Ameen Jakoet, Curwin Nomdoe and especially Stephano De Grandis for making the voltage recording software.

I would like to extend my thanks to everyone else who has helped me along this journey, the support from my family and friends, the constant wastewater treatment works operations knowledge from Prof George Ekama and Dr David Ikumi, the guidance on plant species from Dr Samson Chimphango, the Kirstenbosch and Stark Ayres Nurseries, the WRC steering committee in providing general advice and lastly Prof Sue Harrison for general guidance on MFCs.

Finally, I would like to thank the National Research Foundation of South Africa (Grant number: 11374) and the Water Research Commission for providing me with scholarships so I can undertake this research.

# Table of contents

# List of tables

# List of figures

# Acronyms and abbreviations

| | |
|---|---|
| COD | Chemical oxygen demand |
| EBPR | Enhanced bio-phosphate removal |
| FSA | Free and saline ammonia |
| GAC | Granular activated carbon |
| ISS | Inorganic suspended and settleable solids |
| MFC | Microbial fuel cells |
| MLE | Modified Ludzack-Ettinger |
| OHO | Heterotrophs |
| OP | Orthophosphates |
| OrgN | Organically bound nitrogen |
| OrgP | Organically bound phosphorus |
| PAO | Polyphosphate accumulating organisms |
| PMFC | Plant microbial fuel cell |
| PS | Primary sludge |
| SMFC | Sediment microbial fuel cell |
| TKN | Total Kjeldahl nitrogen |
| TP | Total phosphorus |
| TSS | Total suspended and settleable solids |
| UCT | University of Cape Town |
| VSS | Volatile suspended and settleable solids |
| WAS | Waste activated sludge |
| WWTW | Wastewater treatment works |

# 1. Introduction

## 1.1 Background to the project

### 1.1.1 Overview

Today, the world's population is over 7 billion according to United Nations calculations [1]. In the last century, the population grew by over 300%, from 1.5 billion in 1900 to 6.1 billion in 2000 [2]. The 2015 revision of the UN's world population projections predicts that the population could increase to 9.7 billion by 2050 and 11.2 billion by 2100 [1].

With the increasing population, the energy demand has also been increasing and at a steeper slope (see Figure 1). This is because there is a close correlation between the electricity consumption and the human development index which is based on life expectancy, education and gross domestic product [3]. The United Nations predicts that the consumption of natural resources is expected to triple to 140 billion by 2050. At current energy production rates (assuming no increase), natural gas would be depleted in the next 60 years and oil in the next 40 years [4]. Furthermore, the uneven distribution of natural resources adds an extra strain on the energy challenges.

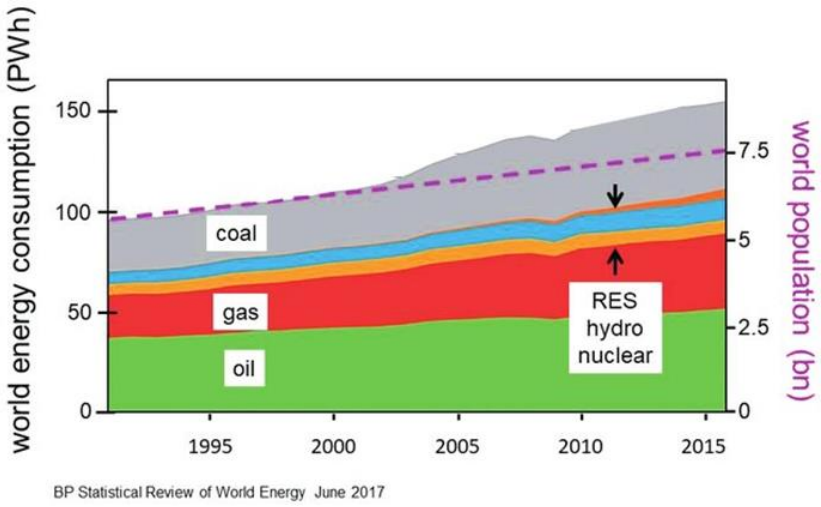



**Figure 1:** Relationship between population growth and world energy consumption [3]. The graph indicates that the energy consumption has been increasing at a faster rate than population growth since 2003.

In addition to the increasing energy demand, waste production has also increased. The world generates 3.5 million tonnes of solid waste a day, which is 10 times the amount it produced a century ago [5]. This is expected to increase to 6 million tonnes per day by 2025 and 11 million tonnes per day by 2100 [6]. The growing waste generation leads to expanding landfill sites.

Furthermore, sustainably meeting food demands will become more challenging with a growing population. Fertiliser production currently increases by approximately 4% annually to meet this growing population trend [7]. Current fertiliser production relies heavily on non-renewable energy and finite resources. Ammonia generated from the Haber-Bosch process requires 35 – 50 MJ of energy per kilogram of nitrogen produced and phosphate mining leads to gypsum production contaminated with heavy metals [7]. In addition, peak phosphate production is estimated to occur by as early as 2030 [7, 8] (see Figure 2).

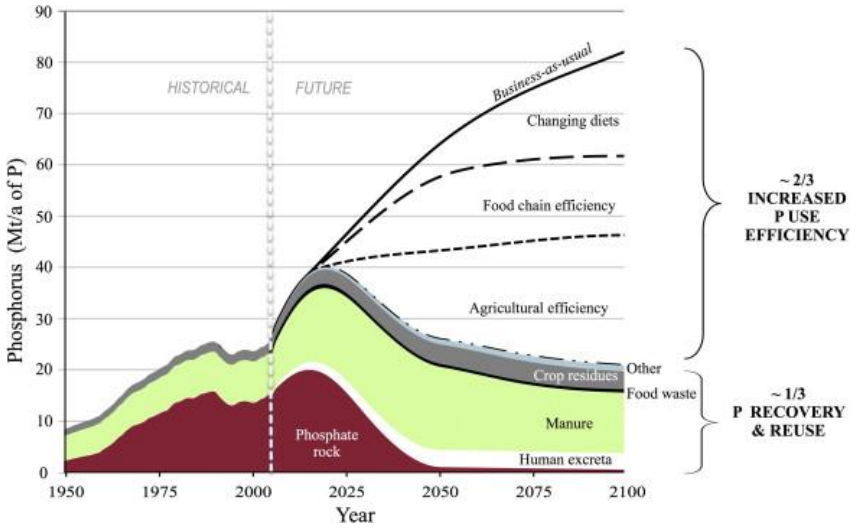



**Figure 2:** Meeting long-term phosphorus demand in a sustainable manner [9].

When considering the world's energy consumption breakdown, it is evident that wastewater treatment currently accounts for 3-5% of the global energy demand [10]. About 77% of this consumed energy is used in activated sludge systems for nitrogen removal in the nitrification process [7]. In addition, sludge produced as a by-product of wastewater treatment is generally disposed to landfill sites. Furthermore, it is estimated that 30% of the nitrogen and 16% of the phosphorus from fertilisers ends up in wastewater treatment works (WWTW) [7]. The three challenges discussed can partly be solved by creating a paradigm shift where WWTWs are converted into resource recovery systems.

### 1.1.2 Potential solution to the growing energy demand

Wastewater resource recovery has seen a growing trend as bio-energy presents a sustainable and environmentally friendly source of energy [11]. Microbial fuel cells (MFCs) are an example of such systems. MFCs are a green, carbon neutral technology that can convert biomass into electricity. Wastewater contains a high concentration of organics, therefore MFCs can be used to extract this energy while also treating the wastewater [12]. Also, the sludge by-product could potentially be stabilised and used as a fertiliser therefore recovering nitrogen and phosphorus and reducing the strain on landfill sites. MFCs generate electricity by taking advantage of anaerobic

microbial metabolism, electron donors such as organics and electron acceptors such as oxygen [13]. The design of a MFC is simple since it only consists of two compartments, the anaerobic anode region where organics are fed and the aerobic cathode region where oxygen is reduced [14].

Organics broken down in the anode release electrons which are transported to the cathode via electron collectors. The electrons move willingly from the anode to cathode given the redox potential. This flow of electrons generates electricity. The anode and cathode are separated by a proton exchange membrane (PEM) which allows only $H^+$ ions to travel from the anode to the cathode. A schematic diagram of a MFC is shown in Figure 3. The anaerobic zone has a low redox potential while the aerobic zone has a high redox potential. The difference in redox potential (known as the redox gradient) is the driving force for the transfer of electrons [15].

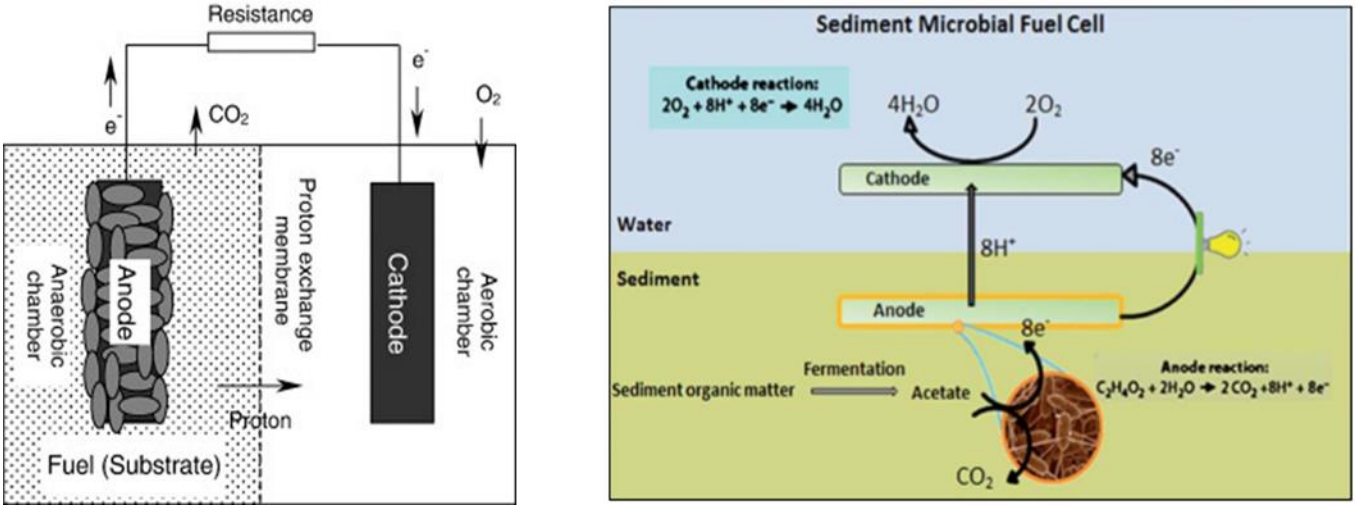



**Figure 3:** Fuel cell schematics. On the left, a MFC [14] and on the right, a SMFC [13].

Sediment microbial fuel cells (SMFCs) operate in the same manner as MFCs. The main difference between them is the use of a PEM. SMFCs use a naturally occurring oxygen gradient to separate the electrodes and therefore, do not require the expensive PEM [13]. This is achieved by imbedding the anode deep into the sediment where dissolved oxygen is depleted while the cathode is placed in the water zone with a relatively higher DO. A schematic diagram of the SMFC is shown in Figure 3.

The embedded anode is advantageous as it allows for easier flow of hydrogen ions, but conversely, the increased distance increases the internal resistance of the system therefore reducing the total power output [16]. Jiang and Li [17] varied the electrode distance from 7.5 cm to 1 cm and the internal resistance decreased by 50%. The maximum power was obtained when the distance was 2 cm. Therefore, a balance needs to be obtained between hydrogen ion movement and internal resistance.

MFCs and SMFCs have two major problems; the first is longevity, and the second is incomplete removal of organics. Over time, the operational efficiency of the MFCs and SMFCs decreases due to electrode clogging [18]. Complete organic removal cannot be achieved when high organic content such as raw sewage is added to the fuel cell [19]. One of the ways these problems can be solved is by growing plants in the SMFC. This system is called Plant Microbial Fuel Cells (PMFC).

Plants grown in the SMFC increase microbial activity and provide oxygen to aid in organics removal [20]. Furthermore, continuously growing roots aid in unclogging the microbial fuel cell. PMFCs are an emerging technology and contain multiple gaps in knowledge, especially when using municipal sludge [13]. This research is therefore aimed at looking at using South African indigenous plant species and also exploring PMFC operation with municipal sludge.

## 1.2 Problem statement

It is evident that WWTWs need to focus on resource recovery instead of only treatment. PMFCs are an emerging and promising bio-technology. Several studies have investigated the role of plants in MFC, but few studies have compared the performance of multiple plant species in terms of power generation, total phosphate removal and volatile suspended solids and settleable solids removal.

Furthermore, most research that has investigated the use of PMFCs in the context of wastewater treatment has only looked at using settled sewage, whereby wetlands are integrated within SMFCs. However, sludge from WWTWs has yet to be investigated. Specifically, waste activated sludge (WAS) and primary sludge (PS), both of which have a very high chemical oxygen demand (COD) relative to settled wastewater. Also, a comparison of different sludge types from WWTWs to determine which sludge is most suitable for PMFCs has not yet been investigated.

## 1.3 Research objectives and approach

The aim of this research was to provide information on the design and operation of a PMFC. Thereafter, three indigenous South African plant species and different sludge types were tested to determine which plant species (of those tested) and sludge type was most suitable.

The objectives of this research were to:

1. Perform a thorough literature study to understand the individual components of a PMFC in order to design one and use it for experimentation.

2. Operate a PMFC to better understand what affects power generation.

3. Source and test three sludge types (thickened WAS, liquid WAS and PS in a PMFC. Thereafter, evaluate the sludge types based on practical application and power output.

4. Choose, source and test three indigenous South Africa plant species when using the different sludge types. Thereafter, evaluate the plant species performance based on power production, assessment of plant health, exudate release, organic removal and fertiliser capabilities. Lastly, choose the most suitable plant for use in a PMFC.

5. Optimise the PMFC such that maximum power is obtained.

## 1.4 Scope

In this research, only batch PMFC was designed and used to test the power production of three different sludge types and three indigenous plant species. The research also only looked at three optimisation aspects: use of a separator, use of multiple electrodes and distance between electrodes.

## 1.5 Limitations of research

The research undertaken has the following limitations:

1. The microbial aspect of the study was not investigated. Microbial study is one of the elements of the PMFC to ensure power generation. Multiple questions raised in this research have been unanswered given the lack of experimental microbial information of the inoculum, substrate used, organisms grown on roots and kinetics of exoelectrogenic bacteria.

2. The sludge after being used in the PMFC could not be fully characterised to evaluate if it can be used as a fertilizer. The guidelines require all three measurements, namely; stability, pollutant and microbial. Stability was classified, but pollutant and microbial were out of the scope of this research.

3. Potassium measurements were not available in the Water Quality Lab and the potential NPK ratio could not be obtained to compare sludge to other organic fertilizers.

4. The study was limited to a batch system. Real plant operation dictates the use of a continuous system as a batch system would require a large area and make the PMFC not feasible in a large scale. The power generation of the sludge and fertilizer properties of the sludge should be evaluated with a continuous system.

# 2. Literature Review

## 2.1 Plant Microbial Fells

The studies conducted on SMFCs, which in itself is a relatively new technology, suggest the use of plants to improve the power production [13]. Plants have been used in constructed wetlands to treat wastewater using bacteria, which is similar to how MFCs operate [21]. MFCs require anaerobic conditions at the anode and aerobic conditions at the cathode. These redox differentials occur in wetlands as well. Therefore, both technologies can be combined to produce electricity while also treating wastewater.

Plants, more specifically, plant roots release dissolved oxygen and organics. Depending on whether the anode or cathode is placed next in the rhizosphere, either product can be used. The choice depends on the PMFC aim and limiting factor, i.e. organics or dissolved oxygen. Liu, et al. [22] used *Ipomoea aquatic* plants in both configurations and found that for COD concentrations of 50 and 100 mg/l placing the anode in the rhizosphere instead of the cathode produced respectively 45% and 8.5% larger power densities. However, it was also noticed as the COD concentration increased from 250 mg/l to 500 mg/l, and then to 1000 mg/l, the cathode placed in the rhizosphere instead of the anode produced higher power densities (see Figure 4). The transition COD determined by Liu, et al. [22] cannot be standardised as it would depend on various factors one of them being the plant species.

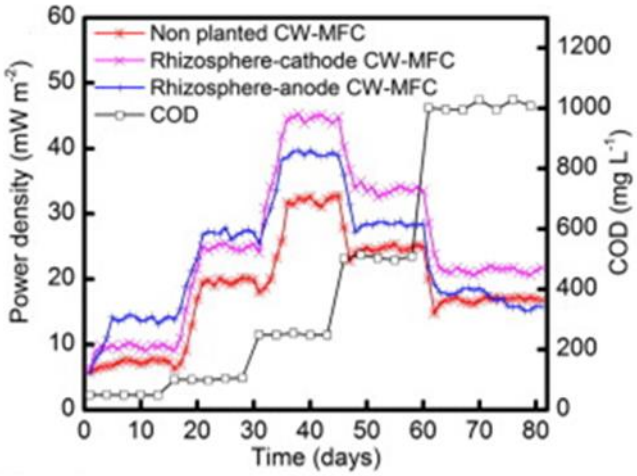



**Figure 4:** Positive correlation between peak power density and COD concentration [22].

The plants present in PMFCs not only provide organics and oxygen, but also aid in treating the wastewater [23]. Plants increase microbial activity at the roots by hosting the bacteria, providing exudates and oxygen [24]. Also, plant roots have the ability to trap organics allowing microbes to feed on them. Furthermore, it has also been shown that increasing microbial activity aids in providing higher power densities [20, 25].

### 2.1.1 Design 1: Plant roots to provide dissolved oxygen

In this design configuration, oxygen is seen as the limiting factor. The plant is placed closer to the cathode in order to increase dissolved oxygen levels and therefore power [21]. The primary focus of this system is to first treat wastewater and secondly, generate power. Fang, et al. [26], Villaseñor, et al. [19] and Chen, et al. [27] used this design in their experiments, with varying organic loads, feeding mechanisms, plant species used, electrodes used and number of electrodes used. This design is viable if sufficient organics are available in the system. Since this system operates like a constructed wetland, sewage can be used with low solid concentrations.

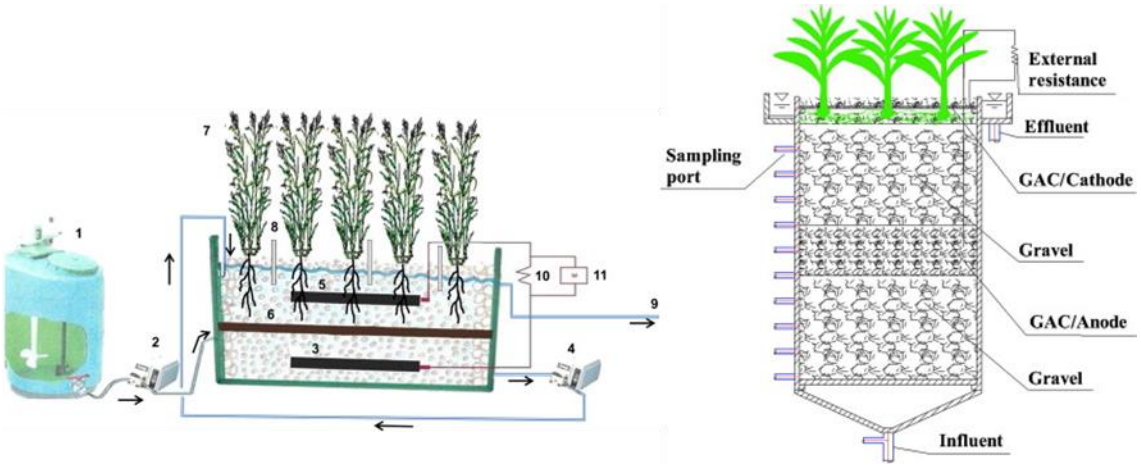



**Figure 5:** On the left, horizontal subsurface flow of synthetic sewage [19] and on the right, up-flow of azo dye [26].

### 2.1.2 Design 2: Plant roots to provide organics

In this design, organics are the limiting factor. The anode is placed at the roots allowing it to utilize organics released [21]. The primary focus of this system is for green electricity production, therefore these systems can be implemented in green rooftops. Moqsud, et al. [28] and Tapia, et al. [29] used this design in their study, with varying garden soils, plant species, and electrodes used (both size and type). Therefore, this design is not limited to organics present in wastewater, as a continuous supply of organics are available from the roots ensuring a continuous generation of power.

PMFCs could also be used in rice paddy fields to convert methane gas that is released into bioelectricity [30, 31]. It is estimated that rice cultivation worldwide accounts for 7 to 20% of total global methane emissions. Methane gas has 25 times more heat-trapping potential than carbon dioxide [32]. Recent calculations suggest that methane emissions have been responsible for approximately 20% of the Earth's warming since the pre-industrial age [32].

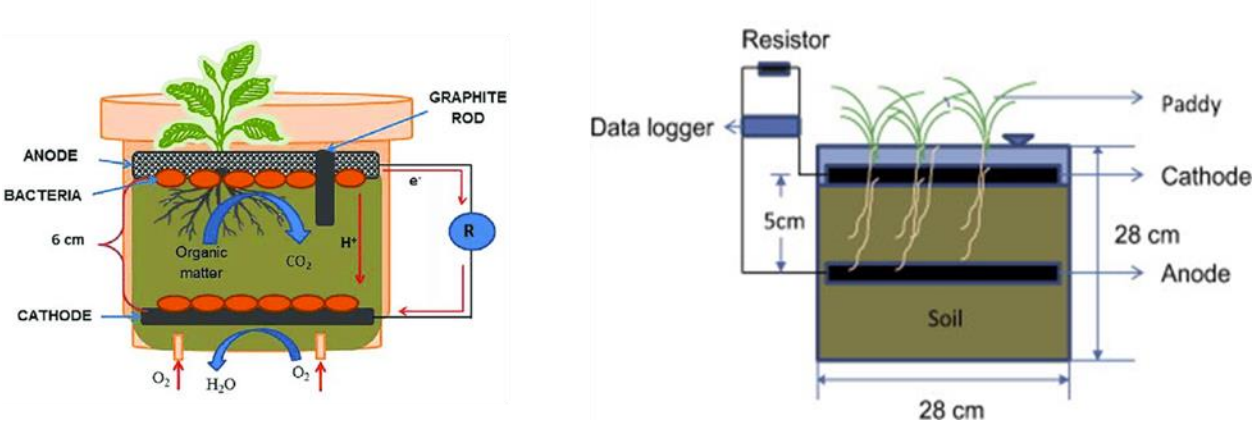



**Figure 6:** PMFC designs, on the left: embedded cathode [29] and on the right: compost mixed with gardening soil [28].

### 2.1.3 The rhizosphere and rhizodeposits

The rhizosphere is defined as the zone of soil influenced by the root of plants [33]. This zone cannot easily be quantified due to the variability in plant species, moisture content, rhizo-deposit variability along the roots and soil properties. However, it was observed that the rhizosphere area was several times greater at a higher soil moisture content than at a lower soil moisture content [33]. This could potentially lead to reduced efficiencies as organics would be carried further away instead of being close to the roots.

Rhizodeposits are organic compounds deposited in the rhizosphere. Plants are autotrophs as they utilize solar energy, carbon dioxide and water to produce biomass in a process known as photosynthesis [34]. Depending on the plant species, environmental conditions and age, part of the biomass created is released into the rhizosphere. The average quantity released varies. Waisel, et al. [33] quoted a 10 to 20% release of total organic substances, while Nitisoravut and Regmi [34] estimated a 40% release and Strik, et al. [35] estimated a 60% release. The method for determining these quantities was not elaborated in their research and therefore the reason for variation could not be established. However, it was seen in all studies that the deposition decreased with increasing plant age.

The rhizodeposits consist of five main compounds, these are [31, 33, 35]:

1) Root exudates: water soluble low molecular weight organics such as sugars and organic acids;
2) Secretions: high molecular weight compounds such as polymeric carbohydrates and enzymes;
3) Lysates: dead cell material;
4) *Gases: ethylene and carbon dioxide; and
5) Mucilage: gelatinous polysaccharide substance.

*Gases are not mentioned by Waisel, et al. [33].

The rhizodeposits are directly linked to photosynthesis. Based on photosynthetic pathways, plants are categorised into: C3, C4 and Crassulacean Acid Metabolism (CAM) plant species [23]. C4 plants have the highest photosynthetic efficiency (this relates to the percentage of total sunlight a plant is exposed to), i.e. 6% while C3 plants have 3.6%. CAM plants have low efficiencies as they are grown in arid environments [23]. This means that C4 plants have the potential to produce higher power relative to C3 or CAM plants if incorporated in a PMFC.

### 2.1.4 Plant oxygen release

Along with the rhizodeposits, roots also release oxygen into the rhizosphere. Oxygen release plays a key role in a PMFCs. Oxygen released aids in increasing the power production efficiency and also aids in nutrient removal. Oxygen is captured by plant leaves in wetlands and transported to the roots through the aerenchyma [36]. Aerenchyma are plant tissues containing air spaces and are found in many aquatic plants. Villaseñor, et al. [19] quoted oxygen released values from different studies done on the aquatic plant *Phragmites australis*. The dissolved oxygen was found to be between 1.5 – 3.0 g $O_2/m^2d$ in one study while another study showed a release of 5 – 12 g $O_2/m^2d$.

Similarly, Zhang, et al. [37] compared multiple studies which tried to estimate dissolved oxygen release of wetland plants. Some researchers used anatomical and physiological studies. Others measured oxygen release rates using methods such as oxygen depleted methods, microelectrode method, oxygen consumption model and mass balanced methods. The values found ranged from 0.014 – 12 g $O_2/m^2d$. The large variation in DO values (1.5 – 3.0 or 5.0 – 12 or 0.04 – 12) can be attributed to:

1) Different experimental conditions such as light, temperature, salinity, atmospheric pressure and hydrostatic pressure. At higher temperatures, salinity, atmospheric pressures and hydrostatic pressures, less oxygen dissolves in solution;
2) Different measurement techniques, given the sensitivity and the small quantities of oxygen released by plants, a variation in technique would cause a large variation in dissolved oxygen measured; and
3) Different plant species used.

It therefore becomes necessary to experimentally measure oxygen release when comparing quantities of oxygen released from different plants. Literature comparing oxygen release from different plant species can only be used if the experimental technique and conditions are kept the same.

Even though there was a large variation between measured oxygen amongst different researchers, the release of both oxygen and rhizodeposits are directly dependent on photosynthesis and irradiance [38]. Photosynthesis activity is increased during the day, and as such, the deposition of

oxygen and organics in the rhizosphere is increased [21, 37-39]. The peak photosynthesis rate, and therefore, the peak oxygen release rate varies between plant species and also depends on the stage of plant growth [37]. This means that the power produced in the fuel cell drops during the night and increases during daytime. This phenomenon was also confirmed by Villaseñor, et al. [19] who reported a 200 mV drop during night-time operation. Electricity generation is thus dependant on the photosynthetic rate, resulting in a 24-hour cyclic power production.

### 2.1.5 Choice of plant species

Plants possess the unique ability to release both oxygen and organic matter into the rhizosphere [27]. If the plants are used to provide organics, the DO released in the anode region reduces efficiency as anaerobic conditions are required. The converse also applies. Therefore, when choosing plant species, it becomes important to first identify the purpose and then choose the plant species accordingly.

Marshy grasses have been found to work best in PMFCs due to their ability to adapt to anaerobic and high salinity conditions. Also, grass grow dense root biomass which aid in increasing microbial activity [34]. Of the 8100 C4 plant species discovered, 5044 are grasses [40]. Grass species such as *Pennisetum setaceum, Cyprus involucratus, Lolium perennee, Enchinorriea crassipes, Acorus calamus, Ipomoea aquatica, Typha latifolia, Echinochloa glabrescens* and *Canna indica.* have been used in PMFC research [34]. Also, for higher oxygen release Villaseñor, et al. [19] used *P. australis* in their study while Fang, et al. [26] used *Ipomoea aquatica*. The quantitative understanding of oxygen release needs to be further investigated as current knowledge is limited [13, 27]. Substantial work on PMFCs has also focused on using rice plants, *Oryza sativa,* which is classified as a marshy plant [27, 28, 30, 41]. This is because rice fields produce the greenhouse gas methane as mentioned previously.

### 2.1.6 South African indigenous plant species

The plant species used in PMFCs from the previous sections are either classified as wetland or aquatic plants or both. A wetland is an area that is saturated with water [42]. The plants grown in this condition can withstand large quantities of water unlike other species where the root bulb would rot in excess water [33]. Wetland plants behave very similarly to aquatic plants but they have a few terrestrial plant properties [43]. Due to the subtle differences between the wetland and aquatic plants, some plant species are placed in both categories. For example, *Phragmites australis,* that is an indigenous South African plant species, is quoted in both government publications, aquatic plant species [44] and wetland plants [45].

Lakay [42] investigated three constructed wetlands used to treat domestic wastewater in Cape Town, South Africa. The plant species used included *Schoenoplectus scirpoides, Cyperus dives, Cyperus papyrus, Cyperus thunbergii, Calopsis paniculata, Wachendorfia thyrsiflora, Pennisetum macrourum, Chondropetalum tectorum, Orphium frutescens, Juncus krausii, Elegia capensis, Psoralea pinnata, Juncus effuses, Carex clavata, Isolepis prolifer*. The plants listed fall part of

either the South African wetland category [45] or aquatic category [44] or both. Since these plants have shown to work well in constructed wetlands, they could be used in PMFCs as well.

### 2.1.7 Plant growing conditions

Optimal plant growing conditions differ from one species to another. Therefore, general wetland plants growing conditions will be discussed in this section. Growing conditions include based on literature findings [33, 34, 44, 46-48] include:

#### 2.1.7.1 Light

Sunlight energy is the source of all life on earth. Plants use this energy to produce organics by the process of photosynthesis. Sunlight emits wavelengths ranging from 250 nm to 1400 nm. Specific wavelength bands define colour. Photosynthesis requires light from two bands only, the red spectrum (640 nm to 740 nm) and the blue spectrum (425 nm to 490 nm). If sufficient lighting is not provided, photosynthesis would slow down, therefore reducing rhizodeposition leading to reduced power production.

#### 2.1.7.2 Water content

Wetland plants require at least saturated soil. Depending on the plant species, they can withstand a certain depth of water above the soil surface (termed flooding). However, for the first plant growing season (when the stalk is 1 to 2 inches high), it is recommended to use saturated soil instead of flooding. Flooding is possible after 2 to 3 months of growth. This is to stop oxygen depletion at the roots during the initial growing phase. An indication of limited oxygen content is the yellowing and dying of lower leaves on the plant.

#### 2.1.7.3 Soil

Different soils are suitable for wetlands, properties such as pH, oxygen, soil organics and nutrient matter and electrical conductivity need to be considered. The pH of the soil should range between 5.5 and 7.5. When the soil pH drops below 5, nutrients leach out of soils more rapidly relative to accepted pH ranges. According to Perry [49], plant nutrients are mostly available to plants in the pH range of 5.5 to 6.5. Similar pH ranges should also be used when growing plants hydroponically. In addition, the electrical conductivity affects plants ability to treat wastewater and therefore should be kept below 4 millimho per centimetre (mmho/cm), in other words 4 deciSiemens per meter (dS/m). In terms of, total dissolved solids, the TDS should be kept below 2560 mg/l (conversions based on [50]).

Furthermore, wetlands plant grown in soils with a high clay content are usually modified through the addition of compost. This is because high clay content soils do not contain required organics for microbial activity.

Wetland plants can tolerate flooded soils with extended periods of oxygen deficiency. Wetland species also possess a specialized metabolism that allows them to gain sufficient energy even with limited soil oxygen levels. In one study, twenty wetland species were tested in complete anaerobic conditions, and thirteen survived at least seven days and regrew when placed in aerobic conditions [33]. For PMFCs, low oxygen concentrations at plant roots is expected due to the anaerobic digestion (not complete anaerobic) of organics, wetland plants can therefore withstand these conditions.

#### 2.1.7.4 Temperature

The minimum temperature for root growth is about 5℃ and the maximum is between 35℃ to 40℃. The broad optimum temperature ranges from 20℃ to 25℃.

## 2.2 Substrate

### 2.2.1 Types

The substrate plays a key role in a MFC. The choice of substrate determines the concentration of organics introduced in the system. The substrate utilised in a MFC can range from pure compounds such as glucose, acetate, ethanol and organic acids to complex mixtures of organic substances present in wastewater [51]. Furthermore, wastewater can be synthetic or real domestic wastewater.

Villaseñor, et al. [19] used synthetic settled sewage. Since the sewage was only liquid, the MFC was filled with gravel to act as plant support. A similar system was also implemented by Fang, et al. [26], the main difference being the substrate utilised was azo dye (different properties compared to wastewater). Glucose, acetate and ethanol have also been individually investigated to observe power variation in the MFC. This is because all three substrates are present in wastewater [52].

Kaku, et al. [30], Schamphelaire, et al. [31] and Tapia, et al. [29] all used gardening soil as substrate. These substrates contained low organics when compared to settled sewage, but the goal of the experiments was to harness organics from root exudates. Compost can be added to such substrates to increase organics and therefore power generation. Moqsud, et al. [28] achieved twice as much power in experiments where 3% of the soil by mass was compost.

### 2.2.2 Sludge from municipal WWTWs

Raw sewage coming into treatment works undergoes various processes to treat it before it can be discharged back into the environment. There are two main types of WWTWs, one that utilises a primary settling tank, the second type that does not. Figure 7 shows these two types. Not all type one WWTWs incorporate anaerobic digesters (AD). Without an AD, the WWTW is no longer called an energy recovery plant and the PS is dewatered and dumped on landfill sites. Figure 7 also includes sludge drying beds before disposal to a landfill site. However, this is an outdated

process, treatment plants today make use of dissolved air flotation (DAF) units and gravity belts to dewater and compress sludge before disposing.

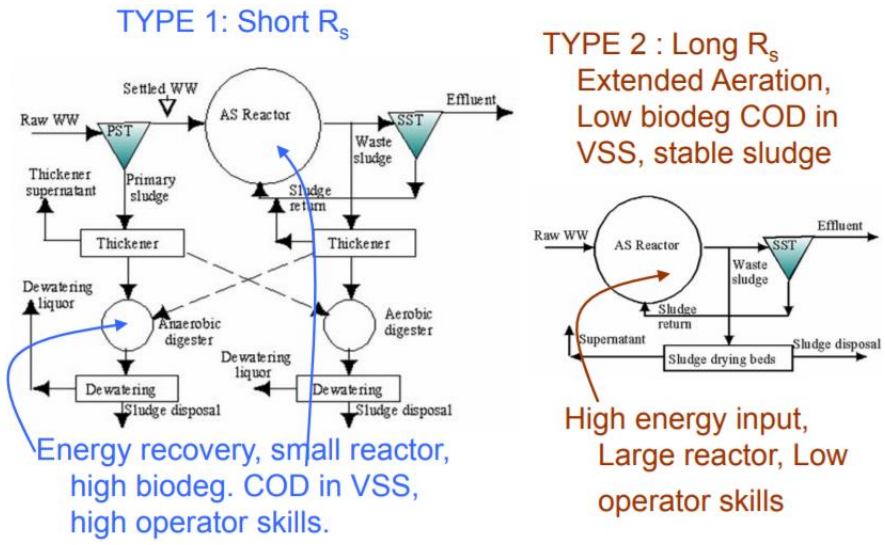



**Figure 7:** General layout of two different types of WWTWs. On the left, a sustainable energy recovering WWTWs and on the right a conventional WWTWs with no energy recovery [53].

**Table 1:** Possible substrate sources from WWTWs [53, 54].

| Source | Organic load (mgCOD/l)* | Comments |
|---|---|---|
| Raw | Low (about 800 – 1500) | This is the raw influent coming from homes. Has a low TSS as the influent has a high water content. |
| Primary sludge ) | Very high (40,000 – 60,000) | Primary sludge comprises of solids which settle out from a primary settling tank. PS has a very high TSS, it can be greater than 45 g/l depending on the settling tank properties. This sludge is usually anaerobically digested, treated with heat or composted. Sometimes the sludge is also dewatered and incinerated or taken to a landfill site. |
| Settled sewage | Low (400 – 700) | This is only obtained when the site has a primary settling tank. Settled sewage overflow continuing past the primary settling tank. It has a very low solids concentration i.e. suspended solids that couldn't settle in the primary settling tank. |
| Waste activated sludge | (4000 – 6000) before thickening and (10,000 – 15,000) after thickening | Waste activated sludge is produced from the activated sludge reactor. WAS is wasted daily from activated sludge systems to maintain the system at a certain mass. Most of the biodegradable content of the sludge is obtained from OHOs and/or phosphorus accumulating organisms (PAO's) (depending on the activated sludge treatment process chosen) as the influent organics are consumed as part of the treatment process. WAS does not digest well and it is therefore aerobically digested or dewatered and sent to a landfill site.<br>The sludge can be dewatered by adding it to drying beds in small plants while in large plants the sludge is dewatered by using DAF units, belt presses or linear screens.<br>The thickened WAS after dewatering is usually sent to a landfill site. However, this sludge can be used in a PMFC and the products used for agricultural purposes. |

*The low and high are based relative to WAS values

** The values quoted are all estimates, these would vary significantly from one plant to another, but the relative (qualitative) comparison would be the same

Substrate for MFCs can be acquired from different stages of the wastewater treatment process. Each stage has different substrate properties. Table 1 below summarises the characteristics of sludge at different sources (table based on Ekama [53]; Noziac and Freese [54]).

From Table 1 it is evident that there are four possible sources of substrate collection. The choice of substrate depends on the design of PMFC and whether this design is economically viable. It is therefore important to compare the energy output from the anaerobic digestion of PS to the power output of a PMFC.

### 2.2.3 Planted drying beds

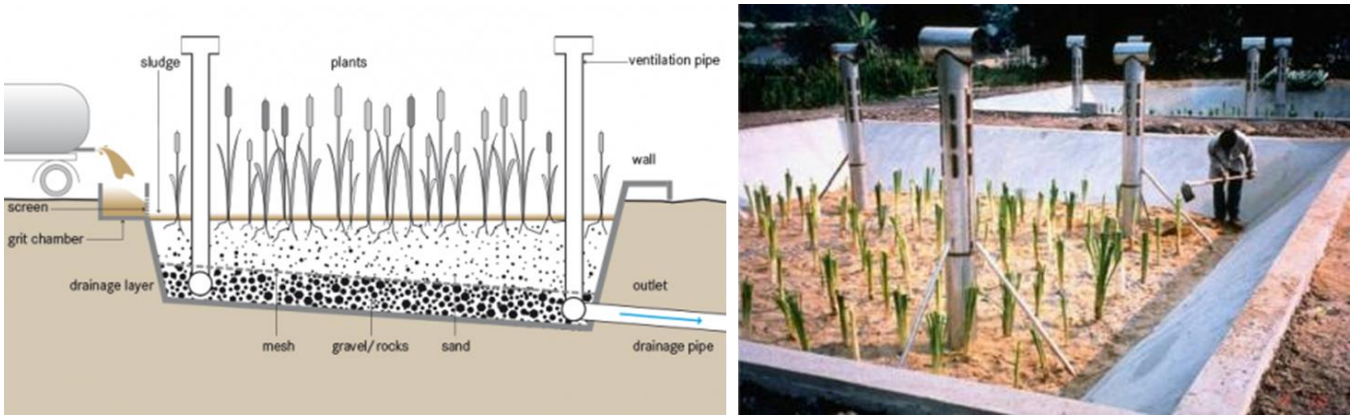



**Figure 8:** Schematic of a planted drying bed used to treat sludge [55].

Sludge from WWTWs may be placed in drying beds for dewatering depending on the size of the plant i.e. the sludge flow rate and available land area. Drying beds usually consist of two types: planted and unplanted. Planted drying beds have macrophytes growing in them and are usually emptied after a certain number of years [55, 56]. Planted drying beds result in stabilised sludge that can be utilised directly as a soil conditioner and fertiliser [57].

Planted drying beds allow for long-term permeability to ensure dewatering of the sludge occurs. The continuously growing root systems of plants in these beds create a dynamic system of percolation canals [56]. Plants also consume some of the water through transpiration. This combined effect allows for an increased bed depth and therefore increases the time duration of sludge accumulation. When comparing the evapotranspiration rate of planted versus unplanted bed, it was found that planted beds achieved over 95% sludge volume reduction and 69% dry matter content while the unplanted bed achieved under 90% sludge volume reduction and 31% dry matter content [56].

Planted drying beds can also increase treatment efficiency depending on the plant species used. Joceline, et al. [58] found a 90% removal of biological oxygen demand (BOD) when the sludge bed was planted with *Andropogon gayanus*, 75% BOD removal when planted with *Cymbopogon*

*nardus* and 76% BOD removal in an unplanted bed. Plants grown in these beds also increase the dissolved oxygen content therefore aiding in nitrification of the ammonia and aerobic degradation of sludge [59]. Therefore, the plant species chosen directly influences the organic removal (see Section 2.1.4 for more information on plant oxygen release).

Macrophytes are used in planted drying beds. Macrophytes are plants found in wetlands, swamps and marshes [56]. Macrophytes are different from regular plant species as they can grow in partially or fully submerged conditions. There are four types of macrophytes: freely floating, submerged, floating leaved and emergent. Of these four, emergent macrophytes are most suitable for planted drying beds as their rate of generation of new biomass is very high. Rapid new biomass generation is very important for drainage. The macrophytes used in planted beds require the following characteristics [55, 56]:

- Fast growing under different conditions;
- Tolerant to varying water depths;
- High transpiration;
- Tolerant to anaerobic conditions;
- Deep growing rhizomes and root systems;
- Resistant to changing pH and salinity; and
- Readily available, indigenous and non-invasive.

The most common species used in planted beds include reeds (*Phragmites sp.*), cattails (*Typha sp.*), antelope grass (*Echinochloa sp.*) and papyrus (*Cyperus sp.*). However, *Phragmites australis* is an invasive species and its use has been restricted in the USA and New Zealand.

Since planted beds incorporate plants this technology is very similar to PMFCs with the exception of power generation. Also, planted drying beds are already in use and can therefore be modified to include PMFCs. If PMFCs were to be implemented in WWTW in the future, using them in drying beds would be the most suitable option as its design can easily incorporate an anode and a cathode to produce electricity. This would also increase the organic removal efficiencies. In addition, the dry sludge from the bed could be used for agricultural purposes.

### 2.2.4 WAS used as a fertiliser

#### 2.2.4.1 Benefits and dangers of using WAS fertiliser

WWTWs produce large quantities of sludge every day. The sludge produced was commonly sent to landfill sites or incinerated, both of which are harmful to the environment [60]. Sludge resting on landfill sites can release methane gas while incinerating sludge produces carbon dioxide. Given this, the agriculture use of sludge has increased worldwide. The sludge is reused as an organic fertiliser or soil conditioner after undergoing composting [61]. Composting involves decomposition of the sludge organic content under controlled conditions [62]. Fertilisers contain nitrogen and phosphorus which can be found in sludge.

Thickened WAS contains a high nitrogen content which is organically bound. When the organics are broken down and utilised, the nitrogen is released as free and saline ammonia (FSA). This FSA can be converted to nitrates in the MFC in the presence of oxygen [13]. In addition, nitrates are good nitrogen source for plants. Therefore, the MFC can also be used as a side stream treatment instead of sending WAS into anaerobic digestors. ADs have no oxygen available and therefore cannot remove the FSA which means that the dewatering liquor from the AD would require further treatment.

The major benefit of using organic fertilisers is that the nutrients are slowly released into the soil. Organic fertilisers are first broken down by organisms present in the soil and this releases the trapped nitrogen and phosphorus. This slow release of nutrients allows for the full use of organic fertilisers [63]. However, there is a disadvantage to this process because if nutrients are immediately required by the plant organic fertilisers are not suitable.

In addition, organic fertilisers have the added advantage in that they can make the soil more workable. Soils containing organic material contain more air pockets allowing air to reach plant roots easily [63]. The organic material also aids in retaining water and increases fungal activity.

Leila, et al. [60] grew *E. Camaldulensis* in different soil sludge ratios and found that 60% sludge and 40% soil for produced the best plant growth results (see Figure 9). Similarly, Xue and Huang [64] found that 45% of sludge and 55% soil produced the best results for *P. Suffruticosa*. Sludge from Leila, et al. [60] had 20 g TKN/kg sludge while Xue and Huang [64] had 19.4 g TKN/kg of sludge. This means that the optimal percentage of sludge would vary from plant species to another depending on its nutrient requirements.


**Figure 9:** A is from research conducted by Leila, et al. [60] where 60% sludge and 40% soil produced the best results for *E. Camaldulensis.* 5B was a similar study conducted by Xue and Huang [64] on *P. Suffruticosa* resulting in 45% sludge and 55% soil as the best combination.

There are two main problems associated with using WAS as a fertiliser, (1) presence of OHOs and (2) the possible existence of heavy metals. OHOs feed on organics and require dissolved oxygen in the process [53]. If WAS with a high concentration of OHOs is used as a fertiliser, it would deplete the oxygen in the root area and affect crop growth. Stabilising sludge i.e. sludge with a low active fraction (concentration of OHOs $X_{BH}$/concentration of total organic content $X_V < 0.1$ [62]) is key to ensure plant safety [65]. For a stable sludge (low concentration of OHOs), an activated sludge system with a sludge age of over 40 days is required, which is not common. The PMFC (which is a combination of wetland and SMFC technology as mentioned previously) can potentially be used in stabilising WAS.

Heavy metals such as lead, nickel, mercury, arsenic, cadmium, zinc, copper and caesium can be toxic to agricultural plants and also pose a threat to ecosystems [33]. Removal of these metals, if present in the sludge, is therefore important. If the metal content is kept within the prescribed pollution standards, the sludge can be used as a fertiliser. Kim, et al. [66] showed that vegetables grown with domestic wastewater whose metal content was within the prescribed Korean standards produced vegetables safe for human consumption with respect to heavy metal content.

The nutrient content of sludge is commonly lower than commercial fertilisers. Therefore, larger quantities of sludge are required to be applied when compared to commercial fertilisers [67]. Since the sludge may contain heavy metals, the risk of applying high and repeated quantities of sludge may pose a health risk [68].

In order to use sludge for agricultural purposes in South Africa, stipulated sludge guidelines should be adhered to. The South African sludge handling guidelines adopt the concept of sustainability and therefore management options chosen are safe for the environment. There are three sustainable disposal options recommended [54]:

1) Heat generation of sludge i.e. using its calorific energy value;
2) Using the sludge as a soil conditioner and/or fertiliser; and
3) Extracting useful constituents from the soil.

From the options listed above, agricultural use of sludge is the most viable reuse of sludge in South Africa [54]. The challenges involved with this is public acceptance and ensuring the sludge is safe for agricultural use. To ensure safe sludge, Snyman and Herselmen [62] as part of the South African guidelines, identified three key classifications for the sludge, namely microbiological, stability and pollution classifications. The classifications have three further subclasses. If the sludge does not meet the required classification for agriculture use, other appropriate management options such as heat generation would be used. The subsections to follow further expand on these criteria (the information presented in the subsections is derived from [62] Volume 1 and 2).

### Microbiological classification

The microbial classification is based on the concentrations of faecal coliforms and helminth ova present in the sludge. The sludge is classified based on microbial class A, B and C with A having the least microbial activity. Further information on each of the classifications can be found in Appendix H.

With microbial class A, no restrictions and requirements apply. Sludge with class B cannot be applied to vegetables which are consumed raw. Vegetables which do not touch the soil-sludge mixture can only be consumed 30 days after sludge application. Sludge with microbial class C cannot be used for agricultural application unless stability class 1 or 2 is achieved, thereafter, the same conditions as class B are applicable. The sludge is disinfected to ensure microbial removal [69].

### Stability classification

The stability classification measures the potential of sludge to generate odours, attract vectors, sludge oxygen requirement and potential decrease in volatile solids. Similar to microbial classification, the stability classification also has three subclasses, i.e. 1, 2 and 3 with 1 being the most desirable and 3 being the least desirable. There are 10 options available and at least one of the options needs to be met with 90 percentile compliance for stability class 1, 75 percentile for class 2 and 0 percentile for class 3 (see Appendix H for more information).

**Table 2:** Summary of permissible utilisation of sludge based on the South African Classification System [62].

| **South African Sludge Classification** | | **Is agricultural use an option?** | **Any additional restrictions and requirements?** | **Notes** |
|---|---|---|---|---|
| **Microbiological class** | A | Yes | No | Could potentially be used as a saleable product |
| | B | Qualified yes | Yes | General restrictions/ requirements apply |
| | C | Maybe | Yes | Only applicable if stability class 1 or 2 is achieved |
| **Stability class** | 1 | Yes | No | Could potentially be used as a saleable product |
| | 2 | Qualified yes | Yes | Additional management actions required to encourage compliance with class 1. |
| | 3 | No | Not applicable | Stability class 3 may not be used in agricultural practices. |
| **Pollutant class** | a | Yes | No | Could potentially be used as a saleable product |
| | b | Qualified yes | Yes | If the soil analysis is favourable. |
| | c | No | Not applicable | Pollutant class c may not be used in agricultural practices. |

Sludge with stability class 1 do not have any agricultural restrictions. However, with class 2, the reliability of the vector reduction options would need to be considered and additional management options would be required. Sludge stability class 3 is not suitable for agricultural applications.

**Pollutant classification**

The pollutant classification is based on the presence of heavy metals. These include arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc. The quantity of each is required to be kept below a specific value to classify for pollutant class a, b or c with a having the least metal content and c having the most (see Appendix H). The listed metals are most commonly found in wastewater. However, a full elemental analysis of the sludge should be conducted when possible.

Sludge with pollutant class a do not have any agricultural restrictions. Class b sludge may also be used for agricultural applications, but this would depend on the metal content already existing in the soil. Sludge class c is not suitable for agricultural application.

### 2.2.5 Heavy metals content and phytoremediation

Snyman, et al. [67] surveyed WWTWs in South Africa and found that plants with an inflow less than 10 ML/day and an inflow > 80 ML/d had zinc concentrations of 2956 mg/kg and 3243 mg/kg respectively. These values are greater than the maximum allowed pollutant class A (Zn < 2800 mg/kg). Similarly, Shamuyarira and Gumbo [70] analysed sludge from five towns in Limpopo and found that silver was present in the wastewater, however since guidelines for silver quantity is not present, the impact of silver on the pollutant class could not be evaluated. Shamuyarira and Gumbo [70] also found that two towns produced higher concentrations of lead than what was permissible. Also, all plants surveyed exceeded the allowed zinc and copper concentrations.

Along with the aforementioned heavy metals, some WWTWs add metal salts such as iron and aluminium, because (1) they are coagulants and they significantly improve the settling of PS in primary settling tanks and (2) iron precipitates phosphate out of solution therefore aiding in phosphorus removal [54]. Iron and aluminium have also been added in constructed wetlands (CW) for phosphorus removal [71]. Wood and McAtamney [72] observed 85% and 98% phosphorus removal when aluminium and iron were dosed in a pilot scale CW plant.

Iron present in the sludge plays an important role in wetlands. Iron supresses methanogenesis, removes sulphides and aids in decomposing organic matter [73]. On the other hand, if iron concentration in sludge is too high, it can inhibit plant growth in wetlands. Batty and Younger [74] found that iron concentration exceeding 1 mg/l significantly affected growth of *Phragmites australis*. The maximum iron concentration allowed varies from one plant species to another.

To ensure that the sludge can be utilised for agricultural purposes, the metal content should be kept below the maximum allowed concentrations [62]. Various physical and chemical methods such as encapsulation, solidification, electro kinetics, vitrification and vapour extraction for metals exist

[75]. These methods are expensive and do not make sludge suitable for plant growth. Alternatively, a biological approach, bioremediation, which uses plants and microorganisms for metal extraction is a more sustainable, efficient and economical alternative as it is a more natural process [75].

Heavy metals are not degraded using bioremediation, but rather are transformed from one organic complex or oxidation state to another. This change in oxidation state of metals makes them either less toxic, more water soluble or easily volatilized [75]. Certain plants are able to tolerate higher metal concentrations compared to others. Baker [76] outlined three mechanisms which allow plants to tolerate and extract high metal contents, namely; (1) accumulators – metals are taken up and concentrated in plant parts, (2) excluders – metal concentrations in the shoots are maintained at a constant level even with changing metal concentration in soil and (3) indicators – where metal uptake and concentration inside and outside the plant is maintained at a constant level.

Phytoremediation is cost effective and applicable when pollutants are close to plant roots. Zhuang, et al. [77] studied the phytoremediation ability of eight plant species in a paddy field contaminated with lead, cadmium and zinc. The plant *V. baoshanensis* accumulated up to 28 mg/kg of cadmium and *S. alfredii* accumulated 6,300 mg/kg of zinc. Similarly, Saraswat and Rai [78] conducted a pot experiment with six plant species to test the removal of zinc, nickel, chromium and cadmium. It was found that *A. thaliana* accumulated maximum concentrations of zinc and cadmium while *B. juncea* and *C. dactylon* accumulated maximum concentrations of nickel and chromium showing that the maximum accumulation of each metal is plant dependent.

The common phytoremediation methods are phytoextraction, phytostabilisation, rhizofiltration and phytovolatilization [75, 79]. Figure 10 summarises the phytoremediation mechanisms.

Phytostabilisation uses certain plant species to immobilize metals in soil and ground water therefore reducing their bioavailability through erosion and leaching. Phytoextraction is the absorption of metals in the roots and shoots of plants. This is the most common phytoremediation technology. Metals are recovered from the plant through incineration of the roots and shoots. Rhizofiltration involves absorption or precipitation of metals onto plant roots. It also involves the absorption of metals that are in solution form around the roots. This is applicable in constructed wetlands to extract metals from wastewater. In phytovolatilization, contaminants are taken up, converted to volatile forms and released from the shoots into the atmosphere. The three phytoremediation techniques could potentially be used to extract metals from sludge when used in the PMFC to ensure the metal content is within the agriculture use of sludge guidelines.

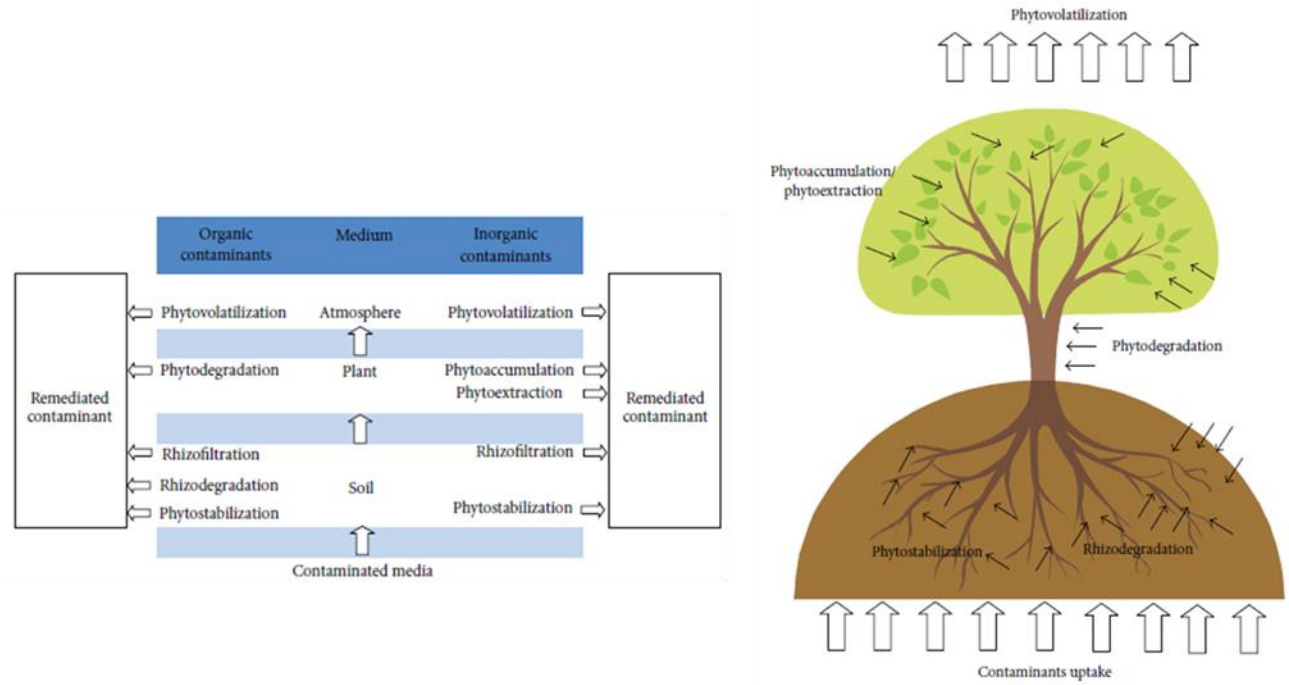



**Figure 10:** Schematic of different phytoremediation metal uptake mechanisms [79].

## 2.3 Substrate feeding mechanism

The PMFC substrate feed mechanism could be either continuous or batch. Continuous flow is further split into surface flow and sub-surface flow [13] The choice of feed system depends on the PMFC design and substrate used. Meaning, if the design is based on using gardening soil as a substrate (where plant roots provide organics), it is physically impossible to have a continuous system. However, if the substrate is a liquid or liquid with a low solid concentration (example WAS with solids concentration of 4 g/l), a continuous flow is possible.

Studies done by Villaseñor, et al. [19] and Fang, et al. [26] used continuous systems. The main difference being the direction of flow. Villaseñor, et al. [19] used a horizontal flow system while Fang, et al. [26] used a vertical up-flow system. The benefit of using a continuous system comes from the enhanced redox gradient between the electrodes [21]. Also, an up-flow system allows the substrate to move from the deeply embedded anaerobic anode to the cathode area allowing time for the complete treatment of substrate before it reaches the cathode [21].

## 2.4 Inoculum source, application and start-up time

Anodes are inoculated to increase the presence of active bacteria and decrease start-up time. This becomes especially important when a pure substrate such as glucose or acetate is used. Three main inoculum sources have been used in previous studies, namely; anaerobic digestor sludge, from an operating MFC and wetland/swamp soil [19, 23, 26, 31, 34-36, 80-85].

Liu, et al. [80] showed that a higher PPD is generated when inoculum is sourced from an operating MFC compared to AD sludge. Furthermore, Nitisoravut and Regmi [34], showed that inocula from different sources did not change PPD with time, but showed that substrate used significantly affected PPD's. Interestingly, Aelterman, et al. [82] mixed sludge from an AD and activated sludge system (contains oxygen) and used it as inoculum. They achieved a PPD of 228 $W/m^2$ with 12 stacked MFC's.

The application of inoculum changes from one research to another. Fang, et al. [26] and Liu, et al. [83] used a continuous operation fuel cell and inoculated with AD sludge by running the system on the sludge for a few hours. Villaseñor, et al. [19] also used a continuous system, but injected substrate from an operating MFC on the electrode. Regmi, et al. [23] coated the anode with inoculum before placing it in the PMFC when using a batch system.

The start-up times differ significantly from one research to another. Start-up time essentially measures the duration it takes for the voltage to obtain maximum stable values. Based on a literature study, Liu, et al. [80] found that start-up times vary from 10 seconds to a few months with the longest time taken in PMFCs using soil as substrate and depending on exudates for power generation. It was also found that using AD sludge as inoculum required 50 hours start-up time while using effluent from a primary settling tank required 140 hours. When using AD sludge as inoculum, Fang, et al. [26] required several days for the PMFC to start-up when using a continuous system while Santoro, et al. [85] required 24 hours to a few days when using a batch system.

From the above, it is apparent that even though sludge is taken from AD's or MFC's which are meant to be anaerobic, exposing them to oxygen does not affect performance given suitable time to acclimatize. Also, the studies which used AD sludge did not see a decline in performance as sludge is taken from operating in 37℃ to on average 25℃ to match lab conditions. Given the large variation in start-up time, it is impossible to predict the exact duration, but when using sludge as substrate and AD sludge as inoculum, start-up time should be within a few days.

## 2.5 Electrodes

As discussed previously, MFCs compose of two electrodes, namely, a cathode and an anode. The anode collects electrons produced through anaerobic breakdown of organics, while the cathode releases electrons in an aerobic environment to form water [86]. The chemical equations below give an example of the reactions that occur on the anode and cathode. The anode reaction is not limited to glucose, but rather any organic compound.

| | | | |
|---|---|---|---|
| Anode: | $6CO_2 + 24H^+ + e^- \rightarrow C_6H_{12}O_6 + 6H_2O$ | pH = 7 | E = -0.428V |
| Cathode: | $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ | $pO_2$ = 0.2, pH = 7 | E = +0.805V |

Therefore, the potential voltage from a cell undergoing the above reaction would be :

$$E_{cell} = E_{cathode} - E_{anode} = 0.805 - (-0.428) = +1.233\ V$$

### 2.5.1 Anode

The anode performs two main functions, namely, the collection of electrons and to provide a habitat for the electrochemically active bacteria [87]. The anode material chosen should therefore have these properties [88]:

1) Low resistance (high electron affinity) and good electric conductivity;
2) Anti-corrosive;
3) Strong bio-conductivity; and
4) Large surface area.

Due to their good bio-compatibility and anti-corrosiveness, carbonaceous materials are most commonly used [87, 88]. Metals have also been used in some studies producing low power outputs, i.e. a maximum of 4 $mW/m^2$ has thus far been recorded. This can be a cause of their poor bio-compatibility [89]. The commonly used carbonaceous materials are, carbon paper, carbon fibre, graphite rod, carbon felt, granular activated carbon (GAC) and graphite fibre brush. Traditionally, graphite rods were used as anode material due to their good electrical conductivity [88]. The limitation with using graphite rods arises from their low porosity and surface area for microorganism function. This problem was solved using a graphite felt which produced approximately three times more power than graphite rods [90]. Graphite felt is a porous material with large apertures allowing for an increased surface area [88].

Anodic surface area plays a key role, three-dimensional anodes allow for a greater microbial activity [88]. Two-dimensional anodic materials such as carbon paper and carbon fibre have been stacked in layers in the past to produce a three dimensional material effect, Ahn and Logan [91] did this by weaving carbon fibre on titanium wires (as titanium is non-corrosive). Graphite felt, and weaved carbon fibre can be expensive, a cheaper alternative would be GAC [88]. Apart from being cheap, GAC can greatly improve bacterial adhesion (good bio-compatibility), it can be easily mixed with sediment and can create new connections when the GAC bed is disturbed by growing plants [21, 88]. Scanning electron microscope (SEM) pictures taken by Jiang and Li [17] showed a good adhesion of bacteria on the GAC material.

GAC is greatly affected by granule size and quantity of GAC used. In one study, particle sizes between 0.25 and 0.50 mm produced 77.7 $mA/m^2$, but when the particle size was increased to between 1.0 and 5.0 mm, the power dropped to 37.9 $mA/m^2$ [21]. In another study which varied the mass of GAC used, it was seen that increasing the mass from 400 g to 700 g of GAC increased power from 4.2 to 7.2 $W/m^3$ [88]. Similarly, Jiang and Li [17] showed that increasing GAC volume increased biomass attachment and ultimately increased the power for the system.

The problem associated with increasing surface area as the size of MFC is increased, is the increased electron pathway. Graphite which has a higher resistivity relative to metals (1375 μΩcm versus 42 μΩcm for titanium), has a linear resistance increase with increasing electron pathway; this could be a major concern with scaled up MFCs [21].

Liu [18] achieved 2920 mW/m$^2$ in an SMFC. Their anode composed of an inverted tube filled with GAC and titanium rods as electron collectors. The inverted tube allowed water to enter the tube flushing out the trapped sediment to increase the efficiency and longevity of the anode. Titanium which has a higher resistivity when compared to copper ($4.2x10^{-7}$ verses $1.68x10^{-8}$) is used as it does not corrode. A cheaper alternative to titanium would be nickel coated copper where the nickel acts as a protective layer. However, the challenge with this is that nickel coated copper cannot be easily sourced.

Liu, et al. [92] also found that using multiple electron collectors in the anode connected to multiple cathodes significantly increased voltage output of the benthic MFC. Also, An, et al. [16] found that voltage values can be further increased by placing electrodes in both, the sediment phase and the liquid phase. However, in both of these studies, the electrode surface area was doubled and/or tripled instead of observing the power output when keeping the overall electrode area constant. It would be beneficial to study the difference in voltage output if the total electrode area in a multiple electrode system is the same as a in the single electrode system.

### 2.5.2 Cathode

This driving force behind the movement of electrons in a MFC is the difference in redox potential [15, 88]. Oxygen is the most widely used electron acceptor and its concentration should be kept above 3 mg O/l [93]. The most commonly used cathode materials include graphite, carbon cloth and carbon paper.

The performance of a cathode can be enhanced by coating it with a highly active catalyst such as platinum (Pt). Cheng, et al. [94] showed that varying the Pt loading from 0.1 mg/cm$^2$ to 2.0 mg/cm$^2$ only slightly varied the power produced. The performance of the a MFC can also be affected by the area ratio of the anode to the cathode. Hong, et al. [93] varied the ratio of anode/cathode area and observed a linear decrease in current density as the ratio decreased (Table 3)

**Table 3:** Ratio of anode surface area to cathode.

| Ratio | Current density (mA/m$^2$) |
|---|---|
| 1:1 | 35.1 |
| 1:1/2 | 21.9 |
| 1:1/5 | 11.4 |
| 1:1/10 | 8.0 |

## 2.6 Power measurement

The power generated in MFCs can be measured in multiple ways. The simplest way is to measure the voltage across an external resistance connected between the electrodes and using Equation 1 to calculate the power.

$$P = \frac{V^2}{R} \qquad \text{(Equation 1 [82])}$$

The maximum power density can be achieved by using the optimal resistor. This value can be determined by doing a polarisation test to obtain a polarisation curve.

## 2.7 Polarisation curves

The performance of an MFC can be evaluated from polarisation curve. The polarisation curve presents the voltage and power as a function of current. Polarisation curves can be used on each electrode individually to evaluate its performance. More commonly, polarisation curves are used to evaluate the performance and maximum power output for the entire fuel cell using a potentiostat [95]. The risk with measuring the performance of both electrodes together is that it does not give information on which is the limiting electrode i.e. the anode or cathode.

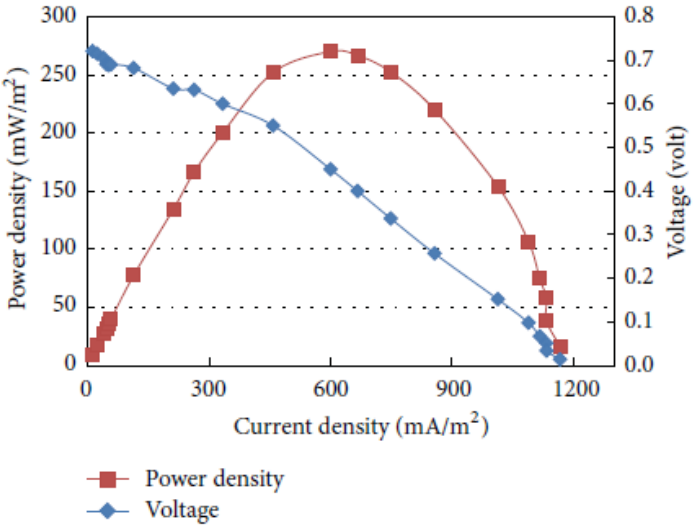



**Figure 11:** An example of expected polarisation test results. This was taken from Ismail and Jaeel [12] where a PPD of 260 mW/m$^2$ was observed.

The polarisation test should be conducted from low resistance values (about 10 ohms where the voltage reading is close to zero) up to high resistance values (about 1 megaohm where the voltage value converges to open circuit voltage) [95]. For each resistance, the voltage reading should be taken after a few minutes to ensure a pseudo-steady state voltage is reached. Figure 11 shows an example of a polarisation curve.

The gradient of the voltage-current graph gives internal resistance (see Equation 2). The internal resistance is the sum of resistances caused by electrode distance (increase in electrode distance increases resistance), solution resistances, quantity of exoelectrogenic bacteria present (less bacteria increases resistance) and electrode reaction resistances (depends on the redox potential and solution conditions) [96, 97]. When the external resistance connected, matches the internal resistance of the cell, peak power is obtained (maxima of power-current graph). Keeping internal resistances low is essential to ensure increased maximum power densities.

$$R_{internal} = \frac{\Delta V}{\Delta R} \qquad \text{(Equation 2 [82])}$$

# 3. PMFC design and methodology

## 3.1 Introduction

PMFCs are an emerging technology in which plants are grown to increase the efficiency of the slightly better-known SMFCs. From literature, it is known that plants provide oxygen that can be used by the cathode. Plants also provide root exudates containing organics that can be used by the anode. It is important therefore to understand the role plants play in a fuel cell by comparing PMFCs power outputs to those of SMFCs.

Since these are the beginning stages of PMFCs and its ability to treat wastewater at the University of Cape Town (UCT) and around the world, a batch system was investigated in this research instead of a continuous system. In this chapter, the design of a PMFC and operating conditions are discussed. The design and materials chosen were based partly on literature describing material performances and partly on past experiments conducted. This chapter also expands into the different plant species chosen. Lastly, this chapter provides summary tables of the experiments conducted during the course of this research.

## 3.2 Plant species chosen and sourcing

For this research, three indigenous plant species were chosen. As explained in Section 2.1.5, it was important that the species chosen would be able to withstand anaerobic conditions, a feed high in COD and FSA and also survive in waterlogged conditions as a batch system was used in the lab. Therefore, wetland plants were seen as suitable option for this research.

The plant species chosen were, *Cyperus papyrus nanus*, *Wachendorfia thyrsiflora* and *Phragmites australis* (see Figure 12). The *C. papyrus nanus* (hereon referred to as *C. papyrus*) is more commonly known as the miniature papyrus or dwarf papyrus because of its resemblance to *C. papyrus L.* [98]. The *C. papyrus* belongs to the *Cyperceae* family and is found in the Eastern Cape and KwaZulu-Natal in South Africa. The *C. papyrus* has a fibrous root system and is best grown in neutral pH soils. Also, this plant can grow in palustrine, lacustrine and riverine wetland types [45]. This makes it very suitable for use in the PMFC and its potential use in potential future continuous systems.

The *W. thyrsiflora* more commonly known as the blood root or red root belongs to the *Haemodaraceae* family [99]. It is found in the Eastern and Western Cape in South Africa. The *W. thyrsiflora* has a fibrous root system and prefers growing in acidic to neutral soils. According to the Stark Ayres nursery staff, the *W. thyrsiflora* exhibits rapid root growth making it suitable for use in PMFCs. This plant has also been used in constructed wetlands to treat wastewater [42].

The *P. australis* more commonly known as the common reed is a robust wetland plant species that has been used to treat wastewater [42] and in PMFCs [24]. *P. australis* belongs to the *Poaceae*

family and grows in all provinces in South Africa. It also has a fibrous root system and can tolerate any soil condition. Similar to *C. papyrus* this plant can also grow in palustrine, lacustrine and riverine wetland types [45]. This makes it very suitable for use in the PMFC and its potential use in potential future continuous systems.


**Figure 12:** Shows the three-plant species used in this research. A – *C. papyrus* [45] B – *W. thyrsiflora* [100] and C – *P. australis* [101].

The *C. papyrus* and *W. thyrsiflora* were sourced from Kirstenbosch National Botanical Garden Centre located in Cape Town. The *P. australis* was sourced from a bird sanctuary called Intaka Island which is also located in Cape Town. All plants were at vegetative growth stage, a stage that occurs after the seeding and before budding. Also, a single root bulb for each plant per set-up was used.

## 3.3 Indoor green house

For the purposes of this research, the experiments were conducted in the Water Research Lab located in the New Engineering Building at the UCT. This was done to prevent a potential health hazards as the lab was built for wastewater research. Since the research involved growing plants indoors, an indoor greenhouse, open on one side, was built to provide plants with the required growing conditions i.e. light, water, air, substrate and temperature as outlined in Section 2.1.7.

### 3.3.1 Plant lighting and duration

As explained in Section 2.1.7 plant lighting plays a significant role in plant growth as it is a key ingredient for photosynthesis. The lighting chosen for the indoor greenhouse is required to provide the same light spectrum as the sun. COB LED lights with a power consumption of 50W were found to contain both the red and blue spectrum and were therefore used for this research (see Figure 13). These lights were purchased from the company COBLED Grow (http://cobledgrow.com).

To increase the performance of the green house and to mimic sunlight, the green house was covered in Sisalation which provided with 90% reflection of the light. The Sisalation was sourced from a local hydroponics store. The light was on for 12 hours during the day i.e. from 06h45 to 18h45.

**Figure 13:** 50W COB LED Grow Light purchased from COBLED Grow. COB LED lights are able to emit both the red and blue light spectrum that are required for plant growth.

### 3.3.2 Temperature

Since the experiments were conducted in the Water Quality Lab, the temperature was governed by the lab thermostat which was pre-set to 20℃. This temperature was seen suitable for plant growth based on literature (see Section 2.1.7).

### 3.3.3 Airflow

Since the plants require $CO_2$, a fan was provided to ensure air recycle and therefore constant supply of carbon dioxide. Apart from air recycle, the fan also served another purpose, it produced wind which allowed the plant roots to grow more vigorously as the roots need to stabilise the plant.

### 3.3.4 Substrate

The substrates used varied from one experiment to another. The plant can be grown in either a solid or a liquid substrate (hydroponics). For the first experiment, a solid substrate i.e. a soil mixture and thickened WAS was used. In the second, fourth and fifth experiment, liquid WAS was used, and the third experiment used PS. Further information on the substrates is provided in the respective Chapters.

## 3.4 Electrodes used

### 3.4.1 Anode

GAC was used as the anode. From literature, it is known that GAC can conduct electricity and therefore it can collect electrons released from the exoelectrogenic bacteria [17, 21, 26, 88, 89]. However, the important aspect to consider when choosing the granular size of the activated carbon is that it can pack well to cover the electron acceptor and host bacteria. To ensure the GAC used was capable of this, a GAC performance test was conducted which is further explained in Section 3.4.2.

### 3.4.2 Performance of GAC when housing bacteria

GAC has previously been shown to house bacteria very well [102]. However, its ability and hence power production varies based on particle size [21]. To understand whether or not the GAC purchased (i.e. particle size of 2-3 mm) was able to house bacteria, a small-scale experiment was set up where GAC as a bacteria host was compared to that of carbon fibre (a material which has been successfully used in the past to host bacteria) and no a no-host scenario i.e. inoculum mixed with substrate without using a carbon material.

#### 3.4.2.1 Experiment design and methodology

The following experiments were run in duplicates the lab:

1) Inoculated GAC
2) Inoculated GAC contained in a porous bag
3) Inoculated carbon fibre
4) Inoculum only
5) Uninoculated GAC to act as control
6) Uninoculated carbon fibre to act as control

For this experiment, acetate was used as the organic source to feed the bacteria. A molar ratio of 10 mmol/l was prepared in the lab using the salt sodium acetate salt. A concentration of 10 mmol/l of acetate theoretically translates to 600 mg/l of acetate.

The experiments were conducted in sealed 600 ml buckets. Each bucket contained a 3 mm internal diameter silicon sampling tube from which 15 ml of acetate was sampled every two days. The concentration of the sample was measured to understand how it decreased with time. The bucket also contained another silicon tube that led to another bucket filled with water to allow for $CO_2$ generated with the consumption of acetate to escape. The water in the second bucket ensured that oxygen would not feed back into the sealed system. Figure 14 below shows the experimental set up used.

The same inoculum as described in Section 3.6 was used. Each set-up used 40 ml of inoculum. In a glass beaker 100 ml of GAC was thoroughly mixed with inoculum. For the experiments with carbon fibre, a mass equivalent of the 100 ml GAC was cut out and inoculum spread over it.

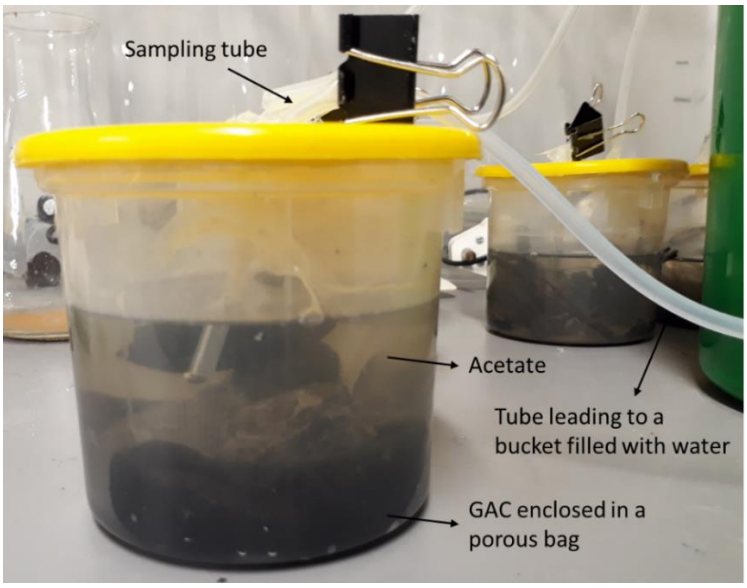



**Figure 14:** GAC performance test experimental set-up. Acetate of 10 mmol/l was utilized by bacteria hosted by different materials and its COD measured every three days.

### 3.4.2.2 Results and discussion

The concentration of each set-up was measured every two days as previously explained. The concentration was measured in the Water Quality Lab using a 5-point titrator [103]. The results obtained are shown in Figure 15. Since the experiments were run in duplicates, an average of the COD was taken for two set-ups to plot the graph shown in Figure 15.

From the results, it was observed that both GAC set-ups (whether enclosed in a porous bag or not) consumed the acetate within six days. This occurred at a faster rate when compared to the other set-ups. The set-up with inoculated carbon fibre reduced the aqueous acetate concentration in under eight days. The concentration results when using carbon fibre were similar to those of inoculated GAC. At day six, inoculated carbon fibre read a concentration of 30 mg/l.

The set-up with the inoculum but no carbon material took the longest to consume the acetate. The concentration reading on day eight showed that the acetate concentration was completely depleted, which was the same for the carbon fibre experiment. However, it can be seen that carbon fibre was better at hosting bacteria since the concentration at day 6 was 40 mg/l while in the inoculum only the was 80 mg/l.

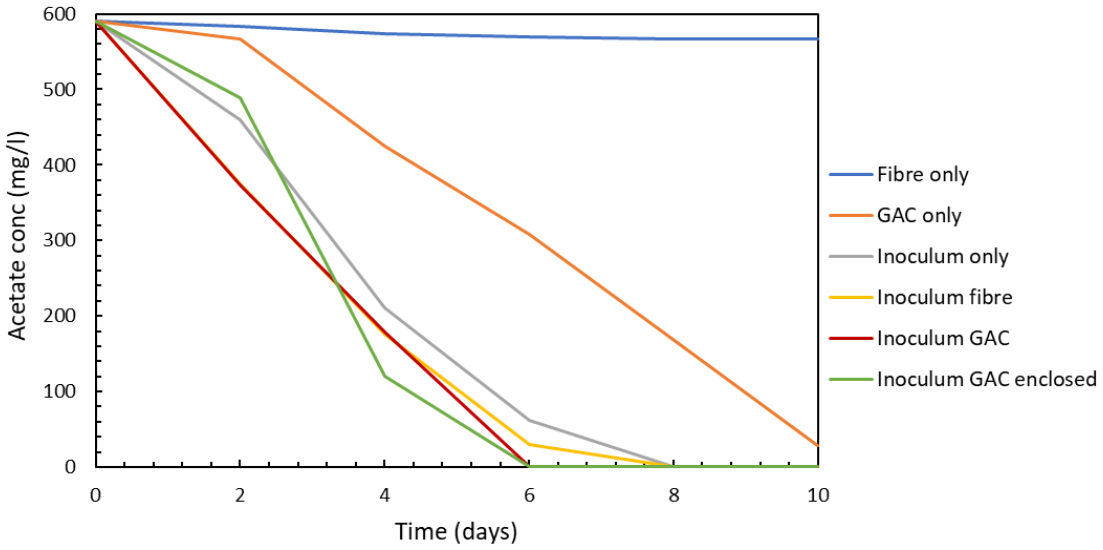



**Figure 15:** Shows how the concentration of acetate varies over the course of ten days to test the performance of GAC as a suitable bacteria host.

Given this information, it becomes important to answer whether or not the rate of acetate reduction by inoculated GAC, was due to its ability to host bacteria or because, along with hosting the bacteria, it was absorbing acetate. This was answered by looking back at the inoculation procedure. Since the GAC was mixed with the inoculum first, it would absorb any soluble organic component of the inoculum before acetate mixture. Therefore, the former i.e. GAC is a good bacteria host must be true instead of the later.

#### 3.4.2.3 Conclusions drawn

From the experiment conducted, it was seen that the GAC with a particle size of 2-3 mm was a suitable host for bacteria and was used in the PMFC. This GAC was then inoculated for all PMFC experiments. Acetate was not added as it was only used in this experiment to test the GAC performance.

### 3.4.3 Cathode

Carbon sheet with a platinum coating of 0.4 mg/cm$^2$ i.e. a membrane electrode assembly was used for the cathode. The carbon sheet was sourced from HyPlat. The area of the cathode was limited to 1/4 of the area of anode chosen. From literature it is known that an anode-cathode area ratio less than 1/5 significantly impacts the current densities [93]. Since this research is aimed at comparing performance of plant species and different wastewater substrates and will only later focus on optimisation, it was decided to limit the cathode area to 1/4 of anode area keeping cost in mind.

Since the area of the GAC (which in this calculation was assumed to be non-granular) covered the diameter of the bucket and the diameter equalled 18.5 cm, the cathode area used was to 67 cm$^2$.

### 3.4.4 Electron collectors

Electron collectors are wires which transport electrons deposited by the bacteria on the anode to the cathode. Since the anode is saturated and in an anoxic/anaerobic environment, copper could not be used as it would corrode. In addition, aluminium, which is corrosion resistant in aerobic environments, could not be used because of the anoxic/anaerobic conditions at the anode. Stainless steel was an option, but nickel coated copper was used instead as it has a lower resistivity when compared to stainless steel.

The nickel coated copper wires used were 0.2 mm in diameter. They were wound three times making the diameter 0.6 mm. This was done twice meaning that each electron acceptor had two 0.6 mm nickel coated copper wires. Even though the diameter was now 0.6 mm, the wires were difficult to work with, therefore they were soldered to copper wires and sealed off with silicon to stop water from entering the connection (see Figure 16). This allowed easier connections to the voltmeter as the copper wire was 1.5 mm in diameter.

To further increase the electron collector surface area, the nickel coated copper wires were soldered to chicken wire (galvanised steel mesh). This was done to increase the surface area of the electron collectors. As shown previously by Liu [18], increasing the number of electron collectors increases the power density. A mesh of 12 cm by 12 cm giving an area of 144 $cm^2$ was used. Galvanised steel was used as it was easy to source. However, it is recommended to use stainless steel mesh as it is more robust.

To collect the electrons from the anode, the electron acceptors were embedded in the GAC. The nickel coated copper wires were stuck on the non-platinum coated side to the cathode to transfer the electrons. Even though the nickel coated copper wire part of the electron acceptor is 0.6 mm, it was difficult to lay the wire flat and therefore from a practical aspect 20 mm of GAC was required to fully cover it.

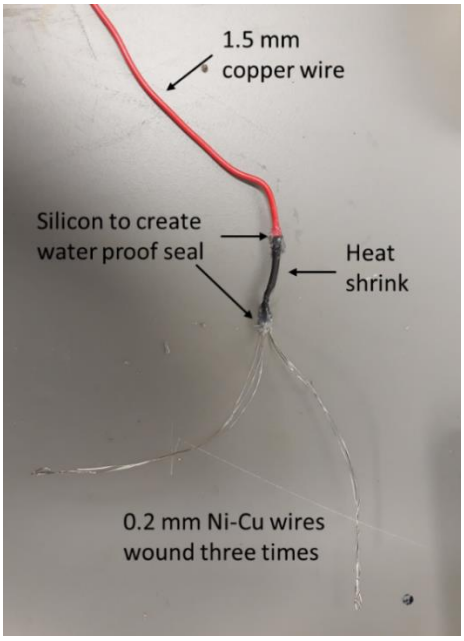



**Figure 16:** Electron acceptor used in the PMFC. Nickel coated copper wires with diameter 0.2 mm were wound three times and soldered to a 1.5 mm copper wire to make connections easy.

## 3.5 Design of PMFC

Having discussed all the materials used in the PMFC, this section describes the construction of the batch system. The design depended on whether the substrate used was a solid or liquid mainly to accommodate the plant. The experiments were done in 5 litre buckets whose dimensions are provided in Figure 17.

The similarity between both solid and liquid substrate was that the plant was placed closer to the edge of the bucket to allow for the cathode to fit. Since *C. papyrus* is bigger than the *W. thyrsiflora* and *P. australis*, it provided less leeway when setting up the PMFC as moving it closer to the centre would mean the cathode would not fit. Also, in both cases, a layer of water on top of the cathode was required to ensure the cathode does not dry out and lose contact with the sediment.

Plant roots are known to increase microbial activity and therefore increase power generation [20, 24]. However, the roots penetrating the anode can also increase the internal resistance [96]. In this research, all the experiments were done without the use of a separator between the electrodes (except for one experiment) to allow root growth and therefore increase microbial activity.

### 3.5.1 Solid substrate

The plant after collection was removed from its pre-existing pot and transferred directly into the 5-litre container when setting up the PMFC systems with a solid substrate. The soil that was encasing the roots was not washed out as the substrate used in the PMFC was a solid. This was done based on the advice received from the growers at the nursery.

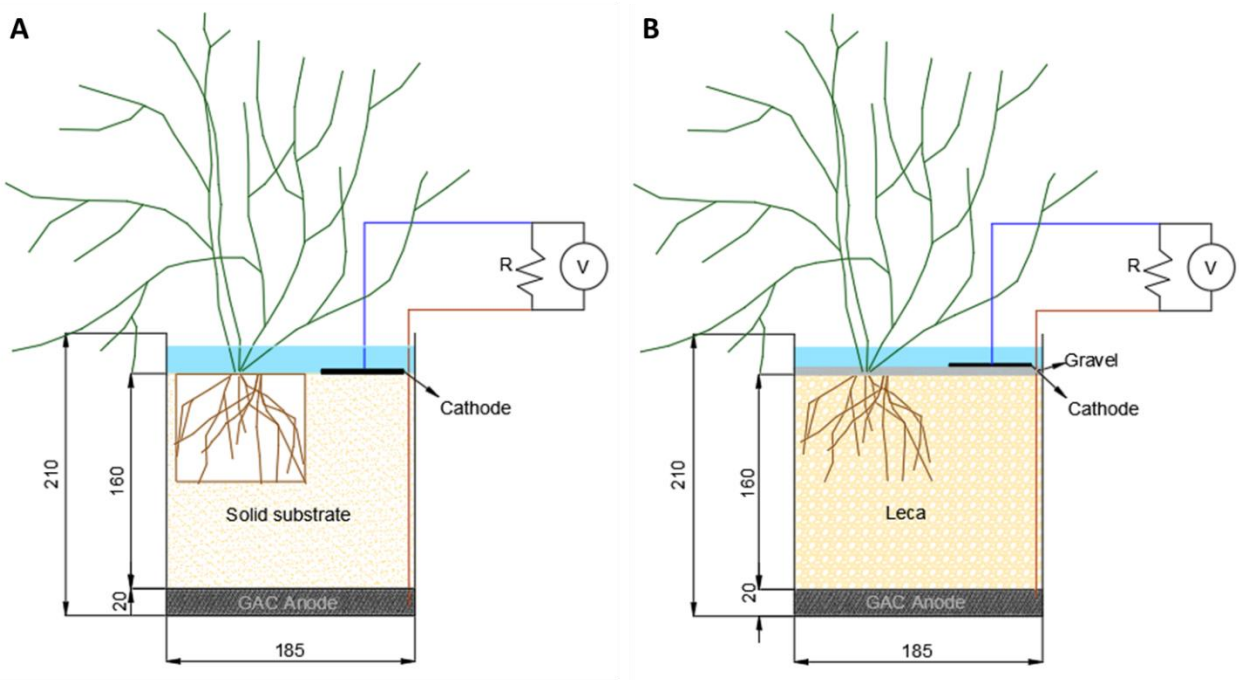



**Figure 17:** Design of PMFC when using a solid (A) and a liquid (B) substrate.

### 3.5.2 Liquid substrate

When using a liquid substrate, the plant was grown hydroponically. In this method, the plant was not supported by soil but rather by expanded clay aggregate (leca). Past research have also made used of gravel as the support material [26]. The leca was sourced from a local hydroponics store. The leca size ranged from 8-16 mm. The problem encountered with leca was that it floats in water. This caused the cathode to lose contact with the sediment. To counter this, a layer of gravel was added on top of the to keep it in place.

When using a liquid substrate, the plant bought from the nursery was washed to remove all the soil thus exposing the roots. Before this step however, the plant was removed from its pre-existing pot and was submerged in water for 4 to 7 days as advised by the nursery to help the roots acclimatise to growing in soilless conditions. This plant was then potted into the PMFC.

## 3.6 Inoculum source and application

The inoculum was sourced from an anaerobic digestor operating in the Water Quality Lab at UCT as part of another master's students' research. The AD used a mixed culture and the aim of this particular master's student research was to grow PAO's and measure its growth kinetics. The AD was fed with activated sludge from Zandvliet WWTWs which used the UCT system. The UCT system is an enhanced bio-phosphate removal system used in wastewater treatment to remove phosphorus using PAO's. Please refer to Wentzel [104] for more information on the UCT System.

Watson and Logan [105] showed that using a pure culture of exoelectrogenic bacteria, such as *Shewanella,* produced lower power output compared to a mixed culture. Furthermore, from Section 2.4, it is evident that anaerobic sludge is a good source of inoculum as it achieved high power outputs and short start-up times. Using substrate from an operating MFC may have been a better source but this could not be sourced.

The anaerobic digestor was operated at 37°C in the lab. The PMFC however was operated at 20°C as discussed in Section 3.3.2. This reduced temperature may affect the inocula and increase start-up times, but previous studies using AD sludge [26, 80, 82-84] did not report any impact on the inoculum. Since batch operation was used, the inoculum was mixed with the anode in a separate beaker before being added to the PMFC. This is a similar approach used by Regmi, et al. [23]. For each set-up, 590 ml GAC was required to obtain a 2 cm layer of GAC (see Section 3.7). This volume was inoculated with 250 ml of AD sludge to ensure the GAC is fully 'wet' before adding it to the PMFC. All the PMFC's were inoculated during the course of the research, if any system was not inoculated, it is stated in the relevant experimental design section.

## 3.7 Voltage measurement and polarisation test

To measure the power output of the PMFC, the electrodes were connected across an external resistor and the voltage measured across the resistor was logged using the National Instruments USB-6000 data acquisition device. An external resistor was used for two reasons, firstly open circuits do not provide a measure of power as the resistance is infinite and current is zero. They just measure the electromotive force (emf) of the cell. Secondly, external resistances have shown to increase microbial activity [106].

However, even though the external resistance allows us to calculate power, it does not give the maximum power density that a cell can provide. The PPD provides a real measure of the cell performance and it can be obtained by doing a polarisation test.

The polarisation test also allows for the internal resistance of the cell to be determined. The test was done by measuring voltage across different external resistors as described in Section 2.7. A sample test is provided in Appendix A. Since GAC is a granular material, the peak power density was calculated based on the geometric anode volume, similar to Fang, et al. [26]. The conversion factor of power from the bucket to per $m^3$ was calculated bearing in mind the GAC depth (2 cm) and diameter (18.5 cm). This was done as follows:

$$\text{multiplication factor} = \frac{1}{\pi \times \left(\frac{D}{2}\right)^2 \times \text{depth}} = \frac{1}{\pi \times \left(\frac{0.185}{2}\right)^2 \times 0.02} = 1860$$

$$\text{Power in W per bucket} = \frac{V^2}{R}$$

$$\text{Power in mW per m}^3 = \frac{V^2}{R} \times 1860 \times 1000 \qquad \text{(Equation 3)}$$

The internal resistance of each system is calculated using the slope of the voltage current graph. Since the current is in mA/m$^3$, the conversion factors were applied to it as well.

$$R_{in} = \frac{\Delta V}{\Delta A} \times 1860 \times 1000 \qquad \text{(Equation 4)}$$

## 3.8 Organic, FSA and OP removal efficiencies

### 3.8.1 Measurement

The organic removal was measured in terms of the volatile suspended and settleable solids (VSS), COD, total Kjeldahl nitrogen (TKN) and total phosphates (TP). The tests for these were done at the start of the experiment (initial) and at the end of the experiment for all systems. In between measurements were not possible as sludge contains solid particles which settle and therefore erroneous results would have been obtained. The removal efficiencies were calculated using the formula:

$$\text{removal efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100 \qquad \text{(Equation 5)}$$

Where *C* represents VSS/COD/TKN/TP/FSA/OP and *i* is initial concentration in mg/l and *f* is final the concentration in mg/l.

For the FSA and orthophosphate (OP) the measurements were done at three-day intervals to provide a removal graph.

### 3.8.2 TKN removal process

The TKN is a sum of the FSA ($NH_4^+/NH_3$) and organically bound nitrogen (OrgN). The OrgN is released into the system as FSA when the VSS breaks and adds to the FSA pool. The TKN is then removed by converting FSA to nitrates. Nitrates are not considered when measuring TKN but do contribute to total nitrogen (TN) of the system.

$$C_xH_yO_zN_a + (2x - z)H_2O \rightarrow xCO_2 + aNH_3 + (y + 2(2x - z) - 3a)H^+ + (y + 2(2x - z) - 3a)e^-$$

$$aNH_4^+ + (5a/2)O_2 \rightarrow aNO_3^- + (2a)H_2O$$

### 3.8.3 TP removal process

TP is a sum of the OP ($H_2PO_4^-/HPO_4^{-2}/PO_4^{-3}$) and organically bound phosphorus (OrgP). The OrgP is released into the system as OP when the VSS breaks and adds to the OP pool. The TP is removed from the system in a combined effect of filtration media, plants and bacteria [20]. OP is one of the

three key elements for plant growth; therefore, plant growth contributes to OP removal which in turn contributes to TP removal.

$$C_xH_yO_zP_b + (2x + 4b - z)H_2O \rightarrow xCO_2 + bH_3PO_4 + (y + x + 8b - 2z)H^+ + (y + x + 8b - 2z)e^-$$

$$aNH_4^+ + (5a/2)O_2 \rightarrow aNO_3^- + (2a)H_2O$$

Furthermore, OP is removed from solution by PAO uptake. PAOs store the OP. This means that the overall TP content of the sludge does not decrease, but rather, the TP and OP of a filtered sample decreases. This is implemented in WWTPs to meet effluent standards set by governments.

### 3.8.4 Linking it to fertilisers

The agricultural application of sludge depends on three classification criteria i.e. microbiological, stability and pollutant removal classification [62]. Given the limitations of this research, the microbiological class of the sludge before and after it was used in the PMFC could not be evaluated. However, activated sludge treatment of wastewater has shown to remove up to 99% of bacteria, but there still remains a high concentration of pathogenic micro-organisms which require further treatment [54]. The pollutant removal was not quantified as well because of equipment limitation. However, given previous studies (see Section 2.2.5) it is suspected that plants would aid in phytoremediation.

#### 3.8.4.1 Stability classification

The stability classification requires any one vector reductions to be met. The vector reduction that this research focused on the ability of the PMFC to reduce volatile solids by 38% after the use of sludge in PMFC.

#### 3.8.4.2 Nitrogen and phosphorus ratio

After excluding systems that did not meet above criteria, their nitrogen to phosphorus ratio was calculated as the measurement for TKN and TP was already done. This ratio is a very basic measure of the expected nutrient ratio and is incomplete. Since two of the three parameters that need to be assessed before sludge can be classified have not been measured, the sludge cannot be used as a fertilizer even if the stability class is met.

## 3.9 Summary

A total of six experiments were conducted in this research. The first used a solid substrate the remaining ones used a liquid substrate as operating on a liquid substrate is more practical in the context of WWTWs. The aim, experimental conditions and durations are provided in Table 4.

**Table 4:** Summary of experiments conducted during the course of the research.

| Experiment name | Substrate tested | Systems (x n)[1] | Duration (days) | Aim |
|---|---|---|---|---|
| Solid substrate | Thickened WAS sourced from Zandvliet WWTW | *C. papyrus* x 2<br>*W. thyrsiflora* x 3<br>Control (no plant) x 2 | 52<br>Except for *C. papyrus* in soil which was run for 60 days | 1. Understand the operation of the PMFC based on the following criteria:<br>• Importance of having a wet cell<br>• Difference between using distilled water and tap water<br>• Cathode contact area<br>• Size of resistors connected<br>2. Obtain the voltage output, peak power densities and internal resistance of the substrates used.<br>3. Assess the plant health grown in soil versus being grown in sludge. |
| | Soil mixture | *C. papyrus* x 2<br>*W. thyrsiflora* x 3<br>Control (no plant) x 2 | | |
| Liquid WAS | WAS sourced from Zandvliet WWTW | *C. papyrus* x 3<br>*P. australis* x3<br>*W. thyrsiflora* x 3<br>Control (no plant) x 3 | 44 | 1. Obtain the variation in voltage output, peak power densities and internal resistance of the substrate used, and the plant species tested.<br>2. Obtain the variation in organic removal, FSA and OP removal efficiencies of the plant species tested.<br>3. Assess the N:P ratio of the systems with a VSS removal > 35% |
| Primary sludge | Primary sludge sourced from Potsdam WWTW | *C. papyrus* x 3<br>*P. australis* x3<br>*W. thyrsiflora* x 3<br>Control (no plant) x 3 | 28 | 1. Obtain the variation in voltage output, peak power densities and internal resistance of the substrate used, and the plant species tested.<br>2. Obtain the variation in organic removal, FSA and OP removal efficiencies of the plant species tested.<br>3. Assess the N:P ratio of the systems with a VSS removal > 35% |

| Experiment name | Substrate tested | Systems (x n)[1] | Duration (days) | Aim |
|---|---|---|---|---|
| Optimisation 1 | WAS sourced from Zandvliet WWTW | *C. papyrus* with a separator x 3<br>*C. papyrus* x 3 | 44 | 1. Obtain the variation in voltage output, peak power densities and internal resistance when using a separator versus not using a separator.<br>2. Obtain the variation in organic removal, FSA and OP removal efficiencies when using a separator versus not using a separator<br>3. Assess the N:P ratio of the systems with a VSS removal > 35% |
| Optimisation 2 | WAS sourced from Zandvliet WWTW | All systems were planted with *C. papyrus* as this was chosen as the most suitable plant species<br>Control x 3<br>Multiple electrodes x 3<br>Root+surface cathode x 3<br>Cathode at root x 3 | 35 | 1. Obtain the variation in voltage output, peak power densities and internal resistance when varying the number of cathodes and their placement.<br>2. Obtain the variation in organic removal, FSA and OP removal efficiencies when varying the number of cathodes and their placement. |
| Optimisation 3 | WAS sourced from an activated sludge system run in the lab | All systems did not contain a plant<br>0.5 x Dist[2]<br>1.0 x Dist<br>1.5 x Dist | 70 | 1. Obtain the variation in voltage output, peak power densities and internal resistance when varying the electrode distance.<br>2. Obtain the voltage results over a long-term operation of the PMFC. |

[1] indicates number of set-ups per system (NB: throughout this document, set-ups refers to the replicate for each system)

[2] Dist indicates the distance between the electrodes compared to the original design distance (see Figure 17)

# 4. PMFC operation using a solid substrate

## 4.1 Introduction

This Chapter focuses on two main aspects, firstly, it tries to understand the operation of the designed PMFC. Since the design was done by bringing different aspects of literature together, this chapter aims to understand if and how all the components fit together. Secondly, it aims to obtain the peak power density when using thickened WAS (a sludge type) from WWTWs. This chapter provides voltage results for each individual set-ups instead of an average of each system as the operation of the PMFC was studied.

## 4.2 Experimental design

Each PMFC was set up as described in Section 3.5.1 (see Figure 17 A). Due to supplier limitation, only two *C. papyrus* were available per substrate contrary to the *W. thyrsiflora* cells which had three cells. Also, the *P. australis* could not be sourced for this experiment and was not included.

### 4.2.1 Substrates used

Two solid substrates were chosen for these experiments, (1) a soil mixture and (2) thickened WAS. The same number of experiments and plant species were used for each substrate.

#### 4.2.1.1 Soil mixture

The main motivation behind using a soil mixture was to provide the plant with ideal growing conditions so its growth could be compared to that of thickened WAS. The substrate was made up of a combination of "Freedom Farms Premium Growing Medium" that was sourced from a local hydroponics store and clay which was sourced from the geotechnical research group at UCT and water.

The ingredients of the growing medium were; coco coir, worm castings, compost, perlite, vermiculite, volcanic rock dust, bone meal, gypsum, dolomite lime, kelp meal and organic nutrients. The concentrations of the nutrients contained within the medium were: nitrogen (9.1 g/kg), phosphorus (7.6 g/kg), potassium (3.1 g/kg), calcium (56.9 g/kg) and magnesium (14.3 g/kg). The growing medium allowed for optimum oxygen exposure and therefore had a high void ratio making it porous. The problem associated was that the growing medium could not contain a layer of water on top. To deal with this, clay was added to the mixture.

Clay is made up of fine particles which can aid in reducing the void space within a medium. Mixing the clay with the gardening soil allowed for a layer of water to be maintained above the mixture. This was important in order to ensure the cathode was always in contact and prevented the cell from drying out. The percentage of clay and growing medium was decided based on two criteria, (1) the mixture was able to accommodate a layer of water and (2) the mixture allowed for enough

seepage to ensure the plant roots do not dry out. Figure 18 shows one of the lab tests done to determine these percentages.

After trying different ratios, the best suited volume based ratio of growing medium, clay and water based was 1.85:1.85:1 respectively.

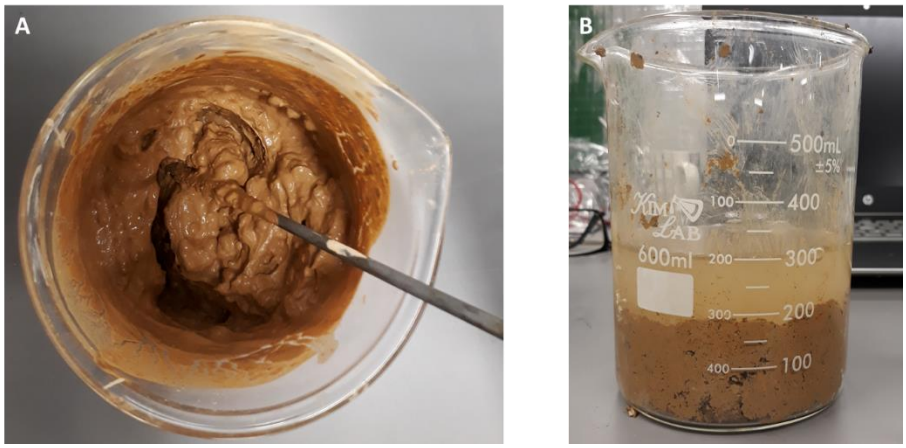



**Figure 18:** A shows the top view of the mixture. The medium and clay were added and slowly stirred through the addition of water. B on the right shows the capability of this mixture to support a layer of water above it. The pores seen allow for some seepage.

#### 4.2.1.2 Thickened WAS

WAS is discharged from the activated sludge system to control the mass inside it [53]. The thickened WAS collected in this experiment was sourced from Zandvliet WWTWs. At Zandvliet, the liquid WAS passes through a dissolved oxygen flotation (DAF) unit after which it is thickened on a gravity belt thickener thus producing sludge with 14% solid and 86% water.

#### 4.2.1.3 Substrate classification

The substrate classification is summarised in Table 5. The substrate was classified per mass instead of the common per litre because it was solid.

**Table 5:** Substrate classification.

| Substrate | TSS | VSS | ISS | COD | VFA | TKN | FSA | TP | OP |
|---|---|---|---|---|---|---|---|---|---|
| Thickened WAS (g/kg) | 136.6 | 99.9 | 36.7 | 174.8 | 0 | 8.2 | 0.13 | 3.18 | 0.13 |
| Soil (g/kg) | - | - | - | 23.43 | - | 9.1 | - | 7.6 | - |

### 4.2.2 Experimental systems

The experiment consisted of six systems, 3 with soil and 3 with thickened WAS. Each of the three were further split between planted and unplanted systems. The systems used were (see Figure 19):

1) *W. thyrsiflora* triplicate growing in gardening soil (1A, 1B and 1C);
2) *C. papyrus* duplicate growing in gardening soil (2A and 2B);
3) Control duplicate in gardening soil of which one was not inoculated (3A) and second was inoculated (3B);
4) Control duplicate in thickened WAS which one was not inoculated (4A) and second was inoculated (4B);
5) *C. papyrus* duplicate growing in gardening soil (5A and 5B); and
6) *W. thyrsiflora* triplicate growing in gardening soil (6A, 6B and 6C).


**Figure 19:** Lab set-up summary when using thickened WAS and gardening soil. 1a – 3b used gardening soil while 4a to 6c used thickened WAS.

### 4.2.3 Experimental timeline

The experiment was started on the 1st of April 2018 and concluded on the 25th of May, except for cells 2A and 2B, which were concluded on the 30th of June 2018. The timeline outlined in Table 6

contains days during which amendments were made to the experiment to better understand each of the PMFC components

**Table 6:** Detailed description of amendments made to PMFC and the associated day the amendment were made.

| Day | Description |
|---|---|
| 1 | Experiment started; all cells connected with a 100 ohms resistor |
| 12 | Stopped adding water to WAS reactors |
| 20 | Stopped adding water to all the cells. Added tap water to 5A, 5B and 6C. This was done to see the impact of a dry cell |
| 29 | On this day:<br>Noticed that cells with sand were very dried out and solidified<br>Added distilled water to all the cells.<br>Noticed WAS *W. thyrsiflora* was deteriorating<br>WAS *C. papyrus* leaves were slightly yellow instead of green when compared to the soil mixture ones.<br>6B cathode damaged (details provided in Section 4.4.6)<br>Noticed voltage in 5A and 5B close to zero even though good initial voltage<br>Connected 1000 ohms to 6A and added distilled water to all cells except for 6A. (no distilled water was added until the 8$^{th}$ of May) |
| 32 | Placed pebbles on all cathodes |
| 36 | Performed polarisation test on all cells |
| 38 | On this day:<br>1A cathode broke. Wire broke from the carbon paper. Reconnected the wires<br>Stopped adding water in cell 1C.<br>2B cathode wires also broke<br>All connections were left as open circuit |
| 39 | Connected 2B again with a new cathode and only growing medium, no clay. |
| 52 | Connected 100-ohm resistors to all cells |
| 55 | Disconnected all cells except for 2A and 2B. 2A was connected with 10000 ohms while 2B was 32000 ohms. |
| 74 | Cells 2A and 2B were left as open circuit |
| 76 | Left 2A and 2B as open circuit |
| 82 | Connected 32000 ohms to both |
| 90 | Disconnected the cells to conclude first experiment |

## 4.3 Voltage results and discussion

### 4.3.1 General results

The general results described in this section, affect all the cells. The results presented here were as a result of the amendments to the cells. These amendments affected the cells voltage results and

therefore it is paramount to understand them before the complete set of voltage results can be analysed. The details of the amendments are provided on Table 6. Even though the sub-sections to follow present figures from cell replicates, the same general result occurred in other cells as well. The figures are limited to replicates for ease of visualisation.

### 4.3.2 Importance of having a wet cell

Having sufficient water serves two main purposes; (1) it allows exoelectrogenic bacteria to grow easily as bacteria cannot grow in dry environments [107] and (2) it allows hydrogen ions to efficiently move from the anode to the cathode.

When the cell starts drying out (such as in this case when water addition was stopped from day 20 to 29), the voltage initially decreases as shown in Figure 20, but later on approaches zero as the top surface of the substrate is dry and the cathode detaches from the substrate (more on this in Section 4.3.3)

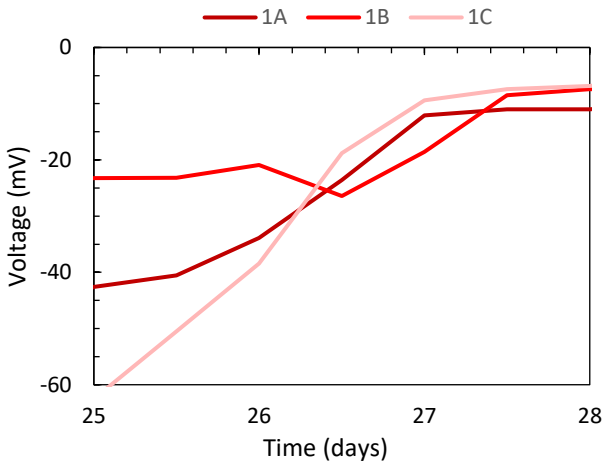



**Figure 20:** 1A, 1B and 1C are replicates of the PMFC where *W. thyrsiflora* is grown in the soil mixture. The Figure focuses on voltage values from day 25 to 28 (a period during which no water was added) to show the effect of a dry cell.

### 4.3.3 Cathode contact area

#### 4.3.3.1 Cathode not touching the sediment

Hydrogen ions move from the anode to the cathode as a result of the oxygen gradient between the electrodes [13]. This forms the basis of SMFCs and PMFCs. Therefore, cathodes must be in contact with the sediment for power generation. When the experiment was started, the cathode was placed on the layer of water assuming that it would continuously be in contact. However, because of the tension on the cathode due to the wiring, the cathode in some instances was not in contact with the cell causing cell voltage to go to zero (see Figure 21). The cathode in 5B constantly detached from

the cell. This was due to a combination of tension in the wires and placing the plant slightly more central than towards the edge as outlined in Section 3.5.

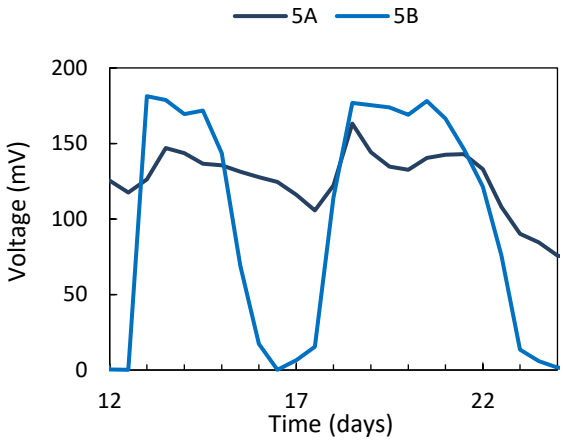



**Figure 21:** 5A and 5B are replicates of the PMFC where *C. papyrus* is grown in thickened WAS. The data presented demonstrates the effect of an 'air' cathode on voltage readings. 5A was not significantly affected by cathode pulling away compared to 5B which had more tension on the connecting wires.

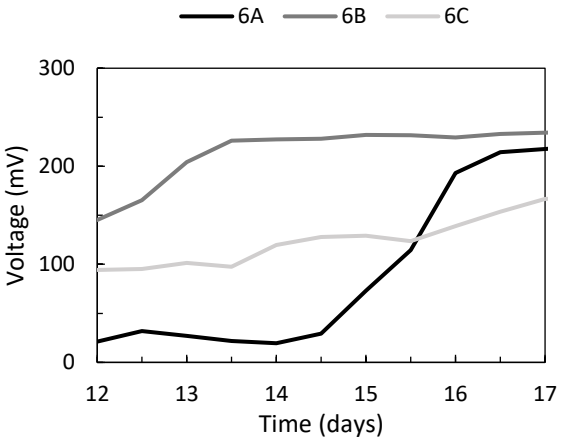



**Figure 22:** 6A, 6B and 6C are replicates of the PMFC where *W. thyrsiflora* is grown in thickened WAS. The data presented demonstrates the effect of reduced cathode contact area with the substrate. 6C which was set up first had the plant in a central position reducing the space for cathode connection.

The cathode being partly pulled away also reduced the efficiency of the fuel cell as it decreased the cathode area in contact with the cell. Decreased cathode contact area is the same as having a smaller cathode area. Hong, et al. [93] showed that voltage decrease correlated with cathode area decrease. This can be attributed to poor set-up where the plant was placed closer to the centre instead of the edge of the cell, as indicated in the methodology in Section 3.5. Figure 22 below

shows this occurring in cell 6C. The voltage produced in this cell is lower than ones produced in 6A and 6B. 6C was the first cell to be set up, and from its set-up, the significance of pushing the plant closer to the edge of the cell was realised.

#### 4.3.3.2 Cathode resting on a layer of water

As explained previously, the water acts as a bridge between the substrate and cathode. Maintaining a constant layer of water between the cathode and substrate however is not necessarily required provided the substrate is 'wet' enough to allow for easy movement of hydrogen ions and moist enough for bacteria to grow. In Figure 20, the cell substrate was soil which dried out when water was not added to the cells therefore resulting in a voltage reading close to zero. In the cells with sludge however, the power was not affected when no water was added because the thickened WAS contained 86% water allowing hydrogen ions to move freely.

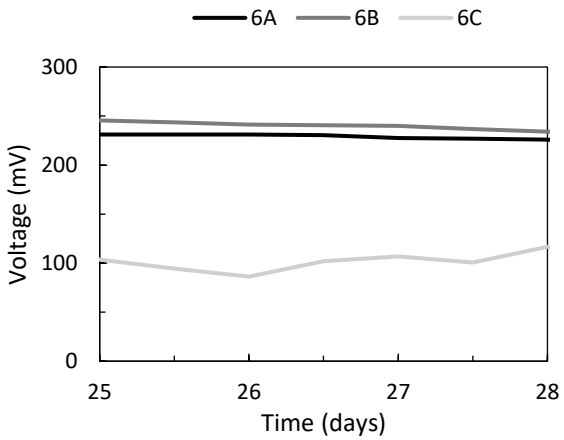



**Figure 23:** 6A, 6B and 6C are replicates of the PMFC where *W. thyrsiflora* is grown in thickened WAS. The Figure focuses on voltage values from day 25 to 28 (a period during which no water was added) to show the effect of having a 'wet' substrate.

The benefit of adding water however is that it provides a leeway in case the cathode lifts from the substrate. The cathode lifting can be avoided however by fixing it on the substrate. The same was achieved in this research by placing pebbles on the cathode on day 32.

#### 4.3.3.3 Cathode resting on substrate, not pressed down versus pressed down

When the cathode was already in contact with the substrate, placing pebbles on the cathode on day 32 did not change the voltage reading. This can be observed from cell 4B's voltage results presented in Figure 24. However, for the cells where the cathode was not fully in contact with the substrate, the voltages readings recorded increased when pebbles were placed on the cathode on day 32. This can be seen from cell 4A's voltage readings.

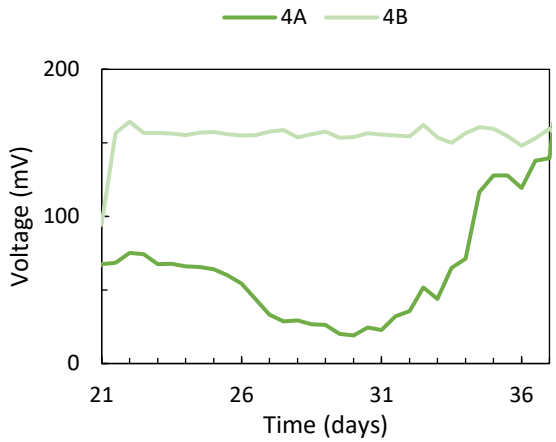



**Figure 24:** 4A and 4B are replicates of the controls with WAS. 4A was not inoculated while 4B was inoculated. The data show the effect of placing fixing the cathode onto the substrate that was done on day 28. The effect is more significant on 4A compared to 4B.

### 4.3.4 Resistors connected

The choice of resistors connected across the electrodes is crucial. According to González del Campo, et al. [106], connecting a low external resistance aids in microbial growth therefore increasing overall power. However, if the resistance chosen is significantly lower than the internal resistance of the cell, voltage readings recorded are close to zero. This was observed in cells with the soil mixture. Given the very low organic content, the internal resistance was high and therefore connecting a 100 ohms resistance produced voltage readings under 100 mV. Changing the external resistances connected across cells 2A and 2B to10000 ohms and 32000 ohms respectively gave a higher voltage reading.

The role of resistance can be further emphasised in Figure 25 where the voltage readings in 6A increased from 240 mV (providing a power of 1070 mW/m$^3$) to 450 mV (providing a power of 377 mW/m$^3$) after changing the resistor from 100 ohms to 1000 ohms. Subsequently, when left as an open circuit on day 38, the voltage, increased to 650 mV (providing a power of 0 mW/m$^3$). The same open circuit increase is noticed in cells 6B and 6C. It therefore becomes very important to ensure the same resistors are connected across cells if they are to be compared. A better comparison can be drawn by comparing peak power densities derived from polarisation tests. Since the same resistor (i.e. 100 ohms) was connected across all cells in this experiment, a comparison of one cell to another can be made.

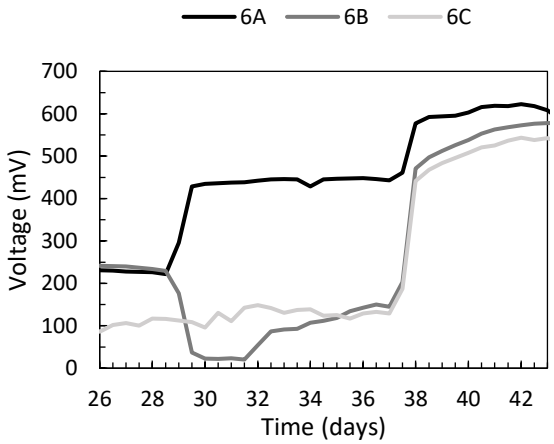



**Figure 25:** 6A, 6B and 6C are replicates of the PMFC where *W. thyrsiflora* is grown in thickened WAS. The data shows the effect on 6A when the resistor was changed from 100 ohms to 1000 ohms on day 29 and also the effect on all three replicates when left as an open circuit on day 38.

### 4.3.5 Understanding negative voltage

For the cells with the soil mixture, negative voltages were observed when connected across a 100 ohms resistor. When left as open circuit, some cells gained positive voltages while others remained as negative (see Sections 4.4.1 to 4.4.3). Loss of bacterial activity causes negative voltage readings [95]. The bacteria loss can be as a result of fuel starvation or dry conditions in which the bacteria cannot grow. In this case the microbes used to inoculate the systems were acclimatized to a high biodegradable organic content in the anaerobic digester and the change to soil meant the bacteria required time to gain positive voltages. The same was observed by Strik, et al. [35] where the PMFC required 50 days before voltage production occurred.

## 4.4 Complete results and comparison between replicates

The experiment ran for over two months as explained above and various amendments were made to the cells. The subsections to follow group the results based on repeats of the same cell conditions.

### 4.4.1 *W. thyrsiflora* in soil mixture

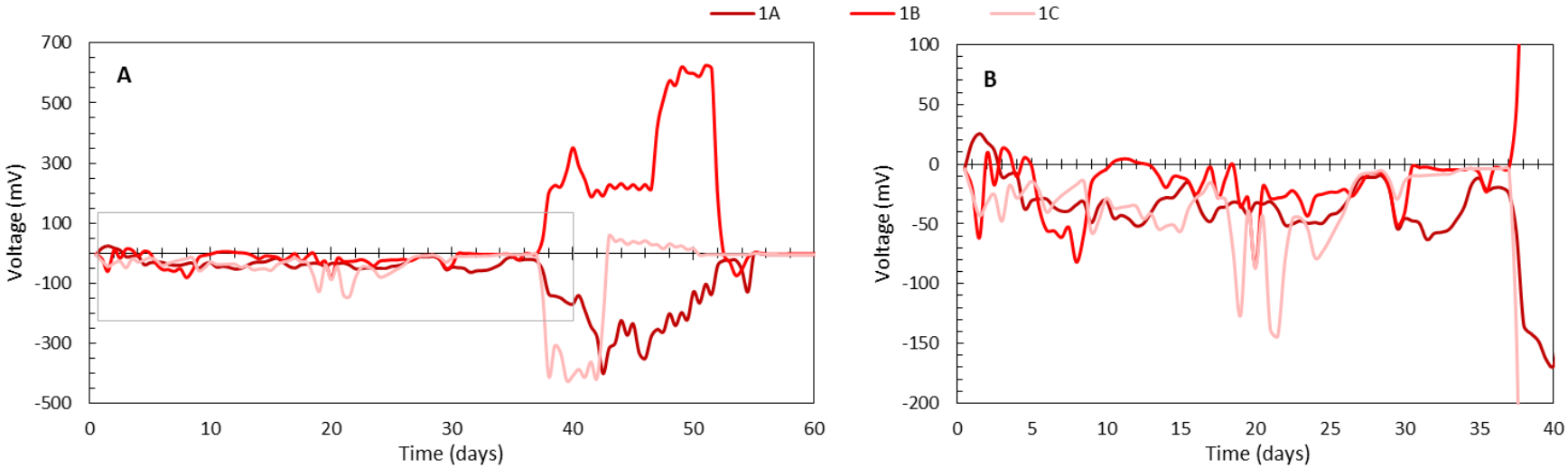



**Figure 26:** 1A, 1B and 1C are replicates of the PMFC where *W. thyrsiflora* is grown in the soil mixture. Graph A represents the full data set while B is a zoomed in section of A that looks closely at the voltage data before the cells were left as open circuit.

From the results shown in Figure 26, it can be observed that voltage readings were less than 100 mV when the 100 ohms resistor was connected across the electrodes. These low voltage readings made it difficult to deduce the exact power reading, especially in instances where tap water was added which in itself gives a voltage reading. Credible voltage readings may be obtained after day 20 when tap water was no longer added to the cells. The voltage reading in the cells was close to -60 mV. This voltage approached zero when the cells dried out by day 28.

Negative voltage readings when the 100 ohms resistor was connected could have been as a result of bacteria being starved or the resistor connected being too low for sufficient voltage measurements as explained in the next section. To negate the resistor as a reason for negative voltage, the cells were left as open circuit. Cell 1A and 1C produced negative voltages which means that the bacteria were starved in the fuel cell. This is as a result of the low biodegradable organic content in soil when compared to anaerobic digestor conditions that these bacteria were accustomed to. Cell 1C does eventually start producing positive voltage, however this was the same cell where

no water was added to see the effect on power. Its voltage production went close to zero as bacteria could not survive dry conditions. Cell 1B readings correlate to the controls, meaning that the plant might not have been releasing exudates. Cell 1A produced positive voltage in open circuit conditions indicating that bacteria were not starved. Since the cell was initially connected with a 100 ohms resistor, it is difficult to determine when the bacteria were acclimatized in cell 1B.

### 4.4.2 *C. papyrus* in soil mixture

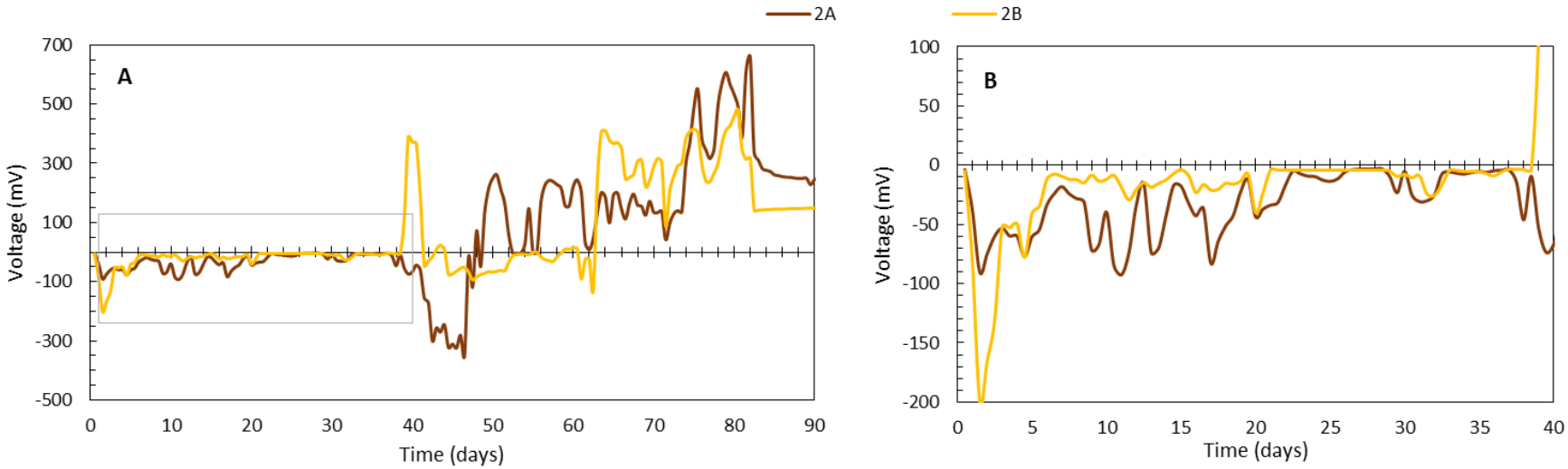



**Figure 27:** 2A, 2B and 2C are replicates of the PMFC where *C. papyrus* is grown in the soil mixture. Graph A represents the full data set while B looks closely at the voltage data before the cells were left as open circuit.

Following from the previous section, voltage reading after day 20 was -40 mV as shown in Figure 27. The effect of adding tap water can be observed by comparing day 20 (when tap water addition stopped) voltage results to those of day 0 – 19. The voltage readings quickly approach zero as water dried up in the cells.

On day 38, the nickel coated copper wires of cell 2B broke and were replaced. On the same day, the substrate used i.e. a mixture of clay and growing medium was changed to only growing medium. This was possible because it was learnt during the course of the experiment that a layer of water, which the growing medium was not able to accommodate, was not required as pebbles placed on the cathode ensured cathode-substrate contact is established.

On the same day 38, the resistors were disconnected from all the cells to record open circuit voltages. Cell 2A continued producing negative voltage as the bacteria required a longer period of time to acclimatize. Positive voltage was measured in cell 2A on day 48. Cell 2B on the other hand, which had just been connected to a new substrate, produced positive voltage initially, but resumed to produce negative voltages. Cell 2B required more time to acclimatize and only started producing positive voltage on day 60.

Open circuit voltages of cell 2A were greater than cell 2B. This was as a result of the plant performing better in cell 2A. The roots had grown through the GAC anode and the exudates could directly be used by the anode (see Figure 34). The roots in cell 2B had grown less relative to those in cell 2A and therefore less organic content was provided by the roots. The same was observed when a 32000 ohms resistor was connected across both cells from day 82 to 90. The difference in voltage is not as a result of a possible higher organic content in the prepared soil itself (i.e. soil used in cell 2A) because the organic content in the soil was used up before day 60 as explained in the next section. These results demonstrate the importance of increased root anode contact area.

### 4.4.3 Soil mixture controls

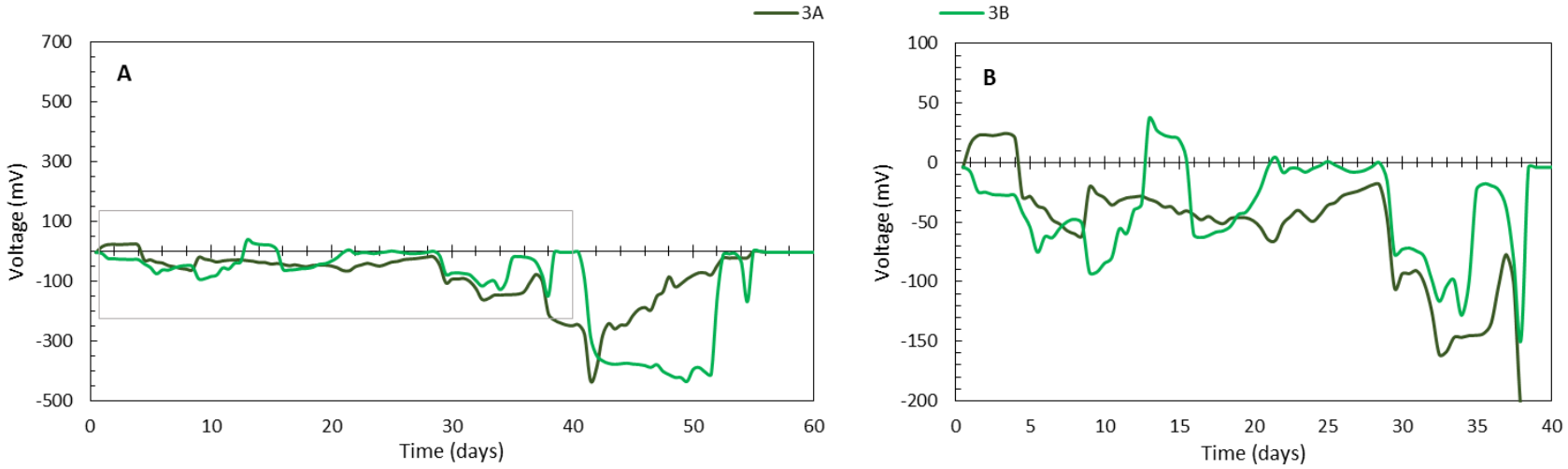



**Figure 28:** 4A and 4B are replicates of the controls with WAS. 3A was not inoculated while 3B was inoculated. Graph A represents the full data set while B looks closely at the voltage data before the cells were left as open circuit.

Voltage readings after day 20 for cell 3A was -40 mV. Cell 6B on the other hand was close to zero. The reason could be related to the cathode detaching from the surface of the cell. Cell 6A produced voltage even though it was not inoculated. This indicates that exoelectrogenic bacteria were present in the soil.

When the cells were left as open circuit, the voltages recorded were still negative until the experiment was stopped. This meant that the bacteria were continuously starved since a plant was not present to provide exudates. From the results it was also noticed that organic content in cell 3A was used up by day 52. If the cells were left as an open circuit during the time when organics were abundant in soil, the voltage readings might have been positive after some time.

### 4.4.4 Thickened WAS controls

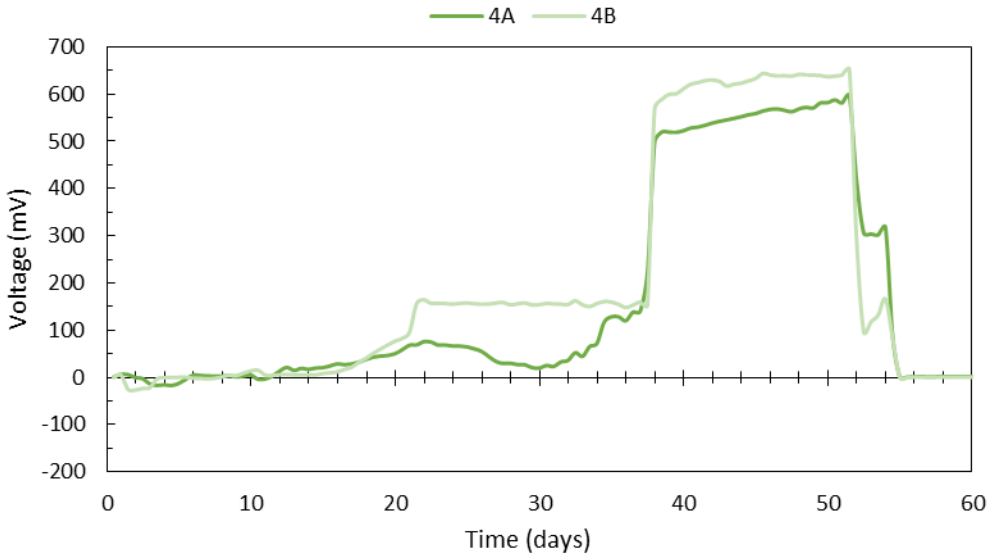



**Figure 29:** 4A and 4B are replicates of the controls with WAS. 4A was not inoculated while 4B was inoculated.

From the results, it can be observed that voltage production stabilised after day 20 for cell 4B. 4A also produced voltage after day 20, but the magnitude was lower. 4A reached a voltage value close to 4B on day 35.

The dip in voltage between day 22 and 34 in cell 4B could be attributed to cathode slowly detaching from the substrate and reducing effective contact area. Once pebbles were placed on top of the cathode, the voltage slowly started increasing and peaked at day 36.

Cell 4B, which was inoculated, produced approximately 150 mV across a 100 ohms resistor from day 20 until it was left as an open circuit. Once open circuit conditions were discarded, and a 100 ohms resistor reconnected (from day 52-55), the same 150 mV was recorded.

For cell 4A, which was not inoculated, the voltage value recorded between day 22 and 26 was lower than that of cell 4B. The voltage was similar to cell 4B from day 35 and day 38 i.e. 135 mV connected across a resistance of 100 ohms. This may be as a result of cell 4A taking a longer time as it was not inoculated.

### 4.4.5 *C. papyrus* in thickened WAS

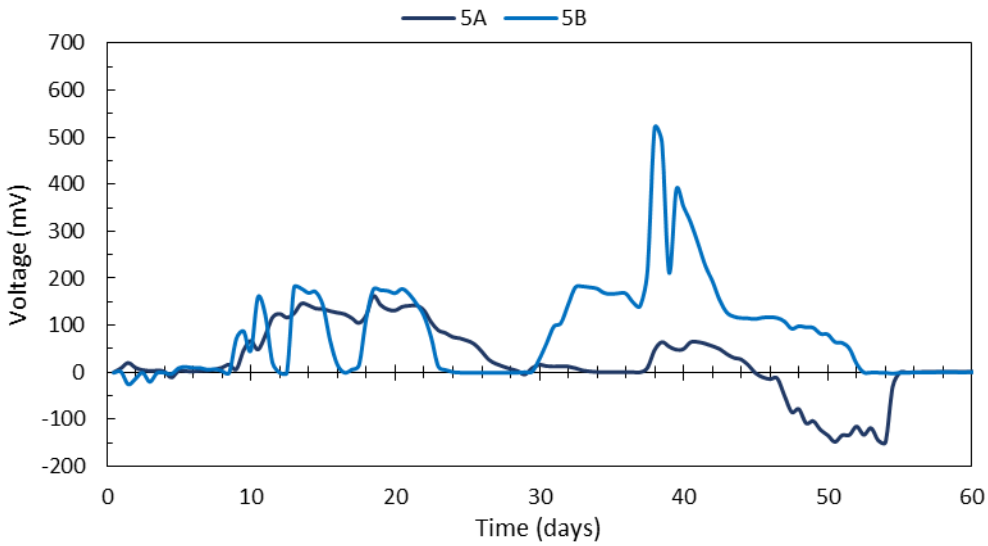



**Figure 30:** 5A and 5B are replicates of the PMFC where *C. papyrus* is grown in thickened WAS.

From the results it can be observed that voltage readings started peaking on day 11 for both cells 5A and 5B. The cathode in cell 5B however kept on lifting away from the substrate which caused the power to drop to zero. This can be observed from day 10 to 32. When pebbles were placed on the cathode on day 32, the voltage reading resumed to the maximum value for 5B. The recorded voltage across a 100 ohms resistor was 175 mV.

For cell 5A, the cathode lifting was not an issue when water was added. The voltage started dropping when the addition of water was stopped on day 20. The voltage reading slowly decreased to 0 mV on day 28 and even after addition of water, voltage did not increase until it was left as open circuit on day 38. The voltage reading after day 47 turned negative indicating that bacteria were staved. The reason for this may be because of lack of water.

The lack of water effect was also be observed in cell 5B where even in open circuit conditions the voltage dropped from 500 mV to 120 mv and finally 0 mV. This may be as a result of *C. papyrus* absorbing more water compared to other cells and therefore experiencing a larger impact of not adding water.

### 4.4.6 *W. thyrsiflora* in thickened WAS

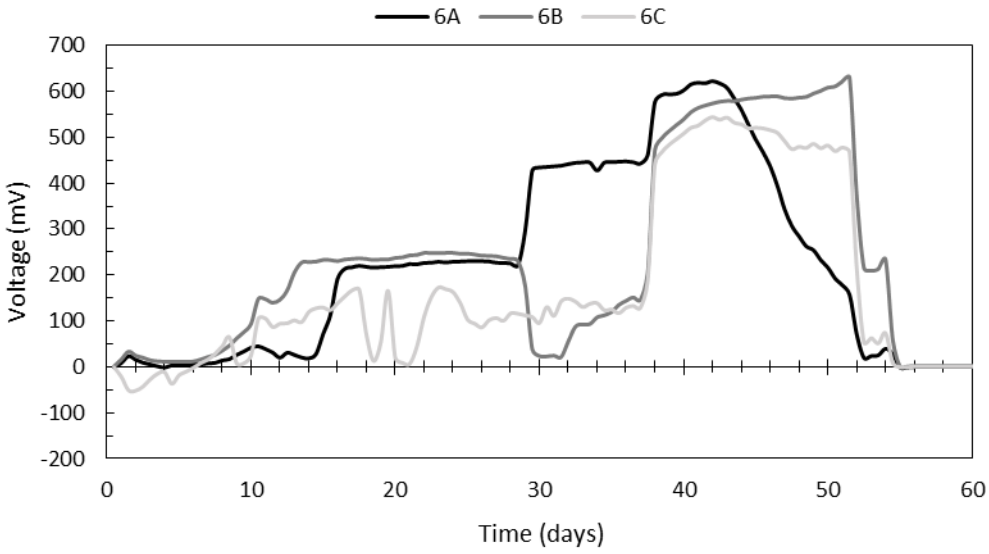



**Figure 31:** 6A, 6B and 6C are replicates of the PMFC where *W. thyrsiflora* is grown in thickened WAS.

From the results above, the *W. thyrsiflora* cells also required 10-12 days to reach maximum voltage. Both cell 6A and 6B produced similar voltage readings of 240 mV connected across a 100 ohms resistor until day 29. This translates to 21.40 $mW/m^2$.

On day 29, cell 6B's cathode broke. This happened as a result of the cathode being stuck on the substrate when no water was added to the cells. In the process of trying to loosen the cathode from the substrate, the platinum peeled away from the carbon sheet dropping the voltage close to zero. Voltage readings increased when pebbles were placed on the cathode fixing the carbon sheet to the platinum.

On the same day, the 100 ohms resistor was replaced with 1000 ohms resistor in cell 6A. The voltage increased to 450 mV. This voltage across 1000 ohms translates to a power value of 377 $mW/m^3$. This value is significantly lower than previous measure of 1070 $mW/m^3$ thus showing the importance of doing a polarisation test to obtain the peak power density which occurs at the optimal resistance.

Cell 6C on the other hand produced lower voltage readings when compared to 6A and 6B. This was as a result of placing the plant towards the centre of the cell therefore reducing the effective cathode-substrate contact area. Cell 6C was the first set-up and the error made when setting up was realised. The voltage on day 18 and 20 also went to zero because of the cathode lifting away from the substrate.

When the cells were left as open circuit, they all produced voltage readings over 600 mV. This voltage however started decreasing in cell 6B, this was as a result of bacteria being affected by the absence of water. Again, SEM pictures of the anode would be recommended to study the bacteria growing on the anode so a comparison between the triplicates can be performed.

## 4.5 Comparison between different set-ups

### 4.5.1 Comparison between substrates

The two substrates used in this set of experiments, as explained in Section 4.2, were soil mixture and thickened WAS. From the onset it can be observed that the COD content of the soil mixture was significantly lower than that of WAS. Lower COD indicates fewer biodegradable organics present in the system. Nonetheless, a COD of 23.43 g/kg of substrate is high but the power produced from cells with clay was low, meaning that most of the COD comes from unbiodegradable constituents. Cells with WAS on the other hand produced on average over 20 times the amount of power produced in soil substrate cells.

### 4.5.2 Comparison between soil mixture cells

A comparison between the cells with soil is challenging as low voltages magnitudes were obtained as explained in Sections 4.4.1 to 4.4.3. When the voltage values after day 20 are compared, *W. thyrsiflora* recorded the maximum voltage of -60 mV while the voltage in both the *C. papyrus* and control was -40 mV.

The controls, even when left as open circuit, produced negative voltages, while those with plants eventually produced positive voltage readings meaning the presence of plants provides exudates which can be used to feed the starved bacteria and therefore produce positive power.

An interesting finding was that when the same volume of water was added to the set-ups, the cells with *C. papyrus* used up more water when compared to the cell with *W. thyrsiflora* which in turn used more water when compared to the controls. This meant that potentially there were times when cells with *C. papyrus* were dry. This means that for future experiments the water added in the cells should be based on the amount consumed (along with evaporated) in the cells with *C. papyrus.*

### 4.5.3 Comparison between thickened WAS cells

When comparing the voltage outputs between the *W. thyrsiflora*, *C. papyrus* and the controls, it was noticed that the *W. thyrsiflora* produced the maximum voltage of 240 mV followed by *C. papyrus* with 175 mV and control with 155 mV as shown in Figure 32.

From Figure 32, the drop-in voltage for the cell with *C. papyrus* was ignored when comparing cells as the drop was as a result of the cathode lifting away from the substrate as explained in previous sections.

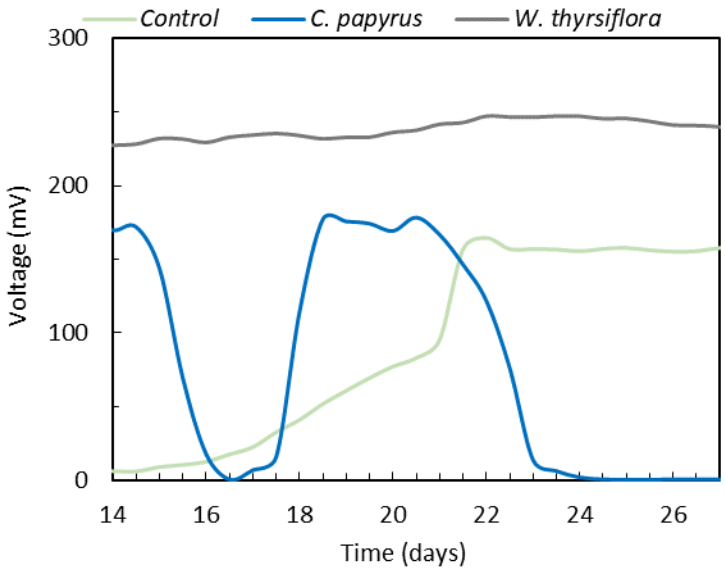



**Figure 32:** Voltage comparison between Control, *C. papyrus* and *W. thyrsiflora*. The line 'negating air cathode' is a hypothetical data line which could have been obtained if the cathode in the *C. papyrus*-WAS PMFC would have stayed connected.

When comparing the acclimatization time between the cells, it was noticed that the cells with plants acclimatized at a faster rate (10-20 days) compared to those of un-planted cells (20 days). In order for the bacteria to acclimatise, a food source must be present. The utilisable organic source in WAS is obtained from biodegradable particulate organics which are introduced in the system as a result of the OHO death (OHOs are bacteria used in the activated sludge system to consume biodegradable material, both soluble and particulate organics). The OHOs are broken down into simple organic compounds through the process of hydrolysis before they can be used [108]. Since the planted cells took a shorter period of time to acclimatize, this could be attributed to roots increasing the microbial activity through providing exudates and oxygen [24, 33].

## 4.6 Polarisation test results and discussion

The polarisation test was done on day 36 on all the cells. The cells with the soil mixture were not yet acclimatized and therefore the results produced from it did not show any trends and were discarded. The polarisation test results done on the cells with WAS are given in Figure 33. By day 36, the both the thickened WAS controls, inoculated and not inoculated were both producing approximately the same power and an average of both systems was possible.

From the polarisation test, the PMFC with *W. thyrsiflora* produced maximum power of 1036 ± 59 $mW/m^3$ followed by *C. papyrus* with 510 ± 92 $mW/m^3$ and finally the control with 392 ± 67 $mW/m^3$.

The highest PPD (twice as high as the other two set-ups) in the *W. thyrsiflora* could be attributed to roots dying and releasing the exudates therefore further aiding in microbial activity. Increased microbial activity aids in higher power generation.

The internal resistance also followed the same pattern with the lowest in *W. thyrsiflora* 155 ± 7 ohms, followed by *C. papyrus* 186 ± 18 and highest in control with 243 ± 17 ohms (see t-test in Appendix I). However, the PPD of *W. thyrsiflora* was twice as that of *C. papyrus* but the internal resistances did not have the same factor. The internal resistance being lower in the planted systems was similar to research done by Saz, et al. [20] and Wang [25].

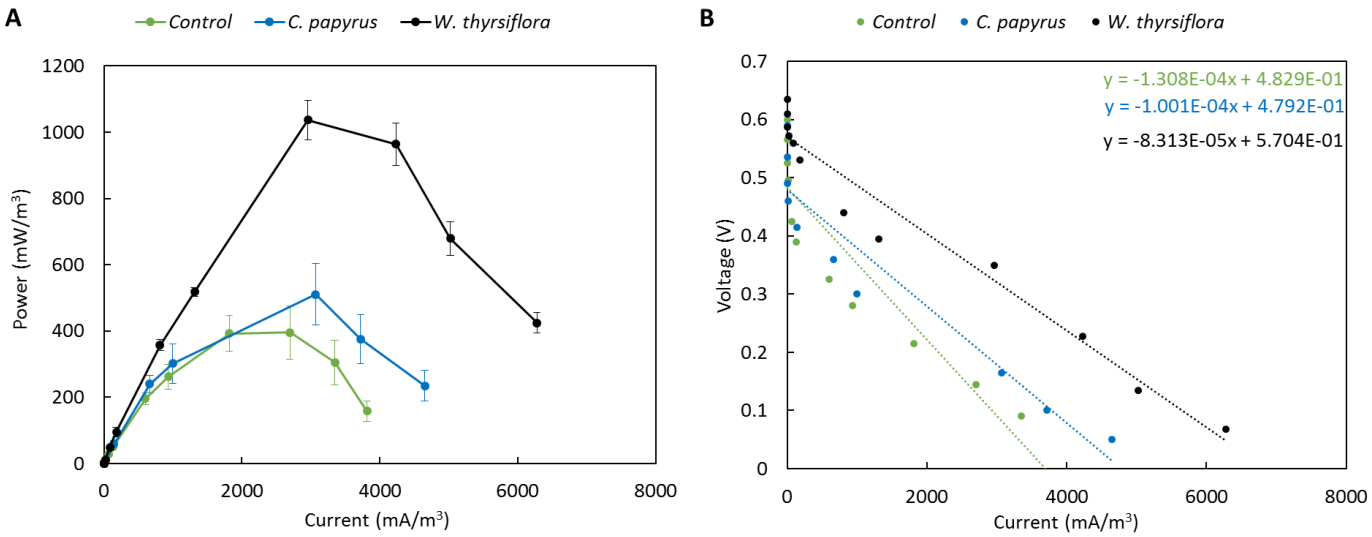



**Figure 33:** Shows the results obtained for the polarisation test done on day 36. A gives the peak power density while B provides the voltage versus current relationship. The error bar represents the standard deviation from the mean of the triplicate setups.

**Table 7:** Peak power density and internal resistance of cells.

| System | PPD (mW/m³) | Internal resistance (Ω) |
|---|---|---|
| Control | 392 ± 67 | 243 ± 17 |
| *C. papyrus* | 510 ± 92 | 186 ± 18 |
| *W. thyrsiflora* | 1036 ± 59 | 155 ± 7 |

## 4.7 Assessment of plant species health

The *C. papyrus* plant performed well and survived during the course of the experiment. The initial height versus final plant height is given in Table 8. The roots of the *C. papyrus* grown in the soil mixture grew through the GAC anode and the shoots were also healthy moving into the budding phase (see Figure 34). The roots growing though the GAC increased the root-anode contact area as discussed in literature [30].

The *C. papyrus* plant grown in WAS also survived the duration of the experiment, however as seen from Table 8, it achieved a 48.3% growth while *C. papyrus* in soil had a 65.4% growth. The *C. papyrus* in WAS also had yellowing of its leaves indicating magnesium deficiency. This was expected as the soil conditions were best suited for plant growth compared to WAS.

**Table 8:** *C. papyrus* and *W. thyrsiflora* initial and final height.

| Cell | Initial height (cm) | Final height (cm) | % increase |
|---|---|---|---|
| W, thyrsiflora in soil | 41.0 ± 1.6 | 56.8 ± 2.3 | 38.5 |
| C. papyrus in soil | 44.3 ± 1.3 | 73.3 ± 3.3 | 65.4 |
| W. thyrsiflora in WAS | 41.0 ± 0.8 | – | - |
| C. papyrus in WAS | 41.0 ± 1.0 | 60.8 ± 8 | 48.3 |

The *W. thyrsiflora* grown in the soil mixture also performed well (see Figure 35), however the *W. thyrsiflora* grown in the WAS died before the completion of the experiment. The death of the plant could be as a result of one or multiple of these reasons:

1) The pH of the substrate.
2) Anaerobic conditions in the cell.
3) The thickened waste structurally inhibits water from reaching the roots.
4) High metal content.
5) The ammonia concentration.

To understand the reason for plant death, the thickened WAS was compared to soil used for plant growth. Also, Dr Samson Chimphango, a researcher from the Biological Science department at UCT, was consulted to discuss possible reasons for plant death.

The measured pH for cells 6A, 6B and 6C were 7.42, 7.56 and 8.08 respectively. From literature (see Section 2.1.7), plants can grow in pH ranges up to 7.5. Except for cell 6C, both cells 6A and 6B were within the acceptable limit but since all three plants died, and not just the plant in cell 6C, pH was discarded as a possible reason for plant death.

The anaerobic conditions hypothesis was also discarded as the same plant species survived and did well when the soil mixture was used as a substrate (cells 1A, 1B and 1C). The same reasoning can be used to discard the possibility of WAS physically inhibiting water to reach the roots as cell 5A and 5B (the ones with *C. papyrus* grown in WAS) would have also died. Also, it was noticed that the plant started dying even before addition of water had stopped (day 20), therefore, WAS physically inhibiting water to penetrate to the roots could not be a possible reason for plant death.

High metal content could be a plausible reason for plant death. Since thickened WAS is 14% solid, it could have a high concentration of metals that inhibit plant nutrient uptake. It is therefore

important to quantify metal concentration and cross-reference it to *W. thyrsiflora* metal allowances to determine if this is a possible reason.

High ammonia concentrations could also be another reason. In normal aerobic plant growing circumstances, organic nitrogen breaks down to form FSA ($NH_4^+/NH_3$) which is oxidised to nitrates ($NO_3^-$) and taken up by the plant. Since this is a wetland plant species, it can withstand higher FSA concentrations relative to non-wetland plant species. However, it is not known at what concentration the plant starts dying. To determine if ammonia concentration was a possible reason, a metal quantification of the sludge should be conducted. If metals concentration is within acceptable limits, then the plant death was a cause of high ammonia concentrations.


**Figure 34:** A shows *C. papyrus* grown in soil while B shows the roots that penetrated to the GAC in soil substrate. C shows the *C. papyrus* grown in WAS.

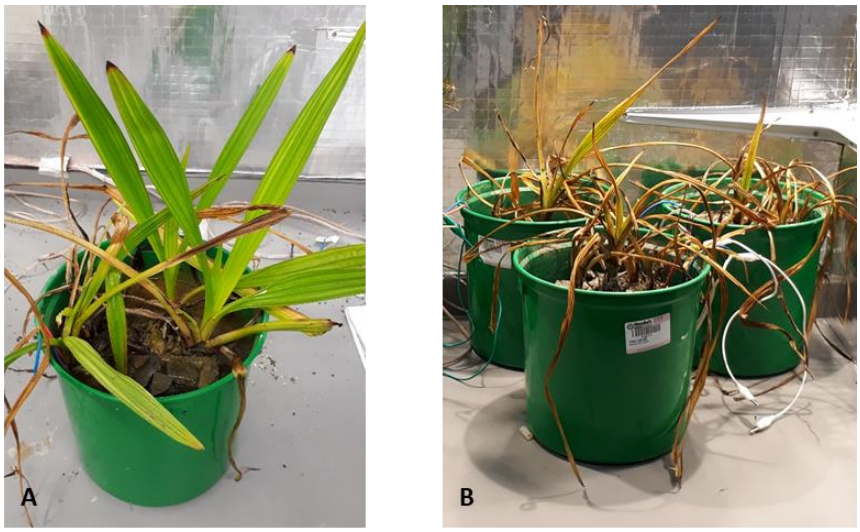



**Figure 35:** A displays the state of the *W. thyrsiflora* that was grown in the soil mixture while B shows the state of the *W. thyrsiflora* that was grown in WAS.

## 4.8 Lessons learnt from this experiment

Multiple PMFC variables were tested to understand the operation of the PMFC. This chapter showed that:

1. PMFC's which use soil as substrate require a very long (about 2 months) to start producing power;

2. a decrease in cathode contact area significantly decreases power production;

3. external resistors connected must be carefully selected as they can give a false impression on the performance of the PMFC; and

4. *W. thyrsiflora* cannot withstand a high COD content and future experiments should be designed bearing this in mind.

# 5. Waste activated sludge as substrate

## 5.1 Introduction

Having understood the operation of the PMFC and obtained the power output when using thickened WAS, the substrate was changed in this experiment to liquid WAS (hereon referred to as WAS). The experiment was started on the 17$^{th}$ of July 2018 (day zero) and concluded on the 1$^{st}$ of September 2018 (day 44) allowing for sufficient time to obtain results. The subsections to follow discuss the voltage output and the organic removal capabilities of the three plants tested.

## 5.2 Experimental design

Each PMFC was set up as described in Section 3.5 (see Figure 17).

### 5.2.1 Experimental systems

Four systems each containing three replicate set-ups were tested. Three systems contained plants while one system did not have a plant (control). The systems were as follows:

1) *C. papyrus*;
2) Control with no plant;
3) *P. australis;* and
4) *W. thyrsiflora*.

### 5.2.2 Substrate used

Waste activated sludge was used for this experiment. WAS is discharged from an activated sludge system at a WWTWs to control its mass [53]. The WAS collected in this experiment was sourced from Zandvliet WWTWs.

Zandvliet has two different activated sludge wastewater treatment operations, the first one uses the Modified Ludzack-Ettinger (MLE) system which is tailored for ammonia removal. The second one uses the UCT system which is tailored for both ammonia and phosphorus removal. . In an EBPR system, the COD is removed using the OHOs, OP is removed using PAOs and FSA is removed using autotrophs (nitrifying bacteria) in the activated sludge system. Therefore, the biodegradable content of WAS comprises mainly of OHOs and PAOs (as autotrophs have a very slow growth rate). The COD stored within them can be utilised after the organism death and breakdown.

After collecting the substrate, it was characterised based on its total suspended and settleable solids (TSS), VSS, inorganic suspended and settleable solids (ISS), COD, VFA, TKN, FSA, TP and OP. The results are summarised in Table 9.

**Table 9:** WAS characteristics.

| Characteristic | TSS | VSS | ISS | COD | VFA | TKN | FSA | TP | OP |
|---|---|---|---|---|---|---|---|---|---|
| mg/l | 13244 | 10538 | 2706 | 16633 | 0 | 626 | 9.9 | 281 | 37.6 |

## 5.3 Voltage results and discussion

The voltage readings across a 1000 ohms resistor recorded from day 35 to 44 for all the systems is provided in Figure 36. The complete set of voltage recordings is provided in Appendix B. Day 35 to 44 was chosen as the previous days had a 100 ohms resistor connected across the cells similar to the previous experiment using thickened WAS (see Chapter 4). The 100 ohms resistor was connected because, according to literature, low external resistors can increase microbial growth [106]. However, in these systems the voltage reading measured was approximately 0.04 V (see Figure 68) making it difficult to compare the performance of one system to another. Therefore, the resistance was changed to 1000 ohms on day 35 providing readable voltage results.

### 5.3.1 *C. papyrus*

The *C. papyrus* system produced approximately 0.15 V from day 35 to 39. Similar readings were recorded across the controls. After day 39, the voltage recorded was higher than the control for one and a half day. Thereafter, higher voltage readings were measured at night and equal readings measured during the day relative to the control. Both set-ups showed a slowly decreasing voltage reading. The slowly decreasing voltage could be attributed to increasing internal resistance in both systems (see Section 8.3.3.2). The *C. papyrus* voltage was observed to decrease at a faster rate than the control, the paragraphs to follow further explain the possible reasons for these results.

From previous work, planted systems generate more power when compared to unplanted controls because of three main reasons; (1) plants provide exudates to increase organic content in the fuel cell, (2) the roots act as a host to increase bacterial growth therefore increasing exoelectrogenic bacteria in the system and (3) plants release dissolved oxygen which can be used by the cathode [19, 20, 26]. Since WAS contains a high content of biodegradable organics, which was given sufficient time to break down, the exudates released by *C. papyrus* do not explain the results obtained. On the other hand, the oxygen released by the roots can inhibit power generation if released in the anodic region which is required to be anaerobic. After the experiment was stopped, it was noticed that roots had penetrated to the anodic region explaining lower than expected voltage recordings (see Section 5.9). The microbial density in the *C. papyrus* system may have been higher but the limitation caused by oxygen release may have provided lower voltage recordings.

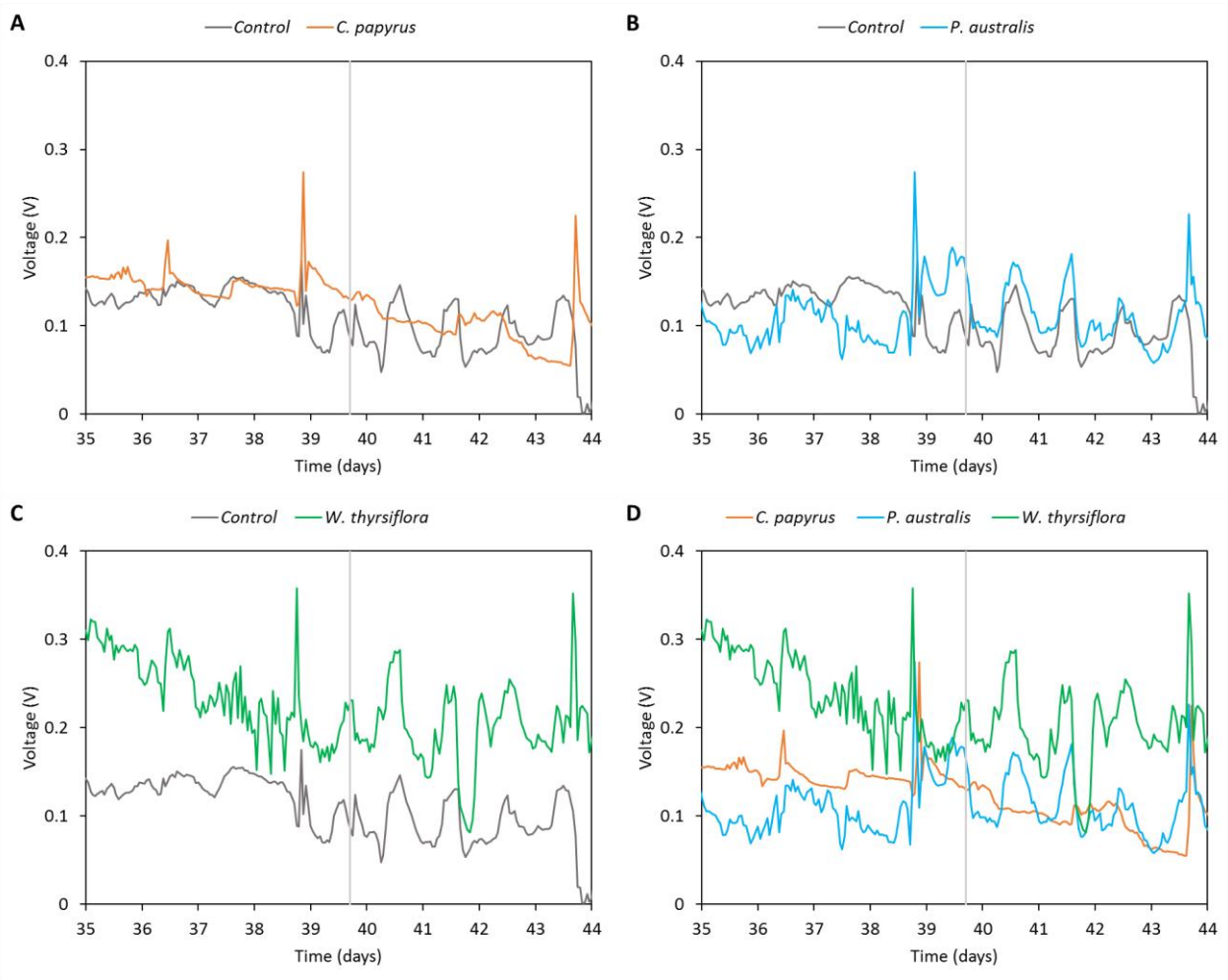



**Figure 36:** Voltage recorded across a 1000 ohms resistor for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) in comparison to the control and a comparison of all plant species (D) investigated when using WAS. Vertical line indicates polarisation test day.

In addition, cyclic voltage data was observed in the control and not in the *C. papyrus* system. The cyclic data in the control was as a result of algae growing on the surface. The *C. papyrus* systems also had cyclic variation, but it was not as significant since there were only a few surface roots and the set-ups had little to no algae growing. Algae, being a plant as well, releases oxygen during the day when photosynthesising and consumes oxygen at night when respiring [39]. Algae present in the control systems increased the oxygen levels during the day therefore producing voltage equal to the *C. papyrus* system. However, at night, this voltage would drop lower than *C. papyrus*. Without algae, the control would have likely produced lower voltage readings (i.e. similar to the night time readings about 0.06 V) than the *C. papyrus* instead of the variation observed.

The *C. papyrus* system was also most efficient in removing FSA (see Section 5.6). FSA is removed by converting it to nitrates. Nitrates have a lower cathode potential compared to oxygen i.e. E’ᵉ

$NO_3/N_2$=0.74 V and E'° $O_2/H_2O$=1.23 V [25]. Therefore the lower voltage readings can also be explained by oxygen being utilised by nitrifying bacteria (autotrophs) to convert FSA to nitrates, and subsequently the nitrate being utilised at the cathode [109]. Since WAS was sourced from a treatment works utilising the UCT system, autotrophs (nitrifying bacteria) were present in the feed. This, coupled with roots increasing presence of microorganisms, potentially grew more nitrifiers and utilised the dissolved oxygen for nitrification. Further research is required to better understand if the cause of low voltage readings was because of nitrates present or oxygen released at the anode or a combination of both.

### 5.3.2 *P. australis*

The *P. australis* system recorded slightly lower voltage readings than the control from day 35 to 39. After day 39, the *P. australis* produced higher voltage readings than the control. Both systems exhibited cyclic data due to the presence of algae in the systems. However, the cyclic variation in *P. australis* increased after day 38. This was also when multiple shoots had grown out of the set-ups. Dissolved oxygen captured from the leaves, travels through the aerenchyma in the shoots and is finally released from the roots [36]. Therefore, an increased number of shoots in the system likely increased dissolved oxygen concentration in the system.

Voltage readings from *P. australis* followed the same trend as the control. This was mainly because the plant mass used in the set-ups was significantly lower compared to those of *C. papyrus* and *W. thyrsiflora* (see Section 5.9). This meant that the *P. australis* mimicked the control, because of the lower root mass until after day 38 where the shoot mass was higher. If the plant had more growth at the beginning of the experiment and/or more plant mass was used, it is speculated that the voltage output would have been higher than the values recorded in this experiment.

### 5.3.3 *W. thyrsiflora*

The biodegradable content of the feed used was obtained from both microorganisms PAO's and OHOs. The organics are released in simple form and consumed by the anode after death and breakdown of these microorganisms. This means that for all the systems, power generation would be dependent on how efficiently these microorganisms are broken down. However, this process was likely expedited for *W. thyrsiflora* system as the anode utilised the exudates released from dying roots.

After the consumption of these exudates, the voltage started dropping and only started producing higher voltage outputs when new roots grew just under the surface. From Figure 36, the voltage difference between *W. thyrsiflora* and the control was about 0.1 V which was also the highest difference when compared to other planted systems. Similar to the control, the voltage decreased which again may be a result of increased internal resistance as the GAC anode gets 'clogged' [92].

The higher voltages in the *W. thyrsiflora* system was as a result of the roots growing just under the surface where the cathode is placed. The roots released dissolved oxygen which was likely

consumed by the cathode. Similar to the control, cyclic data was observed in the *W. thyrsiflora* system. However, the variation was higher which may be as a result of having both, the plant and algae present in the system.

## 5.4 Polarisation test results and discussions

A polarisation test was conducted on day 39. The grey vertical line on Figure 36 indicates precisely when the polarisation test took place. The polarisation was conducted in the evening when the voltage reading was just about to or had started to drop depending on the system (see Figure 36). This is because different plants have different oxygen release and consumption times [39]. The resistors used for the polarisation are provided in Appendix A.

Just before the polarisation test, the *C. papyrus* system was reading higher voltage values than the control system. Therefore, even though the *C. papyrus* voltage readings relative to the control was higher at night and equal during the day, the timing of the polarisation test provided a real representation of peak power densities.

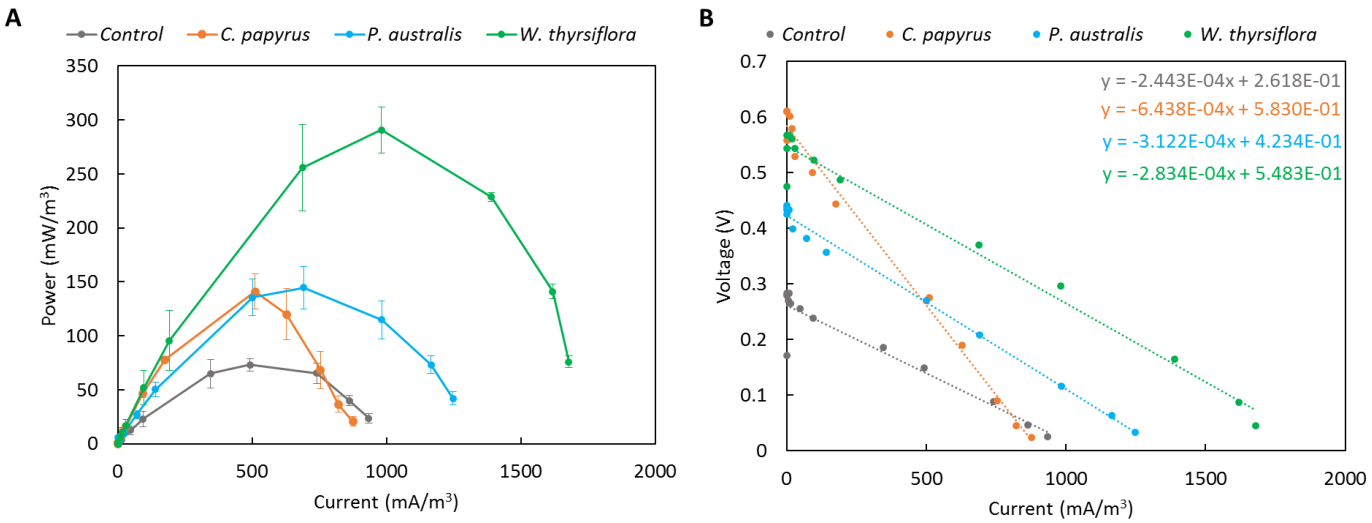



**Figure 37:** Polarisation test results on day 40 when using WAS as substrate. (A) shows PPDs for the three plant species while (B) shows voltage versus current results. The error bar represents the standard deviation from the mean of the triplicate set-ps.

**Table 10:** Peak power density and internal resistance of the set-ups using WAS as feed.

| System | Peak power density (mW/m$^3$) | Internal resistance (Ω) |
|---|---|---|
| Control | 73 ± 6 | 454 ± 143 |
| *C. papyrus* | 141 ± 16 | 1197 ± 109 |
| *P. australis* | 145 ± 20 | 581 ± 34 |
| *W. thyrsiflora* | 291 ± 21 | 527 ± 143 |

Including the standard deviation, the maximum power density followed the order of *W. thyrsiflora* > *P. australis* ≈ *C. Papyrus* > control (see Table 10 and t-test in Appendix I). The *C. papyrus* and *P. australis* produced approximately the same PPDs and these were twice as high as the control, but, half of the *W. thyrsiflora* PPD. This indicates that growing plants in the set-ups increased power generation and complies with previous studies [19, 20, 26, 109].

The *C. papyrus* had a higher PPD compared to the control but also had the highest internal resistance. The PPD is proportional to the square of the current and inversely proportional to the resistance [97]. This means that the high internal resistance in the *C. papyrus* system inhibited it from achieving high power densities. If the internal resistance was half (as recorded for the other systems), the PPD would have doubled (following Equation 1) equalling PPD measured for the *W. thyrsiflora* system.

The internal resistance in a MFC can be explained as the resistance it suffers when the current passes from the anode to the cathode [97]. It can be as a result of cathode overpotential, anode overpotential and current dependant potential losses [96]. Reduced number of exoelectrogenic bacteria can increase these losses thereby increasing the internal resistance. However, since *C. papyrus* had fibrous roots growing, and presence of roots increases microbial activity [24], lack of exoelectrogenic bacteria was not seen as a possible reason for the high internal resistances. The *C. papyrus* roots had penetrated to the anodic region thereby depositing oxygen in an anaerobic zone and effectively reducing the oxygen gradient required for current flow (resistance to current) [13]. Timmers, et al. [96] showed that the internal resistance of a system increased with higher amounts of oxygen released at the anode. This may explain the high internal resistance observed in *C. papyrus* system.

Internal resistances in the control, *P.* australis and W. *thyrsiflora* were similar given the standard deviation. The roots of both of these systems were contained near the cathodic region thereby increasing oxygen supply and therefore PPD. Zhou, et al. [109] achieved lower internal resistances for two planted cells and higher for the remaining two relative to the control. The equal internal resistance could be attributed to root death which increases internal resistance. Similar observations were made by Zhou, et al. [109].

## 5.5 Organic removal

The organic removal was characterised in terms of VSS, COD, TKN and TP removal. The waste classification in terms of the aforementioned characterisation was measured at the start and end of the experiment. This was mainly because the solids in the WAS settled and any samples taken during the course of the experiment would give inaccurate results.

The tests were done using the standard lab procedures [103]. The results are summarised in Figure 38. The graphs present results both in absolute values and in removal percentages.

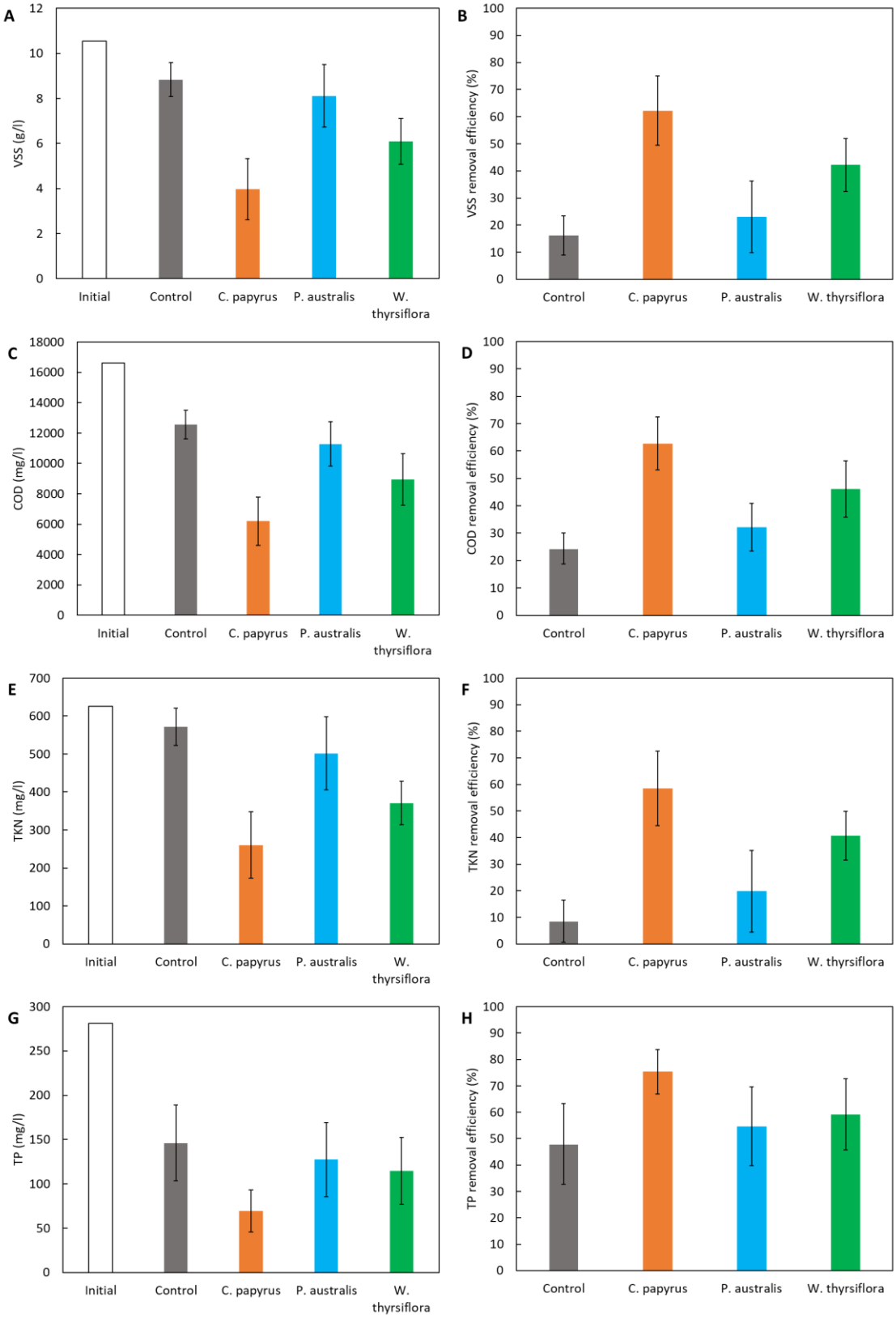



**Figure 38:** Graph showing VSS (A-B), COD (C-D), TKN (E-F) and TP (G-H) removal of different plant species at the end of the experiment when WAS was used as substrate.

### 5.5.1 VSS removal

The VSS removal refers to the breakdown of the solids in WAS. The VSS is removed by death of OHOs and PAOs and their consumption through microbial activity (electricity generation, anabolism and catabolism). The VSS removal followed the order of *C. papyrus* 62.2 ± 12.8% > *W. thyrsiflora* 42.2 ± 9.7 > *P. australis* 23.0 ± 13.2% > control 16.1 ± 7.2% (see Figure 38 (B)). The planted systems achieved higher VSS removal compared to the control. These results were expected as the roots help increase microbial activity through providing exudates and oxygen [24].

The *C. papyrus* achieved the highest removal, i.e. final VSS was 4.0 ± 1.3 gVSS/l. This was because the plant had a thick root structure that penetrated up to the anodic region. The VSS comprises of both a biodegradable fraction and an unbiodegradable fraction. The ratio of the two depends on the plant conditions. The *C. papyrus* achieved over 60% removal which in turn may account for over 90% removal of the biodegradable content.

The *W. thyrsiflora* achieved 20% lower VSS removal compared to the *C. papyrus*. The final VSS was 6.1 ± 1.0 gVSS/l. This was mainly because the roots in this system had died in the early stages of the experiment and new roots only grew out at later stages aiding in increasing the microbial activity in the set-ups.

The *P. australis* achieved the lowest removal of the three planted systems. The final VSS was 8.1 ± 1.4 gVSS/l. Its value was just 6.5% higher than the control system which had VSS of 8.8 ± 0.8 gVSS/l. This low removal can be attributed to low plant mass being used in the system relative to the plant mass of *C. papyrus* and *W. thyrsiflora*. The similarities of the *P. australis* system to the control was also observed in the voltage results (see Section 5.3.2).

### 5.5.2 COD removal

The COD removal followed a similar pattern to the VSS removal. This was because in order for the COD to be removed, the VSS was broken down releasing simpler compounds that were consumed by microorganisms for power generation and other anabolic and catabolic processes.

The COD removal followed the order of *C. papyrus* 62.8 ± 9.6% > *W. thyrsiflora* 46.1 ± 10.2% > *P. australis* 32.2 ± 8.8% > control 24.4 ± 5.7% (see Figure 38 (D)). Again, the planted systems achieved higher removal efficiencies than the unplanted control.

When comparing the removal efficiency to previous work, it was noticed that the removal efficiency was lower. Saz, et al. [20] and Liu, et al. [83] who used the same make of synthetic wastewater achieved over 85% and 94% removal in planted cells while unplanted cells a removal of had 75.8% and 92.1% respectively. Furthermore, Villaseñor, et al. [19] achieved 80% - 95% removal with COD concentration ranging from 250 mgCOD/l – 1100 mgCOD/l. All three systems were operated as continuous systems. The main reason why these systems achieved such high removal efficiencies was because they did not have an unbiodegradable fraction. The

unbiodegradable component contributes to COD but cannot be removed. Therefore 100% removal efficiency with WAS is not possible.

### 5.5.3 TKN removal

The TKN removal followed the order of *C. papyrus* 58.5 ± 14.0% > *W. thyrsiflora* 40.7 ± 9.2% > *P. australis* 19.8 ± 15.3% > control 8.6 ± 7.9% (see Figure 38 (F)). Again, the planted systems performed better than unplanted systems. This is likely because of two reasons, (1) the removal process is initiated with the breakdown of VSS. The VSS breakdown and removal followed the same hierarchy as the TKN removal and (2) the released FSA after the breakdown of OrgN would be removed with presence of nitrifiers and oxygen. As explained previously, plant roots support microbial growth (nitrifiers being one of them) and release oxygen which can be used for nitrification.

When comparing the TKN removal to VSS removal, the planted systems *C. papyrus, W. thyrsiflora* and *P. australis* had a close correlation i.e. 62.2% - 58.5%, 42.2% - 40.7%, and 23.0% - 19.8% respectively. The control however, had 16.2% VSS removal but only a TKN removal of 8.6%. This could be because of the absence of a plant to provide oxygen for FSA removal in the control. It is important to note that a correlation was seen because the initial FSA was equal to 9.89 mgN/l (which does not contribute to VSS but does contribute to TKN). This was very low in comparison to the initial TKN = 626 mgN/l i.e. 1.6% contribution. If this number was high, a correlation between VSS and TKN would be meaningless.

When comparing the removal efficiencies to previous research, Liu, et al. [83] had 90.8% TN removal in planted cells while unplanted cells had 54.4% TN. This removal is greater than that achieved in this research. There are two reasons for this, (1) Liu, et al. [83] had in influent TKN of 150 mgTKN/l which is a quarter of the TKN used in this research and (2) part of the TKN measured in WAS is derived from unbiodegradable organics. An analysis of the biodegradable fraction of the WAS is required to better understand the removal efficiencies to other researches.

### 5.5.4 TP removal

The TP removal followed the order of *C. papyrus* 75.4 ± 8.4% > *W. thyrsiflora* 59.2 ± 13.5 ≈ *P. australis* 54.6 ± 14.9% > control 48.0 ± 15.3% (see Figure 38 (H)). *C. papyrus* produced the highest removal efficiency, however, the difference between the control, *W. thyrsiflora* and *P. australis* was not statistically significant. The high removal efficiency in *C. papyrus* was mainly because of the rapid growth of the plant.

The growth of *W. thyrsiflora and P. australis* was not as significant. In the former, the plant started dying initially and formed new roots at a later stage and the later only had three to four new shoots growing. This could explain the lower removal efficiencies in these planted set-ups. The unanswered question is how the control achieved a high removal efficiency as it was unplanted. The control had algae growing on its surface which may explain the phosphorus removal. Saz, et

al. [20] achieved 97.0 ± 4.4% in the planted system while the unplanted had a removal efficiency of 71.8%. There was no mention of algae growth, also there was no reason provided for high TP removal in the control. Unfortunately, apart from the work done by Saz, et al. [20] there is no information in literature that compares the effects of the different plant species on TP removal.

The TP removal could not be correlated with VSS removal as the initial OP = 37.6 mgP/l (which again being soluble does not contribute to VSS) was high relative to the initial TP = 281 mgP/l i.e. it was 13.4% and would skew the comparisons.

## 5.6 FSA removal

The FSA ($NH_4^+/NH_3$) was measured at regular intervals from two sampling points, top and bottom (bot) as shown in Figure 39. The complete FSA profile is provided in Appendix C. The removal efficiencies provided in Figure 39 is a comparison of initial and final FSA values. The initial FSA was 9.89 mgN/l. This value increased in both sampling tubes as the OrgN broke down to FSA. With time, this value decreased to a higher/lower than initial value depending on the system.

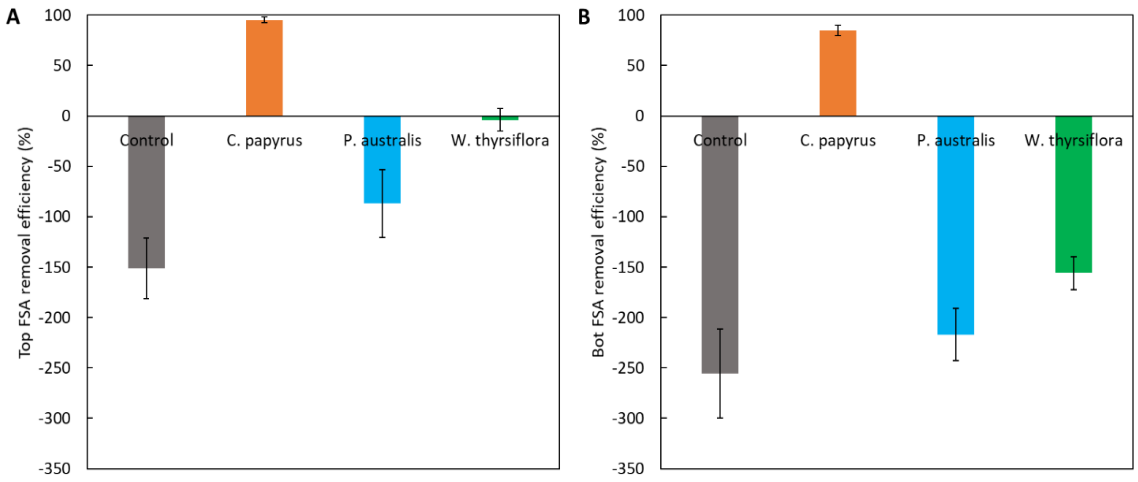



**Figure 39:** FSA removal from the top (A) and bottom (B) sampling tube for different plant species when using WAS as substrate.

### 5.6.1 Top sampling tube

The top sampling tube was placed closer to the roots. This meant that the surrounding area was influenced by oxygen release and therefore produced higher removal efficiencies. The FSA removal followed the order of *C. papyrus* 95.3 ± 2.8% > *W. thyrsiflora* -3.94 ± 11.1 > *P. australis* -86.9 ± 33.8% > control -151.3 ± 30.3%.

The planted systems were found to perform better than the unplanted control. However, a large variation within the planted systems was noticed. The *C. papyrus* performed significantly better

than other systems given its thick root mass. The root mass created a large surface area, provided exudates and oxygen thus promoting microbial growth and therefore nitrification. The *W. thyrsiflora* achieved close to zero FSA removal, this was mainly because the root system initially died out and new roots started growing on the surface of the PMFC halfway through the experiments. If the experimental duration was longer, the new root system could potentially have removed a higher FSA percentage.

The root mass of the *P. australis* as explained previously was significantly lower than the *C. papyrus* and *W. thyrsiflora* and therefore had lower removal efficiencies. Since the *P. australis* is a robust wetland plant that is constantly used for wastewater treatment, even with a lower root mass had about 65% better removal efficiency to the control.

It becomes difficult to compare the obtained results to previous studies [19, 20, 25, 83] because all of them used synthetic wastewater which did not have OrgN. Therefore, their FSA had a continuous decrease whereas in this research, the high concentration of OrgN was released as FSA and then removal occurred. Also, taking the maximum FSA released as a starting point would be incorrect as the *C. papyrus* systems for example continuously consume this FSA at a faster rate than the control and it would skew the maximum values.

### 5.6.2 Bottom sampling tube

The bottom sampling tube was placed closer to the anode; therefore, it was located at more anaerobic conditions. This meant lower oxygen concentrations were available to nitrify FSA. The FSA removal followed the order of *C. papyrus* 84.8 ± 5.0% > *W. thyrsiflora* -156.0 ± 16.3 > *P. australis* -216.9 ± 26.0% > control -255.4 ± 44.1%.

Lower removal efficiency were observed because of slow growth rate of OHOs at the bottom sampling area. The FSA is released into the system with VSS breakdown. The VSS is derived from OHOs and PAO's. The death rate of OHOs is 0.62/d at 20℃, however, in aerobic conditions, OHOs simultaneously grow, offsetting the death rate to 0.24/d at 20℃ [110]. In complete anaerobic zones (absence of an electron acceptor), OHOs cannot grow and therefore the death rate is not offset by the growth rate. Since the bottom sampling tube was located in a more 'anaerobic' region than the top sampling tube, the death rate was offset by a smaller margin relative to the top tube and therefore had a higher FSA release.

Similar to the top sampling tube results, the planted systems performed better than the unplanted systems. However, when the values from the top sampling tube were compared to the bottom, it was observed that *C. papyrus* had a similar removal efficiency, but the remaining systems had significantly lower removal at the bottom tube. The *C. papyrus* achieved high removal rates at the bottom as the roots had grown through to the anaerobic region where the bottom sampling tube was placed.

The *W. thyrsiflora* only had roots growing on the surface and therefore it achieved significantly lower removal at the bottom (-3.9% at the top versus to -156.0% at the bottom). The same was observed in *P. australis* where efficiency dropped from -86.9% to -216.9%. The control on the other hand also showed lower removal efficiencies as the top sampling tube was closer to the surface and oxygen dissolving from the atmosphere to the system could be utilised for FSA removal. The efficiency dropped from -151.3% to -255.4%. This decrease in efficiency is not as significant as observed in the two aforementioned set-ups. This is because the difference in the control system was only the presence of surface oxygen, but the aforementioned systems lost both surface oxygen and root oxygen release.

## 5.7 OP removal

The OP ($H_2PO_4^-/HPO_4^{-2}/PO_4^{-3}$) was measured at regular intervals from two sampling points, top and bottom (bot) as shown in Figure 40. The complete OP profile is provided in Appendix C. The removal efficiencies provided in Figure 40 is a comparison of initial and final OP values. The initial OP was 37.6 mgP/l. This value increased in both sampling tubes as the OrgP broke down to OP. With time, this value decreases to values lower than initial value.

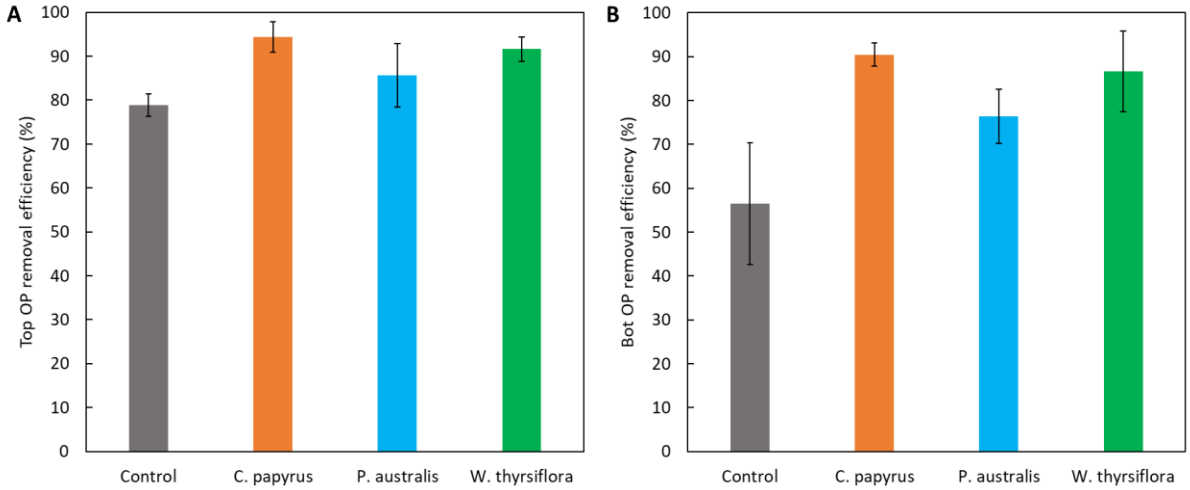



**Figure 40:** OP removal from the top (A) and bottom (B) sampling tube for different plant species when using WAS as substrate.

From Figure 40, it can be seen that the planted systems achieved higher OP removal efficiency than the unplanted systems. Also, the OP removal in both sampling tubes was significantly higher than the FSA removal. The difference in removal within the planted systems was not statistically significant.

The high OP removal was because of two main reasons, firstly, OP being an important nutrient in plant growth was consumed by the three plants (*C. papyrus* in increasing root and shoot mass, *P.*

*australis* in growing new shoots, *W. thyrsiflora* in growing new roots, and in the growth of Algae in all the set-ups). Secondly, the WAS was sourced from a plant running on the UCT system. This means that the WAS had PAO's along with OHOs in the sludge.. Coupled with this, the inoculum that was used came from an anaerobic digestor whose aim was to grow PAOs. This explains the high OP removal in the planted and unplanted control.

## 5.8 Fertiliser capabilities of PMFC waste

To utilise sludge as a fertiliser after PMFC experiment, the microbiological, stability and pollutant classification is required to be met as set by the Department of Water and Forestry South Africa [62] (see Section 2.2.4.1 and Appendix H). The microbiological classification could not be determined in this experiment as it required the quantification of faecal coliforms and helminth ova which was out of the scope of this research. Also, the pollutant removal was not quantified because of equipment limitation.

### 5.8.1 Stability classification

For this research, the stability classification was evaluated based on achieving a VSS removal of 38%. From Figure 38, the 38% VSS removal criteria of WAS after going through the PMFC experiment was only achieved by *C. papyrus* (62.2 ± 12.8%) and *W. thyrsiflora* (42.2 ± 9.7) systems. The control and *P. australis* had lower VSS removals and were therefore not suitable and would require further treatment.

### 5.8.2 NP ratio fertilisers

Fertilisers are commonly based on NPK values. In this research, potassium (K) was not measured, but nitrogen (N) and phosphate (P) were measured. Table 15 summarises the results across all set-ups which passes the Stability Classification.

**Table 11:** N-P ratio of PS.

| System | Nitrogen (mg/l) | Nitrogen% | Phosphorus (mg/l) | Phosphorus% | N:P ratio |
|---|---|---|---|---|---|
| *C. papyrus* | 260 | 0.26 | 69 | 0.069 | 0.26:0.07 |
| *W. thyrsiflora* | 371 | 0.37 | 115 | 0.12 | 0.37:0.12 |

The highest N:P ratio was derived from the *W. thyrsiflora*, which was expected given the slow growth of this plant when compared to *C. papyrus* which had a dense root structure at the end of the experiment. It was also noticed that the N:P ratios were lower than organic fertilisers obtained from animals except for Dairy cow manure. Dairy cow manure, which had the lowest ratios compared to other animals had a ratio of 0.25:0.15: 0.25 (N:P:K) [111]. The N:P rations of the PMFC waste would in fact even be lower than those in Table 15 as the unbiodegradable content was not unaccounted for. This suggests that more fertiliser would require to be applied to

agricultural fields to provide the right amount of nutrients. This can be a problem if the metal content is high (see Section 0).

## 5.9 Assessment of plant species health

Assessment of plant health is a very important factor when choosing the plant species. All three plant species were qualitatively assessed based on shoot and root growth. Even though wetland plants were chosen for this experiment, the species can survive harsh conditions to different extents. The subsections to follow explain the performance of the three species.

### 5.9.1 *C. papyrus*

The *C. papyrus* had a significant growth in both the roots and shoots from the start to the end of the experiment (see Figure 41). The initial thin roots at the start of the experiment turned to thick and dense white roots. Compared to the other two plant species, the *C. papyrus* showed the highest growth. This also explains the high treatment efficiencies obtained in the *C. papyrus* relative to the other plant species.

The roots of the *C. papyrus* had grown out to the anodic region and GAC stuck at the base of the roots can be seen in see Figure 41 B. The roots penetrating into the GAC and releasing oxygen in an anaerobic zone could be linked to voltage results and internal resistance obtained.

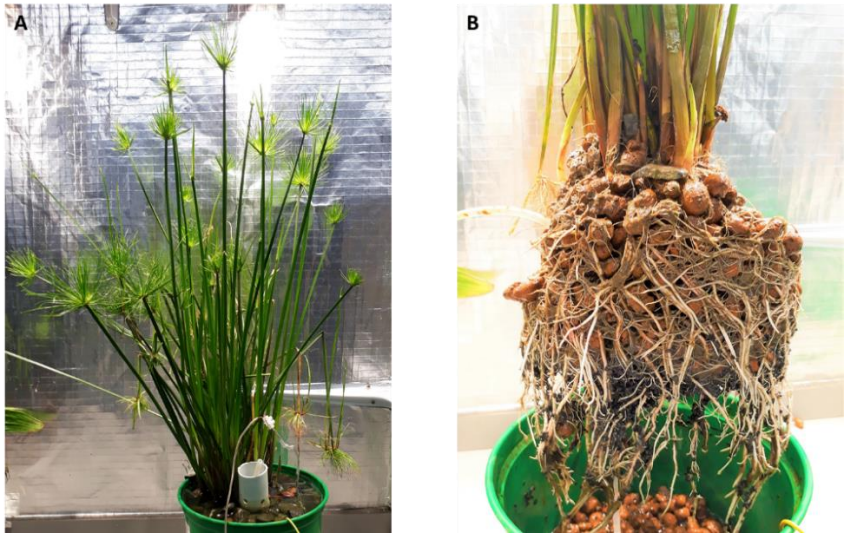



**Figure 41:** Assessment of *C. papyrus* shoots (A) and roots (B) at end of experiment.

### 5.9.2 *P. australis*

The *P. australis* at the time of sourcing from the bird sanctuary was healthy. After it was left submerged in water and added to the set-up, the shoots started drying out (see Figure 42 A). This may be as a result of transportation shock and change from a solid substrate to a hydroponic

system. However, unlike the *W. thyrsiflora*, the root structure did not have dead root mass (see Figure 42 B).

As the experiment progressed, new shoot mass was seen growing at different rates from all three *P. australis* set-ups on day 16 (see Figure 42 A). Since all other conditions were same, the rate of growth was governed by the initial root mass used. The shoots grew to about 0.7 – 0.8 meters in length towards the end of the experiment.

The *P. australis* plant mass used in the experiment was significantly lower when compared to the *C. papyrus* and *W. thyrsiflora*. This may explain why the voltage and removal efficiencies mimicked the controls and only performed slightly better.

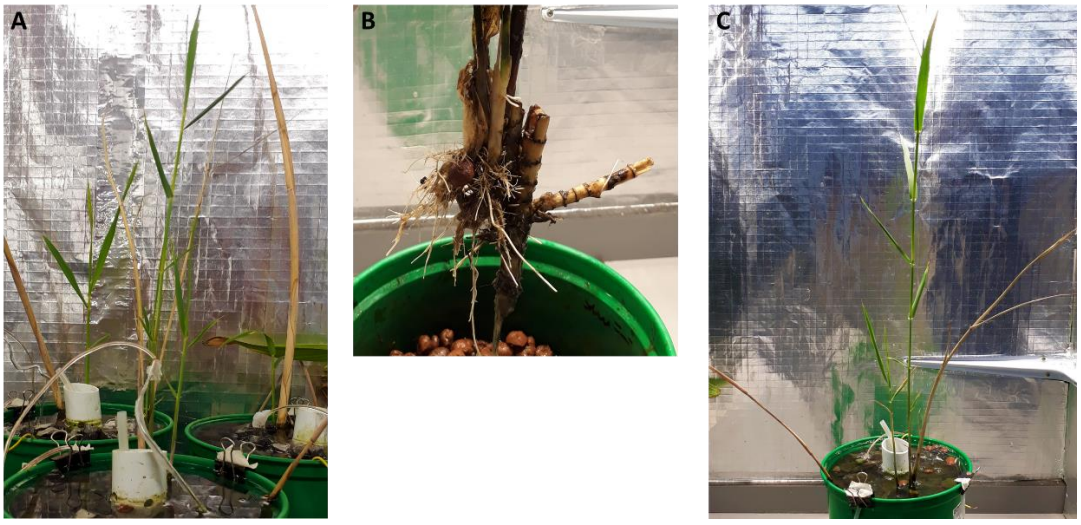



**Figure 42:** Assessment of *P. australis* roots and shoots on day 16 (A) and day 45 (B-C).

### 5.9.3 *W. thyrsiflora*

The *W. thyrsiflora* indicated variability in its ability to survive the harsh PMFC conditions. During the initial phase of the experiment, i.e. on the third day of operation, it was noticed that some of the leaves in all three *W. thyrsiflora* set-ups had started wilting (see Figure 43 C). This indicated that the water lost by evapotranspiration was not replaced and taken up by the roots. The wilting also suggest potassium deficiency in the plant. This may be linked to root mass dying (see Figure 43 B).

Furthermore, the leaves started exhibiting yellow colour and their tips turned brown. This indicates an insufficient consumption of phosphorus and potassium [112]. The lack of phosphorus uptake could again be linked to the roots dying. Yellowing leaves could also be caused by a lack of dissolved oxygen present at the roots [48].

As the experiment progressed, it was noticed on day 16 that new roots had grown in the water later of the system (Figure 43 A). This may be linked with low organic content and higher dissolved oxygen concentrations on the surface of the set-up. The new root growth allowed the plant to absorb water and nutrients and the wilting had stopped. Also, the yellowing of leaves was reversed but the brown tips were maintained until the end of the experiment (see Figure 43 C). The surface roots were thick and dense towards the end of the experiment and the oxygen released directly in the cathodic area may explain the high voltage readings obtained.


**Figure 43:** Assessment of *W. thyrsiflora* roots and shoots on day 16 (A) and day 45 (B-C).

## 5.10 Lessons learnt from this experiment

This experiment focused on using WAS as substrate with the same 3 plant species to obtain the PPD and organic removal. This experiment showed that:

1. the selected external resistance should also be changed when the substrate is changed. It was observed that a 100 ohm resistor was not suitable even though the experiment was run for 34 days using a 100 ohm resistor. The voltage reading did not increase past 0.04 V making measurements difficult. Changing to the resistance to 1000 ohms produced measurable voltages;

2. even though the COD decreased from 175 to 16.6 gCOD/l, the *W. thyrsiflora* still could not grow in the high organic load and the leaves started going brown in the start before improving with time. When the roots were examined after the experiment was completed, it was noticed that roots at the start of the experiment had died and new roots grew on the surface.

3. *W. thyrsiflora* produced the highest PPD in comparison to other systems when the plant acclimatized to the growing conditions. However, the best organic removal was achieved with *C. papyrus*.

# 6. Primary sludge as substrate

## 6.1 Introduction

Primary sludge was the second feed tested in the PMFC. The PS experiment was started on the 17th of July 2018 (day zero) and concluded on the 10th of November 2018 (day 28) allowing sufficient time to obtain stable results. The subsections to follow discuss the voltage output and the organic removal capabilities of the three plants tested.

## 6.2 Experimental design

Each PMFC was set up as described in Section 3.5 (see Figure 17). Triplicates of each experiment were set up and an average of the results was calculated.

### 6.2.1 Experimental set-ups

Four systems each containing three replicate set-ups were tested. Three systems contained plants while one system did not have a plant (control). The systems were as follows:

1) Control with no plant
2) *C. papyrus*;
3) *P. australis* and
4) *W. thyrsiflora*

A 1000 ohms resistor was connected across the set-ups. The choice of 1000 ohms was based on the results obtained in the previous experiment (see Chapter 5).

### 6.2.2 Substrate used

Primary Sludge used in this experiment was sourced from Potsdam WWTWs. PS is a biodegradable rich organic substrate that settles out in a primary settling tank. PS is commonly treated in anaerobic digesters to generate electricity and simultaneously treat the wastewater in terms of COD removal.

The collected PS was characterised based on its TSS, VSS, ISS, COD, VFA, TKN, FSA, TP) and OP. The results are summarised in Table 12.

**Table 12:** PS characteristics.

| Characteristic | TSS | VSS | ISS | COD | COD BPO | COD UPO | TKN | FSA | TP | OP |
|---|---|---|---|---|---|---|---|---|---|---|
| **mg/l** | 8995 | 7806 | 1189 | 10518 | 6662 | 755 | 560 | 13.9 | 61.5 | 14.8 |

## 6.3 Voltage results and discussion

### 6.3.1 General results obtained across all set-ups

The voltage readings across a 1000 ohms resistor recorded from day 5 to 28 for all the set-ups is provided in Figure 44. The cells were left as open circuit from day 0 to day 5 to allow for the system to stabilise. For all the systems it was noticed that voltage readings exponentially increased after day 20. The same results were obtained when thickened WAS was used as a substrate (see Sections 4.4.4, 4.4.5 and 4.4.6).

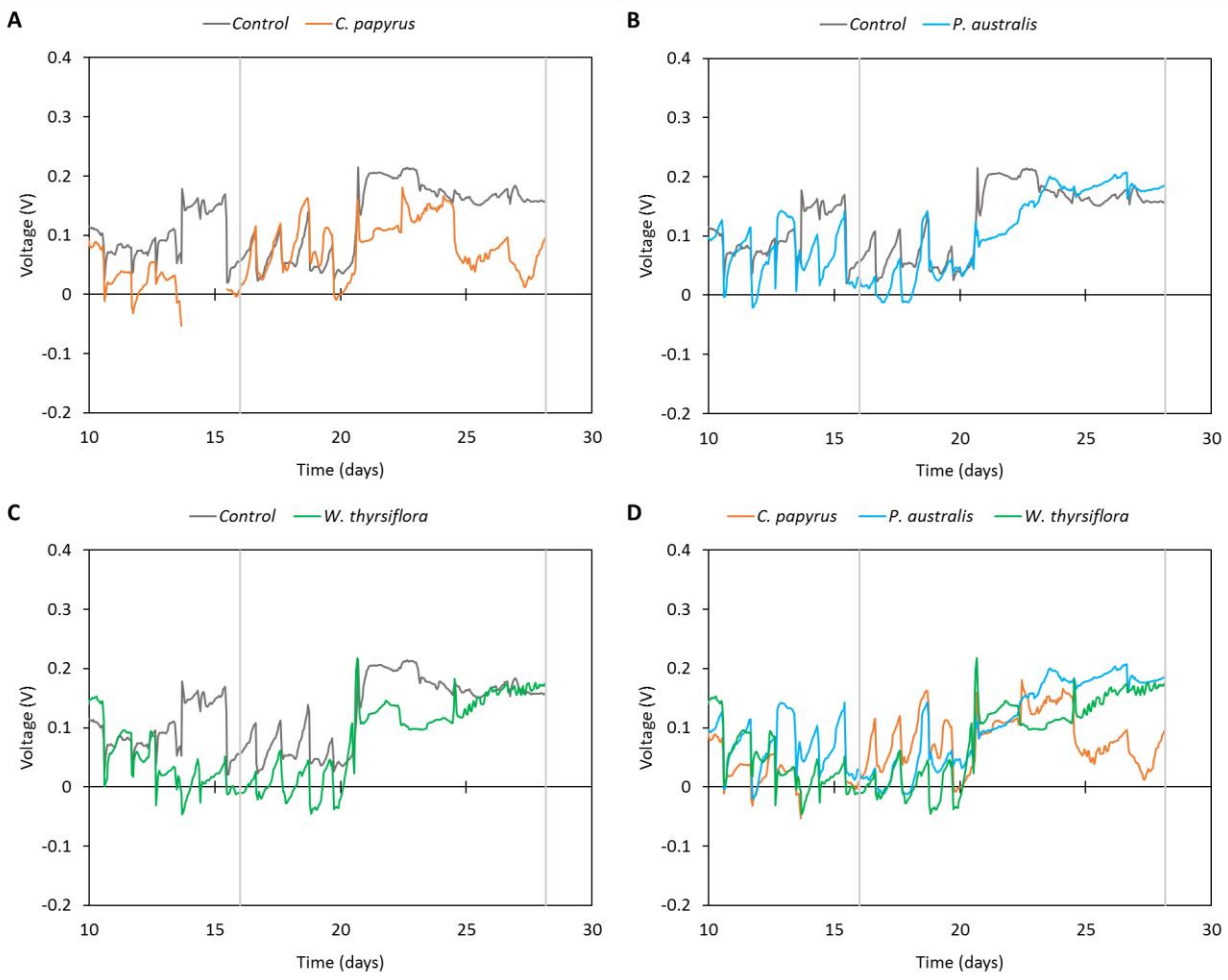



**Figure 44:** Voltage recorded across a 1000 ohms resistor for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) in comparison to the control and a comparison of all plant species (D) investigated when using WAS. Vertical lines indicate polarisation test days.

This may be as a result of a mismatch between the inoculum and the feed used in this experiment. The inoculum was sourced from an anaerobic digester which was fed WAS from Zandvliet WWTWs as explained in Section 3.6. However, the feed used in the PMFC was PS. The

biodegradable organics in the PS are available in both forms i.e. soluble and particulate, while the biodegradable organics in WAS are stored in the biomass and are released after biomass death. This may be the cause of the systems requiring a longer time to prioritize the growth of exoelectrogenic bacteria.

In addition, the longer time to acclimatize may also be a result of a higher microbial activity in the WAS compared to the PS. The WAS underwent biological processing where OHOs, autotrophs and PAOs removed COD, FSA and OP respectively from the wastewater. The PS in comparison was obtained through physical processes only.

Interestingly, after day 20, all the planted systems showed increasing voltage readings, but their magnitude was lower than the control. The voltage in the planted systems increased steadily in the next few days to match the control. Planted systems have shown to increase microbial activity which in turn aid in power generation and organic removal [19, 20, 24, 26]. However, the results obtained in this experiment indicate that the control achieved a higher voltage magnitude than the planted systems. Since the control, similar to all the other system was connected to an external resistance, it promoted the growth of exoelectrogenic bacteria [106]. The 15-day closed circuit period may have been enough to reach maximum exoelectrogenic bacteria concentration. Also, since the planted systems increase microbial activity, they may have been responsible for promoting growth of microbes other than exoelectrogenic bacteria and therefore achieved lower magnitudes in the start and quickly caught up with the control as exoelectrogenic bacteria concentration was maximised. This is only a speculation, further research into the microbial aspect of the PMFC is required.

Overall, once the voltage readings were stable (after day 20), it was noticed that the voltage readings whether planted or not were all approximately the same. Therefore, the presence of plants may not have aided in the power generation when using PS. This is partly because the plant health was not as good as it was when using WAS (see Section 6.9). Also, plants aid in increasing microbial activity through exudate release. However, since PS has a high concentration of readily biodegradable soluble organics, the presence of exudates may not have made a significant difference. The benefit of using a planted cell however was more observable in FSA removal (see Section 6.6). The subsections to follow discuss the voltage output of planted systems in comparison to the control.

### 6.3.2 *C. papyrus*

The *C. papyrus* set-ups recorded low voltage readings before day 20. The voltage results closely matched the voltage readings from the control system during this period. This may be a result of the microbes from the inoculum adjusting to the feed (see Section 6.3.1 for more information). After day 20, the *C. papyrus* voltage readings started increasing similar to the control but with a lower magnitude. On day 21, the voltage reading across the *C. papyrus* system read 0.1 V and after

three days, on day 24 had a magnitude of approximately 0.18 V similar to the control. The reason for this is discussed in Section 6.3.1.

After this, the voltage dropped lower than the control the next day, i.e. day 35. This may be as a result of the plants' ability to remove FSA (see Sections 5.6 and 6.6). The FSA is converted to nitrates, a terminal electron acceptor like oxygen, but with a lower cathode potential i.e. 0.74 V for nitrates and 1.24 V for oxygen [25]. There may have been a nitrate build up in the system and on day 25, the electron acceptor at the cathode may have swapped from using oxygen to using nitrates. The same observation was made in the previous experiment i.e. when using WAS (see Section 5.3.1). However, the voltage was decreased gradually in the previous experiment as OrgN was gradually broken down to FSA with OHO and PAO death. The reason for the voltage drop cannot be explained by the roots potentially growing in the anodic region and releasing oxygen as this would also be a gradual process and not a straight drop in voltage. Secondly, the roots were did not reach the anodic region in this experiment (see Section 6.9).

### 6.3.3 *P. australis*

The voltage results of the *P. australis* exhibited a cyclic variation up to day 20. The voltage results ranged from 0.13 V to 0.00 V. After day 20 the voltage readings started increasing for reasons explained in Section 6.3.1. The magnitude of the voltage after day 20 was lower than the control, but, a steady increase in voltage was noticed. On day 23, the voltage reading in *P. australis* system was the same as the control.

When comparing the *P. australis* to *C. papyrus* and *W. thyrsiflora*, it was noticed that voltage readings in the former system was greater than the latter two for the first 15 days. This may be as a result of the low *P. australis* plant mass used in comparison to *C. papyrus* and *W. thyrsiflora* plant mass. This meant the voltage readings matched the control, which performed better than the planted set-ups. After day 20, the voltage results in all set-ups were approximately equal.

### 6.3.4 *W. thyrsiflora*

The *W. thyrsiflora* produced the lowest voltage results before day 20. The voltage results ranged from -0.04 V to +0.06 V. Apart from the *P. australis* which produced negative voltage values thrice in all the cycles, no other system produced negative voltages. The negative voltage readings can be linked to microbial underperformance. The voltage started picking up when the exoelectrogenic bacteria had stabilised.

The voltage results after day 20 was less than the control. The reasons for which are provided in Section 6.3.1. The voltage readings not being higher than control as observed in the previous experiments may be as a result of the roots not growing as significantly as they had done when using WAS as substrate. Lower root mass meant that less oxygen released into the surface layer where the cathode was placed.

## 6.4 Polarisation test results and discussions

Two polarisation tests were conducted during the course of the experiment. The first test was done on day 16 and the second on day 28. The results are provided in Figure 45 and Table 13.

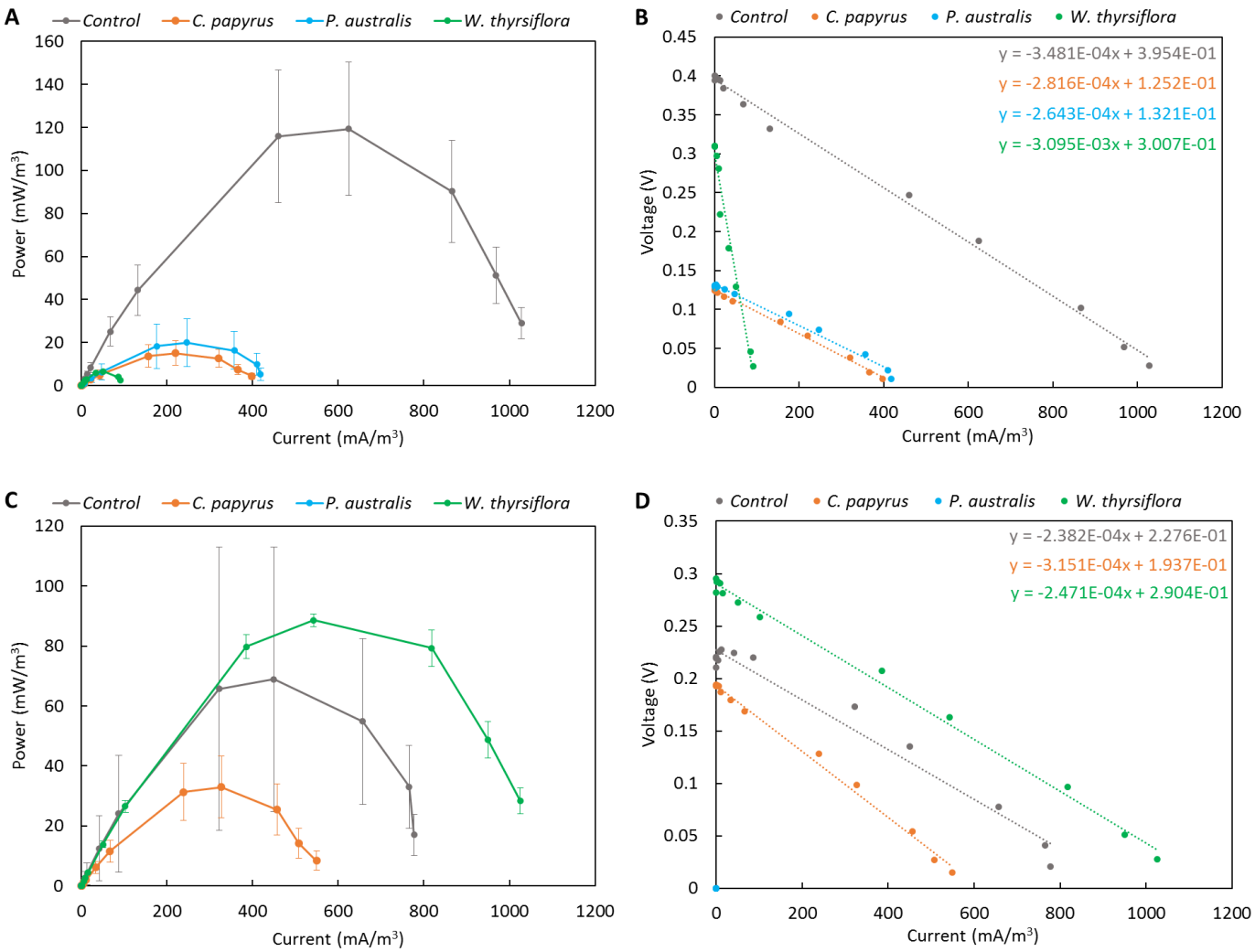



**Figure 45:** Polarisation test results on day 16 (A and B) and day 28 (C and D) when using PS as substrate. A and C show PPDs for the three plant species while B and D show voltage versus current results. The error bar represents the standard deviation from the mean of the triplicate set-ups.

**Table 13:** Peak power density and internal resistance of the set-ups using PS as feed.

| System | Test 1 | | Test 2 | |
|---|---|---|---|---|
| | **Peak power density ($mW/m^3$)** | **Internal resistance (Ω)** | **Peak power density ($mW/m^3$)** | **Internal resistance (Ω)** |
| Control | 119 ± 31 | 647 ± 98 | 68 ± 44 | 443 ± 379 |
| *C. papyrus* | 15 ± 6 | 524 ± 130 | 34 ± 9 | 586 ± 127 |
| *P. australis* | 20 ± 11 | 492 ± 171 | – | – |
| *W. thyrsiflora* | 7 | 5757 | 89 ± 2 | 460 ± 20 |

### 6.4.1 Test 1

The purpose of the first test was to obtain the internal resistance of the cells and compare them to the external resistance connected as the voltage readings were very low. If the internal resistance was significantly greater, it would explain the low voltage readings (similar to the very low voltage readings when 100 ohms was connected across the cells in Chapter 5).

The control system had the highest PPD, approximately, 119 ± 31 $mW/m^3$. This was six times greater than the power densities in *C. papyrus* (15 ± 6 $mW/m^3$) and *P. australis* (20 ± 11 $mW/m^3$). The internal resistances of the mentioned planted systems was the same as the control given the standard deviation. Lower internal resistances in planted systems was observed by Saz, et al. [20] and Zhou, et al. [109]. The low PPDs were as a result of low voltage readings across the set-ups at the time of polarisation. From Figure 44, it can be seen that the voltage readings across the *C. papyrus* and *P. australis* were close to zero at the time of polarisation tests. If the test was performed after day 16 where both set-ups had voltage readings similar to the control, the planted systems may have had a PPD equal to if not greater than the control.

The *W. thyrsiflora* recorded a very low PPD (7 $mW/m^3$) relative to the control. Unlike the other two planted systems, the *W. thyrsiflora* system had a very high internal resistance, i.e. 5757 ohms compared to 647 ohms in the control. This may be as a result of the plant roots dying initially before being regrown (see Section 6.9). Zhou, et al. [109] also noticed high internal resistances in planted set-up using *Acorus calamus* relative to the control (5000 ohms and 600 ohms respectively). The *Acorus calamus* had died in the system and resulted in higher ohmic losses. The *W. thyrsiflora* after re-growing new roots had lower internal resistances (see Section 6.4.2)

### 6.4.2 Test 2

Test 2 was done on the last day of the results collection (day 28). There was a malfunction in the voltage recording device and the polarisation test for the *P. australis* could not be done. However, it is speculated that since the voltage readings were similar to *W. thyrsiflora,* the PPD would also have produced similar results.

The *W. thyrsiflora* (89 ± 2 mW/m$^3$) produced the highest PPD. However, given the high standard deviation in the control (68 ± 44 mW/m$^3$), the highest PPD could have been produced in either system (see t-test in Appendix I). This was expected as the voltage readings at the time of the polarisation test were similar in both systems. The internal resistance in *W. thyrsiflora* was significantly lower than Test 1 internal resistance i.e. 460 ohms compared to 5757 ohms. This was because during Test 1, the roots had died out in the *W. thyrsiflora* and in test 2 new roots had grown out.

The *C. papyrus* produced the lowest PPDs i.e. 34 ± 9 mW/m$^3$. This was because the voltage readings after matching the control on day 24, dropped on day 25 potentially due to nitrate reduction at the cathode instead of oxygen reduction. The internal resistances in test 1 and test 2 were the same taking the standard deviation into account. This may be because unlike the previous experiment where roots had penetrated to the anodic region, the roots in this experiment were still further away from the anodic region (see Section 6.9).

## 6.5 Organic removal

The organic removal was characterised in terms of SS, COD, TKN and TP removal. The waste classification in terms of the aforementioned characterisation was measured at the start and end of experiment. This was mainly because the solids in the PS settle and any samples taken during the course of the experiment would provide erroneous results.

The tests were done using the standard lab procedures [103]. The results are summarised in Figure 46. The graphs present results both in absolute values and in removal percentages.

From the onset, the results were very different compared to the previous experiment where WAS was used as feed. The PS produced similar VSS, COD, TKN and TP removal efficiencies in all systems. However, the WAS had a large variation between the set-ups, and for all removals the *C. papyrus* outperformed the other systems by a significant margin. This may be because of the different makeup of the two feeds. As mentioned previously, organics in WAS are released after OHO and PAO death while in PS the organics are readily available. The need for death and breakdown through microbial activity meant that planted systems with their root structure improving microbial activity aided in better removals. The same concept may not have had a significant impact when using PS because of the readily biodegradable organics.

### 6.5.1 VSS removal

The VSS removal refers to the breakdown of the solids in PS. The VSS is removed by the breakdown and consumption of biodegradable particulate organics. Approximately equal VSS removal was observed across all set-ups. The VSS removal followed the order of *C. papyrus* 59.4 ± 9.7% ≈ *W. thyrsiflora* 60.1 ± 2.6 ≈ *P. australis* 60.3 ± 2.7% ≈ control 59.4 ± 8.3% (see Figure 46 (B)).

The planted systems, even though they have been shown to increase microbial activity [20, 24], did not make a difference in PS. This may be as a result of readily biodegradable organics present in the PS as explained previously.

### 6.5.2 COD removal

The COD had two contributors, biodegradable soluble organics and biodegradable particulate organics. The COD removal followed the order of control 56.5 ± 11.0% ≥ *C. papyrus* 45.7 ± 10.4% ≈ *W. thyrsiflora* 49.3 ± 7.4 ≈ *P. australis* 51.0 ± 3.0 (see Figure 46 (D)). Slightly higher COD removal was observed in the control. This may be as a result of it producing consistent voltage readings while the planted systems produced low voltage readings until the system acclimatized. Power generation occurs as a result of COD consumption, therefore higher voltage readings translated to more COD consumption.

It was also noticed that VSS removal was higher than COD removal for all set-ups. This means that not all the VSS that was broken down was consumed in the system. This may be as a result of a high biodegradable soluble organics content available in PS. The soluble organics, which do not contribute to a VSS were likely consumed first as they are available in a simple form.

### 6.5.3 TKN removal

The TKN removal followed the order of *C. papyrus* 82.0 ± 3.3% > *W. thyrsiflora* 76.0 ± 4.1 ≈ *P. australis* 75.0 ± 3.9% > control 71.3 ± 1.7% (see Figure 46 (F)). The *C. papyrus* outperformed the other two planted systems. This may be a result of its thick root structure which potentially increased the dissolved oxygen concentration in the system. Higher oxygen meant better removal efficiencies.

The planted systems performed slightly better than the control. This was expected as the planted systems released oxygen which aided in the conversion of FSA to nitrates therefore removing TKN. Also, the conversion of FSA to nitrates is dependent on the presence of nitrifiers in the system. Since plants have shown to increase microbial activity [24], planted systems would have a higher concentration of microorganisms. However, without the presence of plants, the control still had a high TKN removal i.e. 71.3%. This is significantly higher when compared to the removal observed with WAS i.e. 8.7% even though the initial TKN were similar i.e. 560 mgN/l in PS and 626 mgN/l in WAS (see Section 5.5.3). However, the FSA peak value in WAS was greater than in PS as the breakdown occurred.

Also, it was noticed that the OrgN was converted to FSA in PS at a faster rate than in WAS (see Appendix C). This allowed a longer time duration to convert the FSA to nitrates. Saz, et al. [20] also noticed high ammonium removals in non-planted cells, i.e. 88.1 ± 7.15 and 97.3 ± 1.57 in planted cells. The possible preference of converting oxygen to nitrates instead of using the oxygen for power production may explain the lower than expected voltage readings observed in PS in comparison to WAS [109].

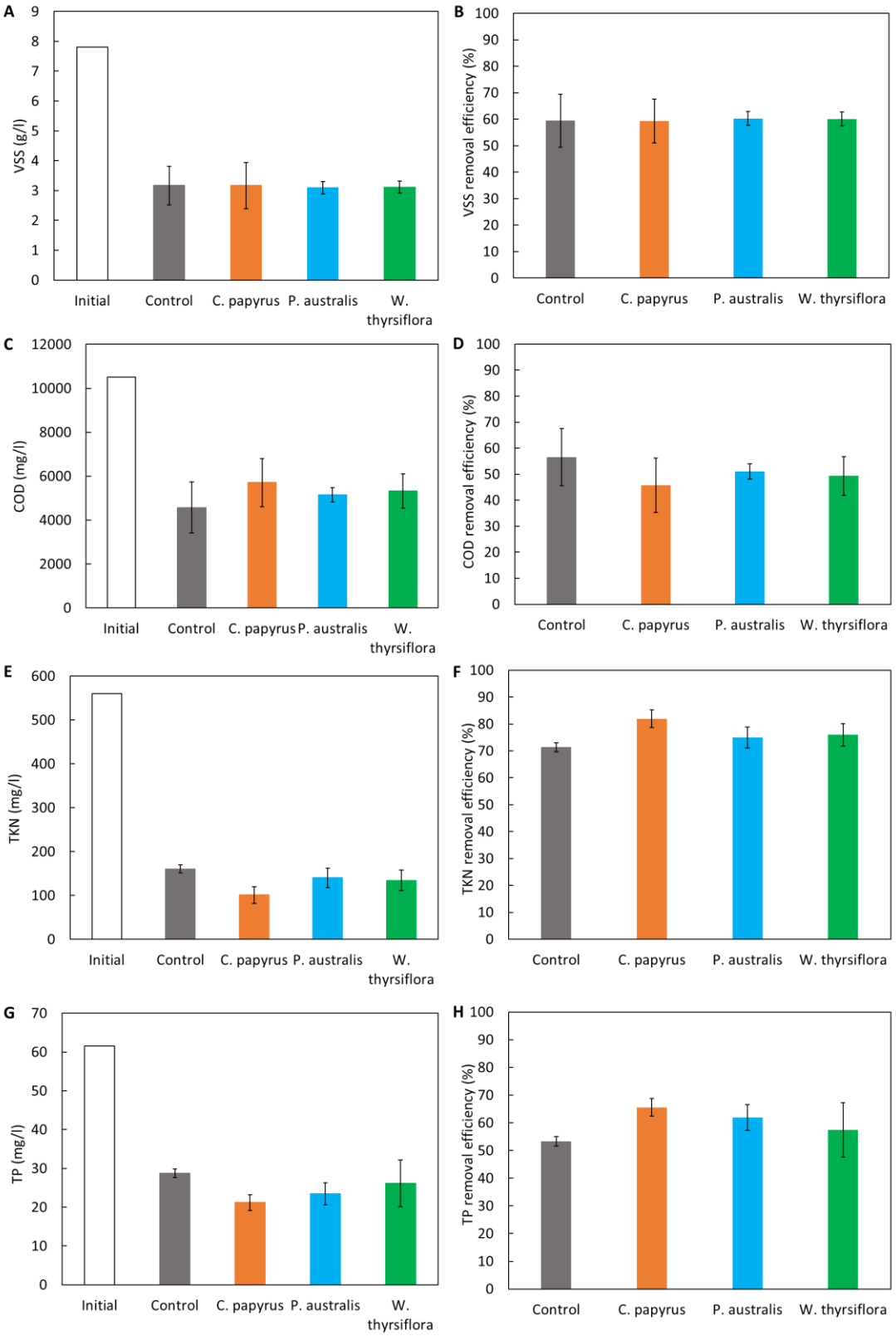



**Figure 46:** Graph showing VSS (A-B), COD (C-D), TKN (E-F) and TP (G-H) removal of different plant species at the end of the experiment when PS was used as substrate.

When comparing the TKN removal to the VSS removal, it was observed that TKN removal percentages were higher than the VSS removal percentages. This again can be related to the presence of biodegradable soluble organics which contribute to a TKN measure, but do not have a VSS measure.

### 6.5.4 TP removal

The TP removal followed the order of *C. papyrus* 65.6 ± 3.2% > *P. australis* 61.9 ± 4.6% ≈ *W. thyrsiflora* 57.4 ± 9.8 > control 53.2 ± 1.8% (see Figure 46 (F)).

The planted systems again performed better than the unplanted system given their root mass growth. The *C. papyrus* achieved the highest removal while the remaining two planted cells were equal given the standard deviation. However, the difference in planted compared to control was not as significant as observed in WAS (see Section 5.5.4). This was because the initial TP in WAS was significantly higher than in PS, i.e. 281 mgP/l and 62 mgP/l respectively. Lower initial TP meant the difference in planted versus control was not as prominent.

## 6.6 FSA removal

The FSA ($NH_4^+/NH_3$) was measured at regular intervals from two sampling points, top and bottom (bot) as shown in Figure 47. The complete FSA profile is provided in Appendix C. The removal efficiencies provided in Figure 47 is a comparison of initial and final FSA values. The initial FSA was 13.9 mgN/l. This value increased with time in both sampling tubes as the OrgN broke down to FSA. The FSA started decreasing after reaching to a peak value and achieved a final FSA value lower than initial.

Positive FSA removal was observed in both the top tube and the bottom tube. The top tube achieved higher FSA removal rates which was expected as the top tube was in a more aerobic zone relative to the bottom tube that was closer to the anode. The high removal efficiencies in both the top and bottom tube link to the high TKN removal efficiencies observed in PS. Importantly, that these removal values are after most of the OrgN was already converted to FSA which means that the conversion of OrgN to FSA to nitrates was very efficient therefore producing high TKN removals. This high removal efficiency can be as a result of OrgN converted to FSA at a faster rate allowing more time for the systems to convert FSA to nitrates.

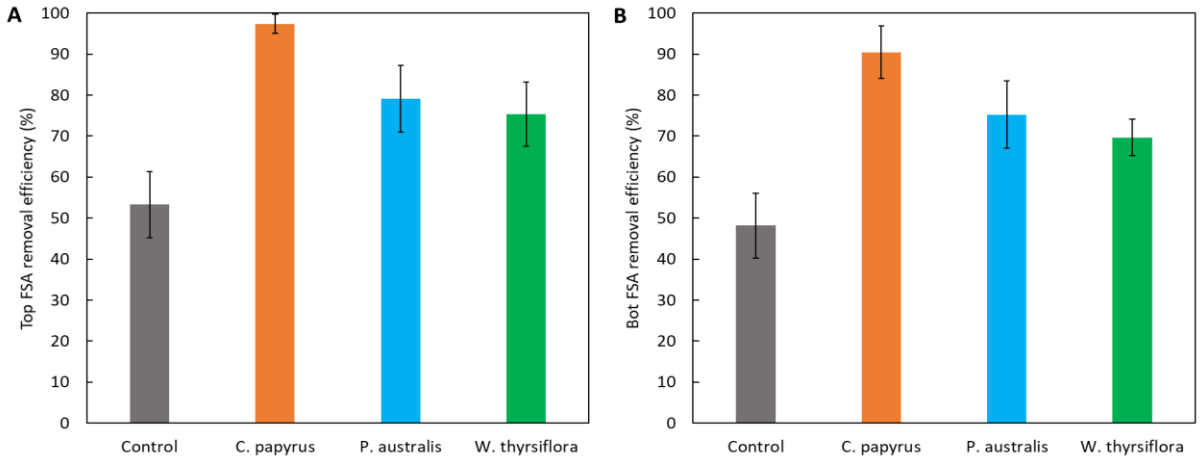



**Figure 47:** FSA removal from the top (A) and bottom (B) sampling tube for different plant species when using PS as substrate.

**Table 14:** WAS and PS FSA peak concentration comparison.

| Sample | WAS | PS |
|---|---|---|
| FSA concentration at the top (mgN/l) | 71 | 18 |
| FSA concentration at the bottom (mgN/l) | 80 | 53 |
| Day when peak FSA was measured | 15 | 5 |

The *C. papyrus* plant again achieved the highest removal efficiencies. This was as a result of its dense root structure releasing more dissolved oxygen and allowing for microbial growth. The *P. australis* and *W. thyrsiflora* produced similar removal rates. For *P. australis* this was because lower root mass was used in comparison to the other two plants. For *W. thyrsiflora* this was because the plant initially died and later grew new root mass.

The FSA removal in PS when compared to WAS, showed significant differences except for the removal in *C. papyrus* which was similar across both experiments. For the remaining three set-ups, the FSA removal was negative in WAS while positive in PS. Negative removal indicates higher FSA concentration at the end of the experiment compared to the start of the experiment. This was because of two reasons, (1) the FSA concentrations measured across the top and bottom tube were higher in WAS compared to the PS even though the initial TKN values were similar and (2) time required to release the peak FSA value required 5 days in PS compared to the 15 days in WAS. The results are summarised in Table 14.

## 6.7 OP removal

The OP ($H_2PO_4^-/HPO_4^{-2}/PO_4^{-3}$) was measured at regular intervals from two sampling points, top and bottom (bot) as shown in Figure 48. The complete OP profile is provided in Appendix C. The

removal efficiencies provided in Figure 48 are a comparison between initial and final OP values. The initial OP was 14.8 mgP/l. This value increased with time in both sampling tubes as the OrgP broke down to OP. The FSA started decreasing after reaching a peak value to achieve a final FSA lower than initial.

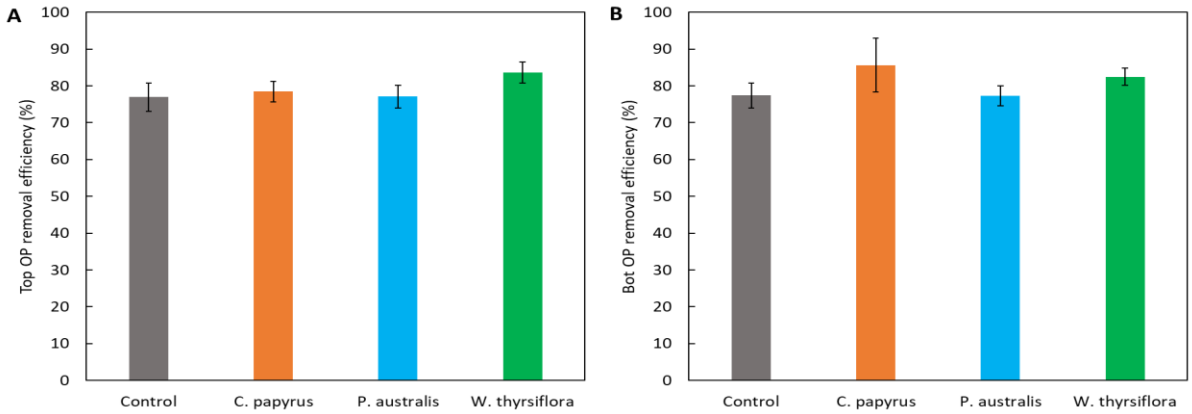



**Figure 48:** OP removal from the top (A) and bottom (B) sampling tube for different plant species when using PS as substrate.

High OP removal was observed across both sampling points. The removals can be accredited to the inoculum which consisted of phosphate accumulating organisms (PAOs). The PAOs as explained previously take up phosphate ions. Also, slightly higher removal was observed in the *W. thyrsiflora* top tube, this may be as a result of utilising phosphates for new biomass growth.

When comparing the OP removal of PS to WAS, it was noticed that higher removal efficiencies were observed in WAS even though WAS TP was greater than PS TP i.e. 281 mgP/l and 62 mgP/l respectively. This was because the WAS used was sourced from an EBPR treatment works which had pre-existing PAOs while PS did not contain PAOs from the onset.

## 6.8 Fertiliser capabilities of PMFC waste

### 6.8.1 Stability classification

For this research, the stability classification was evaluated based on achieving a VSS removal of 38%. From Figure 46, the 38% VSS removal criteria of sludge after going through the PMFC experiment was in all set-ups making them all Stability Class 1.

### 6.8.2 NP ratio fertilisers

Table 15 summarises the N:P results across all set-ups which passed the stability classification.

**Table 15:** N-P ratio of PS.

| System | Nitrogen (mg/l) | Nitrogen% | Phosphorus (mg/l) | Phosphorus% | N:P ratio |
|---|---|---|---|---|---|
| Control | 160 | 0.16 | 29 | 0.03 | 0.16:0.03 |
| *C. papyrus* | 101 | 0.10 | 21 | 0.02 | 0.1:0.02 |
| *P. australis* | 140 | 0.14 | 23 | 0.02 | 0.14:0.02 |
| *W. thyrsiflora* | 134 | 0.13 | 26 | 0.03 | 0.13:0.023 |

The highest N:P ratio was derived from the control, which was expected given the absence of a plant removing the nutrients. It was also noticed that the N:P ratios were significantly lower than organic fertilisers obtained from animals. Dairy cow manure, which had the lowest ratios compared to other animals had a ratio of 0.25:0.15: 0.25 (N:P:K) [111]. The values would in fact even be lower as the unbiodegradable content has not been unaccounted for. This suggests that more fertiliser would require to be applied to provide the right nutrients to grow. This can be a problem if the metal content is high (see Section 0).

## 6.9 Assessment of plant species health

The plants underwent the same processes as thoroughly explained in Section 5.9 (please refer to this section for more information). However, it was noticed that the plants were not grown as significantly as they were in WAS.

Comparing the root structure of the *C. papyrus* and *W. thyrsiflora* grown in PS to those grown in WAS (see Figure 41, Figure 43, Figure 49 and Figure 50) showed that more root mass and root hair was observed when using WAS. This may be as a resulted of two factors, (1) the WAS experiment ran for a longer duration i.e. 44 days compared to 28 days and (2) the faster release rate of ammonia could have been detrimental to plant growth [112]. Research into the ammonia handling capacity of the plant is required to better understand the reason for this observation.


**Figure 49:** Assessment of *C. papyrus* shoots (A) and roots at end of PS experiment.


**Figure 50:** Assessment of *W. thyrsiflora* roots on day 16 (A) and at end of the experiment (B).

## 6.10 Lessons learnt from this experiment

This experiment focused on using PS as a substrate with the same 3 plant species to obtain the PPD and organic removal. This experiment showed that:

1. a 1000 ohms resistor was also required in this experiment.

2. decreasing the COD even further to 10.5 gCOD/l still caused the *W. thyrsiflora* health to deteriorate at the beginning of the experiment, improving with time.

3. interestingly, the highest PPD was obtained from the Control system instead of the planted systems. This means that the plants did not aid in increasing the power when PS is used as a substrate. However, the best organic removal was achieved with *C. papyrus*.

# 7. Feed and plant species choice

## 7.1 Introduction

This chapter focuses on evaluating the different feeds used in this research and the three plant species chosen. The aim of this chapter is to choose the most suitable feed and plant. The subsections to follow evaluate the aforementioned, based on various criteria.

## 7.2 Feed evaluation

### 7.2.1 Power per gram of COD utilised

In order to understand what feed most efficiently converted COD into power, a feed evaluation considering COD consumed, the time duration of experiment and power produced was required. The ratio was calculated by calculating the area under the voltage and time graph using a Riemann's sum and dividing that by COD consumed (see Appendix E). The evaluation was done on control systems for thickened WAS, liquid WAS and PS. The reason for using the control was to eliminate any variabilities as a result of plants. The results are summarised in Figure 51.

Also, the starting COD concentration was different in all three set-ups. The thickened WAS was 208 gCOD/l, the liquid WAS was 16.6 gCOD/l and the PS was 10.5 gCOD/l. However, the biodegradable fraction was different for all the feeds. An augmented bio-methane potential test done on the WAS from Zandvliet by another researcher in the Water Quality Lab at UCT obtained a biodegradable fraction of 0.45. An aeration test done in this research when collecting the feed for the Optimisation 2 experiment resulted in a biodegradable fraction of 0.47 (see Appendix D). Multiplying 16.6 gCOD/l by 0.45 gives 7.47 gCOD/l. Similarly, the PS biodegradable fraction was found to be 0.7. Multiplying 10.5 gCOD/l by 0.7 gives 7.35 gCOD/l. This meant that the biodegradable COD of both liquid WAS, and PS was approximately the same.

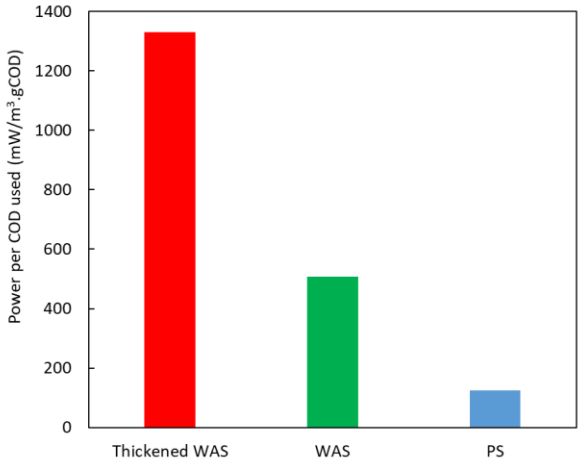



**Figure 51:** Power output per gram of COD utilised for thickened WAS, liquid WAS and PS. The Riemann sum used is provided in Appendix E.

The power output per gram of COD utilised was highest in Thickened WAS followed by liquid WAS. This was because the characteristics of the sludge are the same, the only difference is that thickened WAS had a lower water content. Also, the thickened WAS and liquid WAS were sourced from the same WWTWs, which meant that the activated sludge system design was the same (in this case the UCT system was used). The PS on the other hand produced less than a quarter of the power per gram of COD utilised. This may have been a result of readily available organics which were consumed before the exoelectrogenic bacteria concentration increased in the system. To better understand the power per COD utilised, a microbial study is necessary to understand how the inoculum responds to different sources of COD and power generation.

### 7.2.2 Impact of feed on plant health

### 7.2.3 Anaerobic digestion of PS versus use in PMFC

Conventionally, PS is anaerobically digested to generate electricity. If the PS utilisation was changed to using it in a PMFC as anaerobic digestors are not able to remove FSA and OP, a comparison between the power output is required per gram of COD used. The power/COD of the PMFC was based on the experiment in Chapter 6, but the ratio for AD was based on a theoretical estimation for which the calculation is presented in Appendix F. The results are summarised in Figure 52.

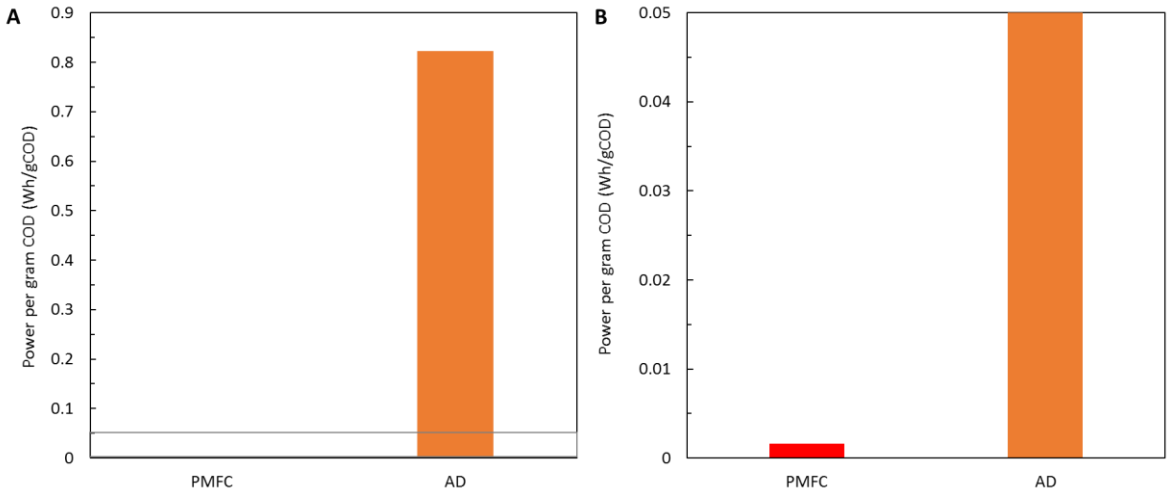



**Figure 52:** Power produced per gram of COD consumed in a PMFC compared to an AD (A and B). Given the very low output of PMFC it is not visible in A, the y-axis was capped to 0.5 Wh/gCOD (B) indicated by grey horizontal line in A.

From the results it was observed that the use of PS in PMFC generated a significantly lower power output when compared to anaerobic digestion. The AD produced over 500 times more power when compared to the PMFC. Figure 52 A shows both power outputs, however the PMFC power output

was so small in comparison, that it cannot be seen. The axis was capped to 0.01 Wh/gCOD to see the PMFC on the graph (Figure 52 B).

This was expected as the internal resistances of the PMFC were extremely high. A normal battery has an internal resistance ranging from 0.1 ohms to 0.9 ohms while the PMFC had and internal resistance of 443 ohms. The PMFC technology still needs significant advancement before it can be used to replace anaerobic digestors. However, the PMFC can be useful in treating the wastewater produced during anaerobic digestion. An AD, depending on the sludge age and other factors does not consume all the COD. Also, since the AD is anaerobic, it does not remove the ammonia. Therefore, the wastewater exiting an AD can potentially be added to the PMFC in a treatment works to treat the waste instead of recycling it back into the influent flow.

### 7.2.4 Anaerobic digestion of WAS?

WAS has a higher nitrogen and phosphorus elemental composition compared to PS [108, 110]. The WAS also has a low concentration COD concentration as it exits the activated sludge system. Given these reasons, Ekama [113] explored the quantitative impact of anaerobically digesting WAS and found that WAS produced a low methane yield, but made the sludge retention time longer and increased the complexity in removal of nitrogen and phosphorus. Therefore, the anaerobic digestion of WAS is undesirable, and it is usually treated in aerobic reactors. However, the PMFC can potentially replace the aerobic reactor saving electricity used in aeration and also produce added electricity on top of that. The PMFC is also capable of nitrogen and phosphorus removal as seen in Section 5.5.

### 7.2.5 Feed chosen

The PS application in a PMFC as discussed in Section 7.2.3 is not viable at this stage. Also, the PS in this experiment was diluted, meaning that if undiluted PS was used, the plant species chosen may not have been able to survive the concentrations and rapid release of ammonia. Other plant species should be investigated to see if they can withstand this.

The WAS on the other hand produced a higher power output per gram of COD consumed when compared to the PS. Also, the plants, specifically the *C. papyrus* performed well in the sludge producing high organic removal as well. Furthermore, the thickened WAS was not chosen as the feed when optimising the design (even though it produced higher power per COD utilised when compared to liquid WAS) because its use is not practical in WWTWs. The WAS would first have to be thickened to a solid and would then require manual application into the PMFC while liquid WAS (which will eventually dry out to form thickened WAS) can be added to the PMFC using gravity flow similar to how WWTWs operate. Bearing these factors in mind, liquid WAS was chosen as the feed for use for the optimisation experiments (see Chapter 8).

## 7.3 Plant species evaluation

### 7.3.1 Planted versus unplanted drying bed (control)

Planted drying beds showed better organic removal abilities and also achieved higher power generation in comparison to the control. Furthermore, if this technology were to be implemented in sludge drying beds, planted beds perform better than unplanted beds as plant roots create pathways for easier drainage and therefore allow for deeper sludge beds [55]. The added benefit of planted drying beds is that the filters do not require desludging after each feeding and drying cycle [55]. Also, unplanted sludge drying beds are only allowed a WAS depth of 0.3 meters and dry sludge depth of 0.2 meters [54]. On the other hand, planted beds having a depth of 1.0 – 1.5 meters have been used previously [114]. Therefore, planted beds, even though they require purchasing of plants are a better alternative than unplanted beds.

### 7.3.2 Peak power density

The peak power density when using both liquid and thickened WAS was achieved by *W. thyrsiflora* (289 $mW/m^3$) followed by approximately equal PPDs in *C. papyrus* (143 $mW/m^3$) and *P. australis* (145 $mW/m^3$) (see Section 5.4 and 6.4).

### 7.3.3 Organic removal

The *C. papyrus* achieved the best organic removal across all characteristics (VSS, COD, TKN, FSA, TP and OP). *W. thyrsiflora* was second in line in organic removal followed by *P. australis*. Also, the waste from *P. australis* was not suitable as a fertiliser as it did not meet the 35% VSS removal criteria (see Section 5.5, 5.6 and 5.7).

Even though the *C. papyrus* achieved highest removal efficiencies, it had lower N:P ratios when compared to *W. thyrsiflora*. Since both met the 38% VSS removal criteria, waste from *W. thyrsiflora* would be a better fertiliser. However, this is solely based on stability classification as microbiological and pollutant removal was not tested across the plant species. Therefore, if higher organic removal is required, *C. papyrus* is preferable and if lower removal but enough to comply with the stability criteria is required, *W. thyrsiflora* suitable.

### 7.3.4 Exudate release

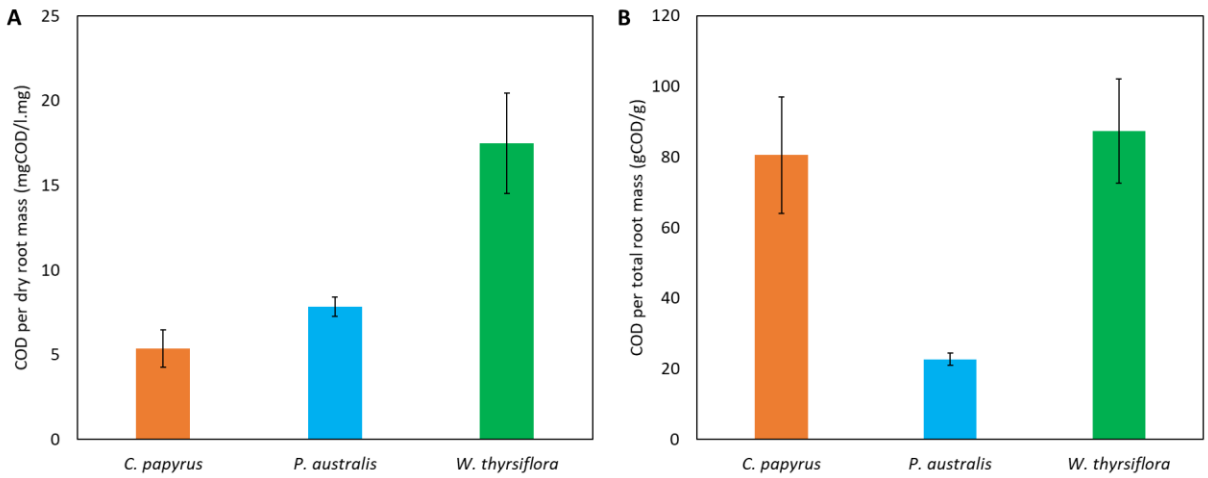



**Figure 53:** Exudate released per dry root mass (A) and exudate released per total root mass. The error bar represents the standard deviation from the mean of the six setups.

The exudate release was measured using the methodology described in Appendix G. From the results shown (see Figure 53), *W. thyrsiflora* had the highest exudate release, followed by *P. australis* and *C. papyrus*. However, when the total plant root mass was considered, the results shift favouring the *C. papyrus* given its dense root structure. The *W. thyrsiflora* exudate release came in second. The *P. australis* achieved very low COD per total root mass as low plant mass was used. From this, it can be seen that plant root mass plays a key role in exudate release and the results could vary depending on root mass.

### 7.3.5 Choice of plant species

Among the three plant species *W. thyrsiflora* died when used in thickened WAS, old roots died when using liquid WAS and PS until new roots grew out. The *C. papyrus* performed well across all feeds. The *P. australis* required a longer time to get used to a hydroponic system but adapted and grew new shoots.

The *W. thyrsiflora* may have produced higher PPDs and acceptable organic removals, however, the plant is not useful if it was implemented in a drying bed as the sludge would thicken and the plant would eventually die. Also, if the PMFC was implemented in the WWTWs as a continuous system, the constant high concentration of feed would not allow new root mass to grow and the plant would eventually die again. Therefore, *C. papyrus* was seen as the most viable plant species and was chosen for further experimentation.

# 8. Optimisation

## 8.1 Introduction

This chapter focuses on optimising the PMFC design. From the previous chapter, liquid WAS was chosen as the most suitable feed and *C. papyrus* as the plant species. Three optimisation experiments were run, all testing different aspects of the design. The first experiment focused on the use of a separator between the electrodes, the second on cathode placement and use of multiple electrodes and the third on the distance between electrodes.

## 8.2 Optimisation experiment 1

The first optimisation test was aimed at understanding voltage generation and organic removal when using a separator between the electrodes. The purpose of the separator was to contain the plant roots in the cathodic region. This was done to potentially encourage surface root growth and supply oxygen to the cathodic region. This experiment was run along with the experiment where WAS as a PMFC feed was tested (see Chapter 5). Therefore, the experiment design and substrate classification were the same as those described in Chapter 5.

The experiment had two triplicate set-ups, both planted with *C. papyrus*. The first had a permeable (to ensure $H^+$ ions can travel from the anode to the cathode) plastic sheet used as a separator (set-up hereon referred to as *C. papyrus* ES*)* and the second did not contain a separator (hereon referred to as *C. papyrus*). The plastic sheet is normally used as a safety net and has a pore size of 1 mm. The sheet covered the diameter of the bucket and was placed 90 mm from the bottom. This allowed another 90 mm for the roots to grow. The sheet was placed on LECA and did not require further support.

The separator versus non-separator followed the same design as described in Section 3.5.2 and illustrated in Figure 54. The subsections to follow provide voltage, peak power density (PPD) and organic removal results obtained from both set-ups.

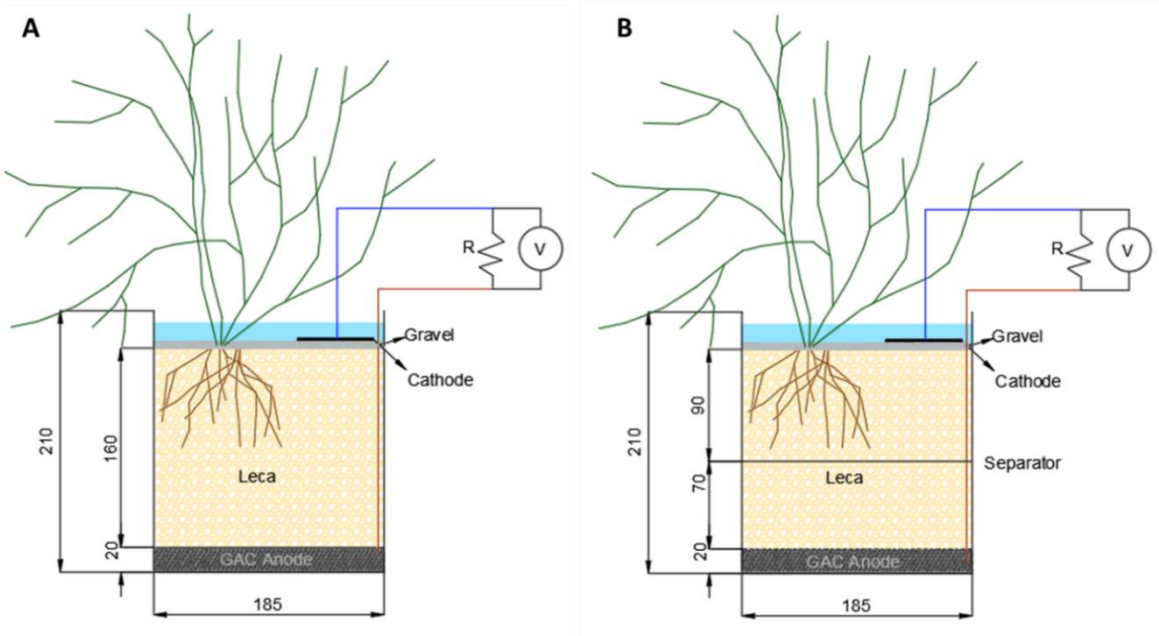



**Figure 54:** Shows the set-up used for the Optimisation experiment 1, Design A did not have a separator while Design B contained a porous PVC separator.

### 8.2.1 Voltage results and discussion

The voltage results from day 35 to 44 are provided in Figure 55. Day 35 to 44 were chosen for reasons described in Section 5.3. The complete voltage results are provided in Appendix B.

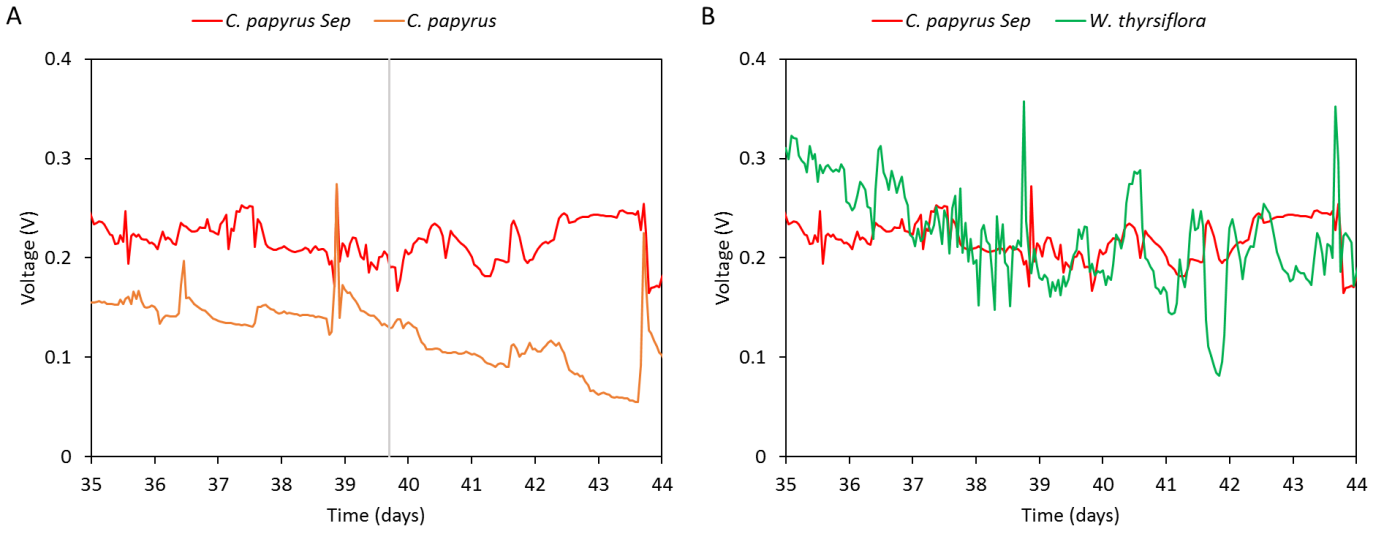



**Figure 55:** Voltage results obtained when using a separator in comparison to no separator (A) and to highest power producing cell in the experiment from Chapter 5 (B). The vertical grey line indicates when polarisation test took place.

The voltage recorded when using a separator was found to be 38% greater than without a separator. The *C. papyrus* ES recorded 0.22 V while *C. papyrus* started with 0.16 V and decreased with time. The *C. papyrus* ES was found to have the same voltage reading in comparison to *W. thyrsiflora*, which had the highest voltage recording in the experiment described in Chapter 5.

The reason for this could be associated with a greater surface area of roots which were visible in the *C. papyrus* ES system as compared to the *C. papyrus* system. High voltage readings recorded for the *W. thyrsiflora* system were also accredited to a greater surface area root growth. The roots release oxygen which likely aided in obtaining higher voltage readings.

Also, when taking down the experiments, it was observed that the roots of the *C. papyrus* ES system did not reach the anodic region the way the roots had in the *C. papyrus* system (see Figure 41 and Figure 56). Figure 56 does not imply that the roots had a more prolific growth when using a separator, rather it implies that more roots grew on the surface as the separator stopped the majority of the roots from growing through it. Oxygen release at the anode, which is required to be anaerobic, reduces the oxygen gradient required to generate electricity [13]. The roots however grew along the wall of the bucket and past the separator, it is therefore recommended to use something like calcium bentonite which was previously used by Villaseñor, et al. [19] or glass wool previously used by Yadav, et al. [115].

**Figure 56:** *C. papyrus* ES roots growing on the surface. Picture taken on day 44.

The polarisation test to obtain PPD was done on day 39 (see grey vertical line on Figure 37). PPD was 35% higher when using a separator. The *C. papyrus* ES produced 191 ± 16 mW/m$^3$ of power while *C. papyrus* produced 141 ± 16 mW/m$^3$. This again can be attributed to release of oxygen in the cathodic region.

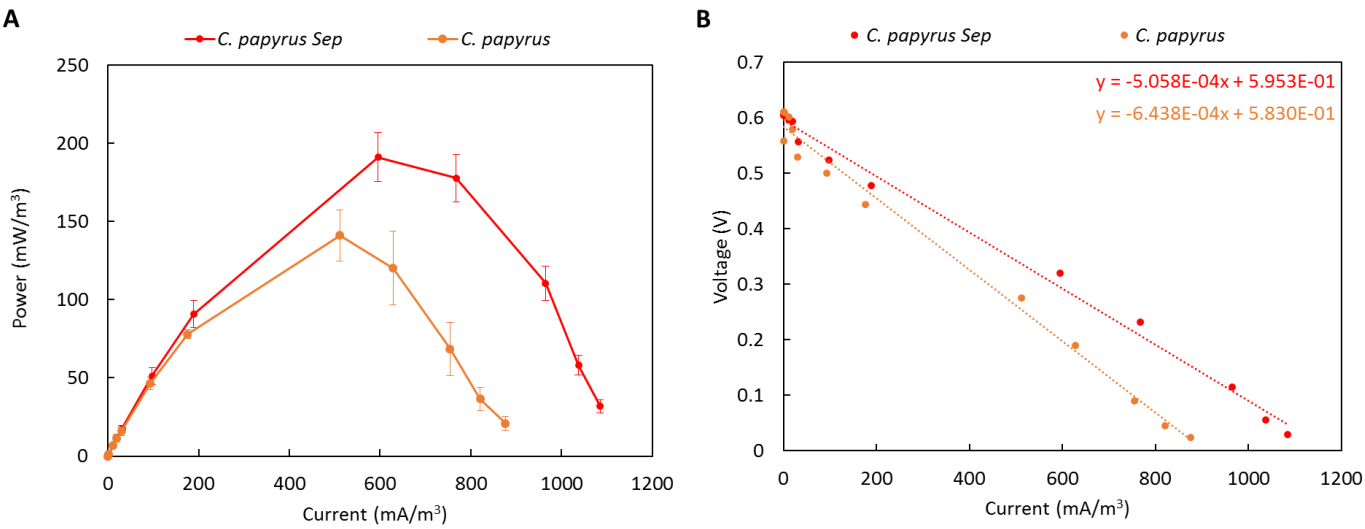



**Figure 57:** Peak power density graph for the separator versus no separator (A) and voltage-current graph (B). The error bar represents the standard deviation from the mean of the triplicate setups.

The internal resistance measured when using a separator was found to be lower than without a separator, i.e. 941 ± 51 and 1197 ± 109 ohms respectively. The lower internal resistance could be attributed to roots being kept away from the anode. Roots growing at the anode release oxygen and can increase the internal resistance [96]. Interestingly, the internal resistance of both systems was still significantly higher in comparison to the internal resistances of the control and other plant species (see Section 5.4). The unplanted control had an internal resistance of 454 ± 143 ohms which is half of what was observed when using the *C. papyrus* with or without the separator. This means that the oxygen release did have a likely contribution to the increasing internal resistance, but its impact was not as significant as previously thought.

However, when the same experiment was run using PS as a substrate (see Chapter 6) or settled sewage as a substrate (Oodally, et al. [116]) similar internal resistances between *C. papyrus* and unplanted controls were observed. Further research is required to fully understand the reasons why the internal resistance is high for *C. papyrus* when using WAS as a feed.

### 8.2.2 Organic, FSA and OP removal

The *C. papyrus* ES performed better than *C. papyrus* in power generation as previously discussed. However, when the organic removal capabilities of both systems were compared, it was seen that the *C. papyrus* achieved better VSS, COD, TKN and TP removal efficiencies (see Figure 58). This again can be related to the *C. papyrus* ES roots being contained in the cathodic region.

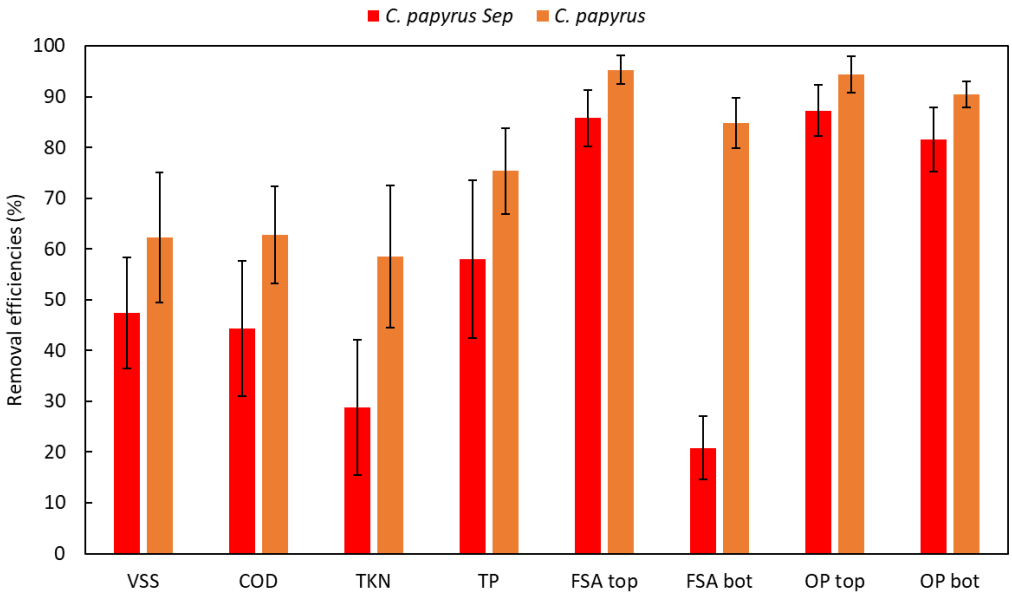



**Figure 58:** Organic removal observed when using separator versus no separator.

The samples collected and tested for organic removal efficiencies were an average of the top and bottom of the system. Therefore, in the top sampling tube, the *C. papyrus* ES may have had the same removal efficiencies as *C. papyrus* but in the bottom section it behaved as a control. This meant that the plant roots did not contribute to the microbial activity of the system and therefore lower VSS and COD removals. Also, less oxygen in the bottom layer meant lower TKN removals and no OP uptake to reduce TP.

The top tube FSA removal was slightly better in *C. papyrus* when compared to *C. papyrus* ES. This may have been as a result of the *C. papyrus* ES system dedicating more oxygen to power generation than for nitrification. It may also be because of the separator inhibiting root growth. The bottom tube FSA removal was significantly lower in the *C. papyrus* ES system in comparison to the *C. papyrus* system. This was again as a result because of the absence of roots in the bottom section to provide oxygen and convert any released FSA to nitrate.

The OP removal in both the top and bottom tubes were over 80% in both set-ups. The *C. papyrus* system performed slightly better than *C. papyrus* ES across both sampling points. The high OP removal was because of the presence of PAOs in the sludge and in the inoculum. The slightly better removal efficiencies in *C. papyrus* system can be attributed to better root growth and higher uptake, as OP is a vital plant growing nutrient.

### 8.2.3 Nitrogen and phosphorus ratio

Both systems had a VSS removal of over 38% and therefore meet the stability classification. Their N:P values are provided in Table 16. The *C. papyrus* ES system had lower organic removal

efficiencies, this meant that the N:P ratio was higher and therefore less sludge would be required to meet the N:P demand of crops grown. However, this is based on the assumption that both the microbial and pollutant removal requirements are met.

**Table 16:** N:P ratios of *C. papyrus* and *C. papyrus* ES.

| System | Nitrogen (mg/l) | Nitrogen% | Phosphorus (mg/l) | Phosphorus% | N:P ratio |
|---|---|---|---|---|---|
| *C. papyrus* ES | 445 | 0.45 | 118 | 0.12 | 0.45:0.12 |
| *C. papyrus* | 260 | 0.26 | 69 | 0.069 | 0.26:0.07 |

### 8.2.4 Conclusions

This experiment showed that using a separator between electrodes generated 35% higher PPD and 38% higher voltage across 1000 ohms resistor in comparison to not having a separator. However, the experiments achieved lower organic removal efficiencies which in turn is an advantage for fertiliser application.

## 8.3 Optimisation experiment 2

The second optimisation experiment focused on the use of single and/or multiple cathodes. It also focused on using multiple anode electron collectors. The key aspect of these experiments was to understand how the power production varied when the number of cathodes and electron collectors in the anode was increased.

### 8.3.1 Experimental design

A triplicate of each system was set-up following the design criteria as described in Chapter 3. All systems were planted with *C. papyrus* and used WAS as substrate. However, the number of cathodes and their configuration was varied. Also, the number of electron collectors was varied in the anode, but, the volume of GAC used was not changed. Further information is provided in the following subsections.

#### 8.3.1.1 Experimental set-ups

Four different systems each having triplicates were set-up (see Figure 59). These were:

i) Control system which had a single electron collector in the anode and single cathode placed on the surface. This system was set-up as shown in Figure 17 B.

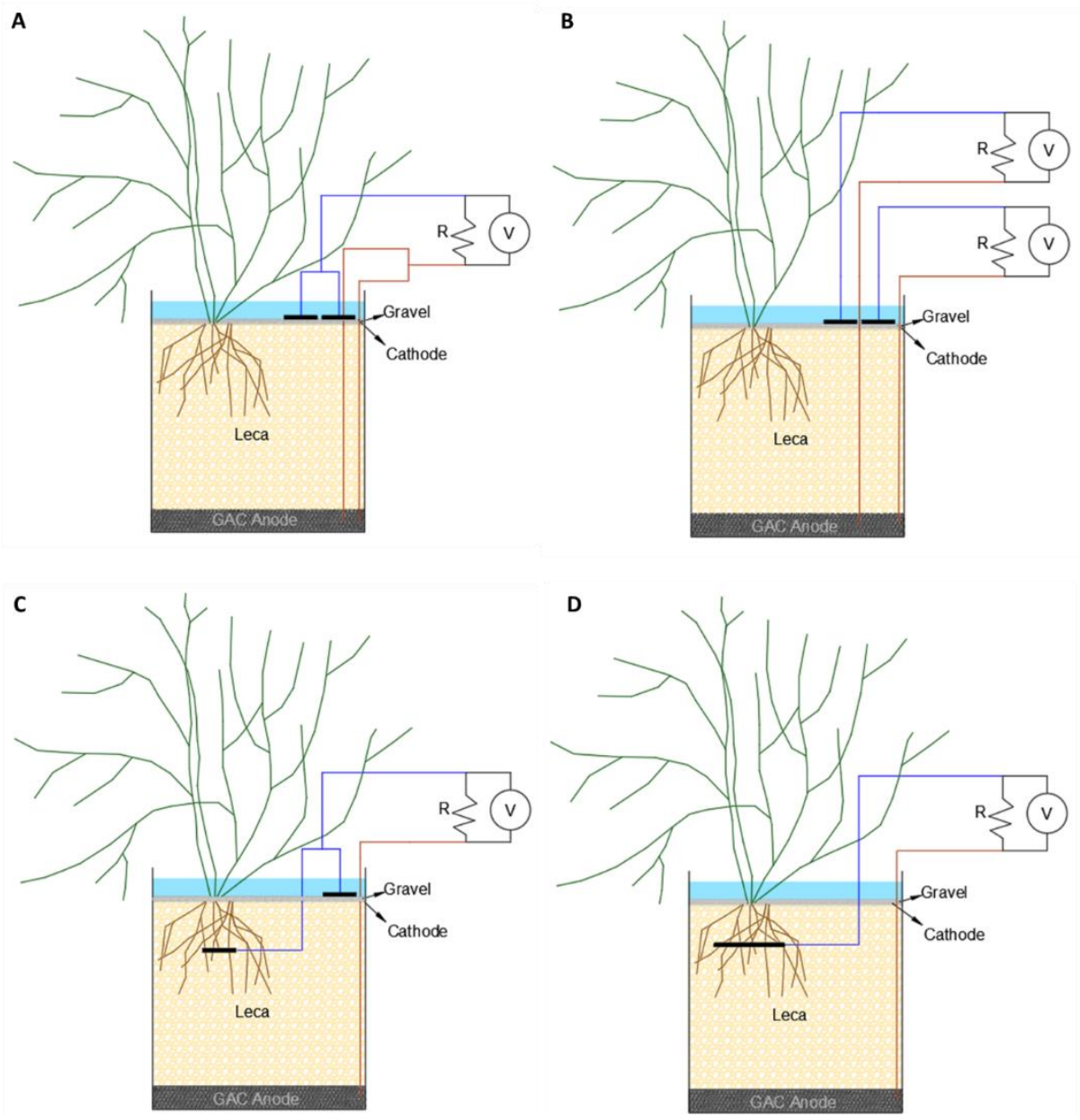



**Figure 59:** Optimisation experiment set-ups where A is parallel connection, B is a single series connection, C is a Surface + Root cathode connection and D is a root only connection.

ii) Multiple electrodes system had two electron collectors in the anode and two cathodes placed on the surface. The triplicate of the multiple electrodes was further split into parallel and series connection. A single set-up of the three had a parallel connection and voltage was recorded across the cell (this system is henceforth termed as Parallel). For the remaining two set-ups, the voltage was recorded per set of electrodes (so basically four voltage readings). This meant that it was similar to the control, but with half the cathode and anode electron collector area

(this system is henceforth termed as Single series or Series connection). Summing the voltages measured across two single series voltage recordings provided a total voltage recording of the set-up connected in series (henceforth referred to as dual series or Seriesx2 connection). Voltages were summed bearing in mind the theory of voltage outputs from cells connected in series (see Section 8.3.1.2).

iii) The surface and root system also had two cathodes. The first cathode was placed on the surface and the second cathode was placed 15 cm from the base of the bucket in the root layer. These two cathodes were connected to a single anode electron collector. This system provided the sum of voltages from a control type system (surface cathode) and root only system (root cathode).

iv) The root only cathode had a single cathode and single anode electron collector. The cathode was placed 15 cm from the base of the bucket in the root layer.

Importantly for systems with multiple cathodes and/or multiple electron collectors in the anode, the overall area was kept constant. For example, the control, which made use of a single cathode, had a cathode area of 67 $cm^2$ (see Section 3.4.3 for reason why this area was chosen). However, each cathode in multiple electrodes set-ups was 33.5 $cm^2$.

#### 8.3.1.2 Electrode connection and expected voltage, resistance and current

The overall voltage, current and resistance of connected cells, depends on the connection type i.e. parallel or series chosen. Understanding this variation becomes important when analysing the voltage results obtained in the parallel multiple electrodes and series multiple electrode. Before explaining the two it is important to note the following equations:

$$V = IR \qquad \text{(Equation 6)}$$

$$P = IV = I^2R = \frac{V^2}{R} \qquad \text{(Equation 7)}$$

P: Power (W)
V: Voltage (V)
I: Current (A)
R: Resistance (Ω)

**Parallel connection**

In this connection type, the positive terminals of the cell (the cathode in this case) are connected to one another and the negative terminals (the anode in this case) are connected to one another. This means that the current values are summed but the resistance is summed reciprocally. The schematic and equations to follow further explain this. Note the equations are limited to two cell

connections as a maximum of two electrodes were connected in this experiment (equations are based on [117]).

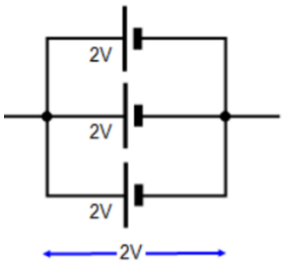



**Figure 60:** Schematic of cells connected in parallel [117].

$I_T = I_1 + I_2$ and since the cells were the same $I_T = I_{avg} + I_{avg} = I_{avg} \times 2$ (Equation 8)

$R_T = \frac{R_1 \times R_2}{R_1 + R_2}$ and since the cells were the same $R_T = \frac{R_{avg} \times R_{avg}}{R_{avg} + R_{avg}} = \frac{R_{avg}}{2}$ (Equation 9)

$$P_T = I_T^2 \times R_T = \left(2 \times I_{avg}\right)^2 \times \frac{R_{avg}}{2} = 2 I_{avg} R_{avg} = 2 \left(\frac{V_{avg}}{R_{avg}}\right)^2 \times R_{avg} = \frac{2 V_{avg}^2}{R_{avg}}$$ (Equation 10)

**Series connection**

In this connection type, the positive terminal of the cell (the cathode in this case) is connected to the negative terminal of the cell (the anode in this case). In this cell connection, the voltage values are summed producing a higher cell electromotive force (emf) but at the same time, the internal cell resistances are summed as well. The schematic and equations to follow further explain this (equations are based on [117]). Note, the equations are limited to two cell connections as a maximum of two electrodes were connected in this experiment. Also, the resistances refer to internal resistance of the cell.

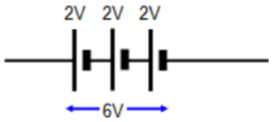



**Figure 61:** Cells connected in series [117].

$V_T = V_1 + V_2$ and since the cells were the same $V_T = V_{avg} + V_{avg} = V_{avg} \times 2$ (Equation 11)

$R_T = R_1 + R_2$ and since the cells were the same $R_T = R_{avg} + R_{avg} = R_{avg} \times 2$ (Equation 12)

$$P_T = \frac{{V_T}^2}{R_T} = \frac{(2 \times V_{avg})^2}{2 \times R_{avg}} = \frac{2 {V_{avg}}^2}{R_{avg}}$$ (Equation 13)

This means that the expected power, voltage and resistance is twice in Seriesx2 compared to Seriesx1. In the equations, it was stated that the voltage $V_1$ and $V_2$ are equal and therefore relabelled as $V_{average}$. This was done because both cathodes were in the same set-up and the voltage readings would be the same for both.

Also, from Figure 59 B, it can be seen that an external resistance of 1000 ohms was connected across each electrode pair. Therefore, a total of 2000 ohms was connected in the series set-ups. Comparing this to all other set-ups, they used 1000 ohms resistances. The reason for connecting 1000 ohms per electrode pair was because in series connection, the internal resistances of cells are summed. Therefore, you need the external resistances to match this summation aspect of the series connection. If only a total of 1000 ohms (making it 500 ohms per electrode pair), the voltage recorded would be lower than expected. As shown in Section 4.3.4, the higher the resistance connected the larger the voltage reading, as it needs a bigger electromotive force to pass through the resistor. So, if only 500 ohms was connected per electrode, the voltage reading across it would be lower than shown in Figure 62 B and therefore give wrong interpretation of the voltage output of single series and double series.

Studying the equations above shows that theoretically, the total power for a series connection (see Equation 13) is the same as power from a parallel connection (see Equation 10). This means that regardless of the way the cells are connected, the power output is expected to be the same. However, the combined internal resistance of two electrodes connected in parallel would be half of the internal resistance of a single electrode and two electrodes connected in series would an internal resistance twice as high as the single electrode.

#### 8.3.1.3 Substrate classification

Waste activated sludge was chosen for the optimisation experiment. The WAS was sourced from Zandvliet WWTWs. The sludge was classified based on its TSS, VSS, ISS, COD, VFA, TKN, SA, TP and OP. The results are summarised in Table 17.

**Table 17:** WAS characteristics

| Characteristic | TSS | VSS | ISS | COD | VFA | TKN | FSA | TP | OP |
|---|---|---|---|---|---|---|---|---|---|
| mg/l | 9549 | 7645 | 1895 | 11518 | 0 | 910 | 15 | 270 | 43 |

The COD of the WAS was lower than when it was collected for the second experiment (see Section 5.2.2). This was because the membrane filters of the plant were under maintenance. Also, the OP was high meaning that the EBPR process was not operating at standard conditions when the sludge was collected.

#### 8.3.1.4 Experimental timeline

The experiment was started on the 21st of November 2018 (day 0) and concluded on the 26th of December 2018 (day 35). However, all the systems were disconnected on day 30 except for the Surface + Root cathode system in which connection was changed and the voltage across each cathode was measured individually.

### 8.3.2 Voltage results and discussion

The voltage readings across a 1000 ohms resistor recorded from day 5 to day 30 for all the systems is provided in Figure 62. The systems were left as open circuit from day 0 to 5 to allow them to stabilise. The voltage results from all the systems show a gradual decrease in voltage with time. This again may be as a result of increasing internal resistance (Section 8.3.3.2 provides further discussion).

#### 8.3.2.1 Multiple electrodes

The voltage was recorded for both connection types, the dual electrodes connected in parallel in one set-up providing single voltage readings (as single set-up) and a voltage recording of the dual cathodes connected separately per set-up providing four voltage readings (see Section 8.3.1). The voltage readings of the separately connected cathode readings were doubled to provide voltage in seriesx2 (see Equation 11).

**Parallel connection**

The voltage recorded across the parallel connected system was highest relative to all systems. The voltage started at 0.5 V decreasing to 0.35 V on day 30 (see Figure 62 A). The control on the other hand recorded 0.35 V on day 5 which decreased to 0.22 V by day 30. The results obtained were expected since multiple electrode connections are more efficient at power production than single electrodes [13, 16, 18].

**Series connection**

The voltage results from a single electrode are provided in Figure 62 A. The voltage readings also showed a decreasing voltage trend from day 5 to day 30. The voltage dropped from 0.31 V to 0.15 V. The voltage recorded was lower when compared to both the control and the dual electrode connected in parallel. This was expected as the single cathode area was half when compared to the control (since the electrode was split into two, but the area was maintained as explained in Section 8.3.1). The same observation was made by Hong, et al. [93] who noticed decreasing voltage recording when the cathode area decreased.

Interestingly, the voltage reading dropped at a faster rate in the single series connection compared to the control. The difference in voltage between the control and single series was 0.04 V initially. This later increased to 0.07 V towards day 30. This may be as a result of the internal resistance increasing at a faster rate in the single series connection than the control i.e. 53.2% and 37.4%

respectively (see Section 8.3.3.1). This effect is more easily observed when studying the data from dual electrodes connected in series.

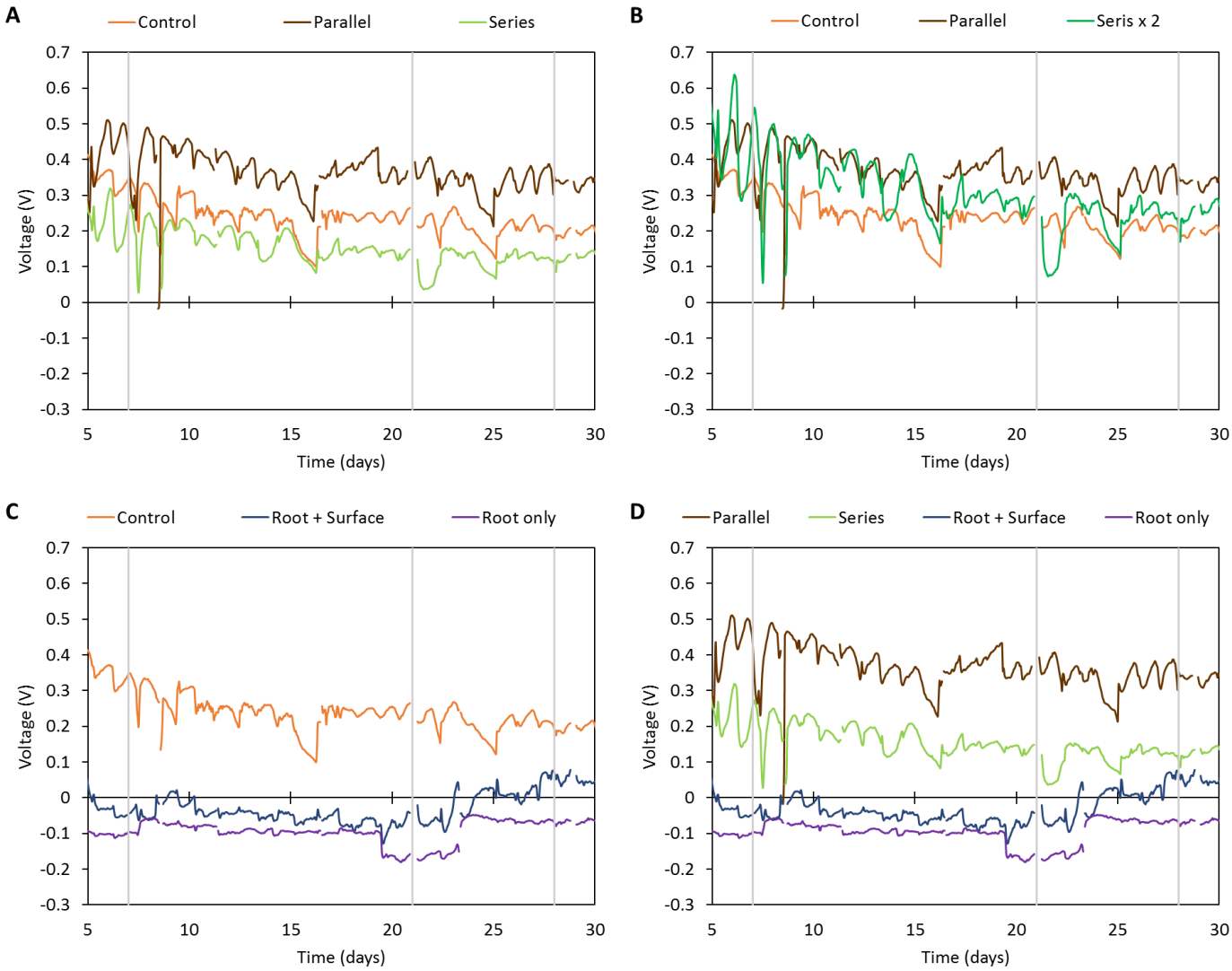



**Figure 62:** Voltage results recorded across a 1000 ohms resistor for parallel versus single series connection (A), parallel versus double series connection (B), root + surface versus root only cathode (C) and all displayed together (D).

The dual series electrode initially started off with 0.62 V which decreased to 0.3 V by day 30. This decreasing voltage and also the decreasing voltage difference between the dual series and control are shown in Figure 62 B. Theoretically, a series connection is supposed to produce higher voltage readings compared a parallel connection ($V_{series} = V_{avg} + V_{avg}$ and $V_{parallel} = V_{avg}$). However, the results obtained show an initial higher voltage for the series x 2 connection compared to the parallel connection, but not double as theory suggests. This implies that not only did the parallel connection produce a higher voltage value, its low (half, given the parallel connection theory in

Section 8.3.1) internal resistance meant that the peak power density would be significantly higher, instead of being equal based on the theory. This was later observed when conducting the polarisation tests (see Section 8.3.3).

#### 8.3.2.2 Root only cathode

The cathode in this system was placed close to the roots. A voltage reading of -0.1 V was recorded on day 5 and it slowly decreased (in terms of magnitude) to -0.08 V (see Figure 62 C). This means that a negative voltage was produced during the course of the experiment. Since the cathode was placed near the roots and not on the surface, the presence of dissolved oxygen was lower and therefore the oxygen gradient was lower.

A lower oxygen gradient meant that a longer time was required to establish what the anode and the cathode in the system was. Negative current (which is as a result of negative voltage) was observed by Wetser, et al. [118] whose systems produced positive voltage past day 26. Chen, et al. [27] observed negative open circuit voltage readings for the first 20 days. Therefore, it is speculated that the voltage would have been positive if the experiment were run for a longer period of time.

#### 8.3.2.3 Cathode placed at root and surface

In this system, a cathode was placed at the plant roots of the system and at the cathode as explained in Section 8.3.1. A negative voltage reading of about -0.05 V was recorded for the first 24 days. The voltage transitioned from a negative reading to a positive reading over the span of 3 days i.e. day 24 to day 26. Thereafter, the voltage gave a positive reading until day 30 (see Figure 62 C).

The low voltage readings and the shift from negative voltage to positive voltage was as a result of the negative voltage recorded by the root cathode cancelling out the positive voltage recorded by the surface cathode.

To further understand this, the cathodes for all three set-ups were split from parallel connection and instead voltage readings across each electrode were taken individually. The surface cathode produced voltage readings of 0.1 – 0.2 V (Figure 63 A) while the root cathode recorded 0.1 – 0.18 V (Figure 63 B). The voltage results recorded were approximately equal to one another cancelling each other out when connected together. This explains why the voltage readings when connected in parallel had such a low reading.

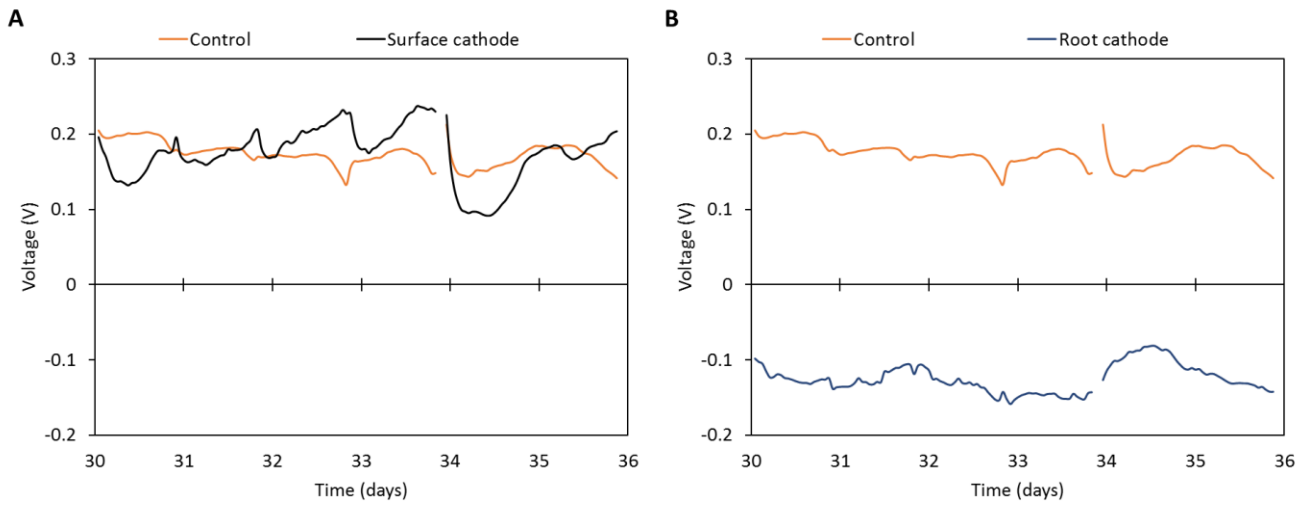



**Figure 63:** Voltage recording across surface cathode (A) and root cathode (B).

### 8.3.3 Polarisation test results and discussions

Three polarisation tests were conducted during the course of the experiment. The first on day 7, the second on day 21 and the third on day 28. The results of the polarisation test are shown in Figure 64. The purpose of doing multiple polarisation tests was to understand how the internal resistance changes with time. The absolute values of the peak power densities and internal resistances are summarised in Table 18 (see t-test in Appendix I).

**Table 18:** Peak power density and internal resistance of the systems.

| System | Test 1 | | Test 2 | | Test 3 | | $R_{int}$% increase |
|---|---|---|---|---|---|---|---|
| | Peak power density ($mW/m^3$) | Internal resistance (Ω) | Peak power density ($mW/m^3$) | Internal resistance (Ω) | Peak power density ($mW/m^3$) | Internal resistance (Ω) | |
| Control | 156 ± 17 | 773 ± 10 | 127 ± 9 | 1057 ± 82 | 97 ± 4 | 1007 ± 5 | 37 |
| Parallel | 443 ± 0 | 523 ± 0 | 306 ± 0 | 631 ± 0 | 272 ± 0 | 700 ± 0 | 34 |
| Single Series | 148 ± 23 | 950 ± 5 | 66 ± 4 | 1346 ± 35 | 58 ± 21 | 1456 ± 144 | 53 |
| Dual Series | 296 ± 46 | 1900 ± 10 | 132 ± 8 | 2692 ± 70 | 116 ± 42 | 2912 ± 288 | 53 |
| Surface + Root | 8 ± 4 | 320 | 13 ± 16 | 170 ± 61 | 32 ± 35 | 552 | 72 |
| Root only | 32 ± 8 | 150 ± 11 | 57 ± 17 | 227 ± 13 | 39 ± 21 | 222 ± 25 | 48 |

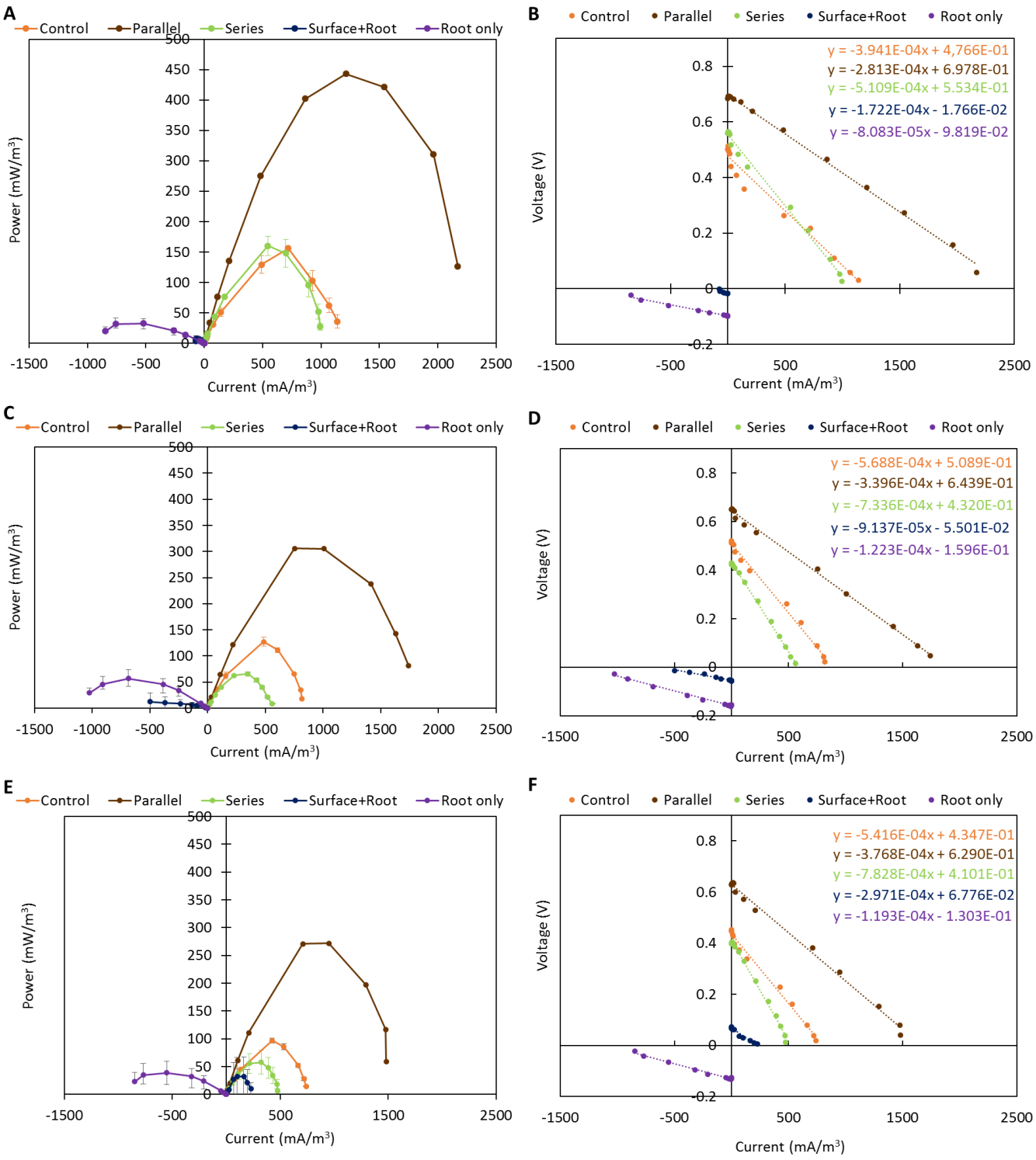



**Figure 64:** Polarisation test results on day 7 (A and B), day 21 (C and D) and day 28 (E and F) when using WAS as substrate. A, C and E show PPDs for the different electrode configurations while B, D and F show voltage versus current results. The error bar represents the standard deviation from the mean of the triplicate set-ps.

**Table 19:** Comparison of measured internal resistance in parallel to calculated internal resistance based on series resistance.

| Test | Series Single | Series Dual | Parallel | Parallel resistance calculated using series resistance |
|---|---|---|---|---|
| 1 | 950 | 1900 | 523 | 475 |
| 2 | 1364 | 2728 | 632 | 682 |
| 3 | 1456 | 2912 | 700 | 728 |

#### 8.3.3.1 Peak power density

Across the three tests, parallel connections had the highest PPD. This was followed by the dual series connection in the first test, however, with the rapidly increasing internal resistance, the dual series produced the same PPD as the control (132 ± 8 versus 127 ± 9 in Test 2 and 116 ± 42 versus 97 ± 4 in Test 3). Liu [18] also observed an increase in PPD with increasing electron collectors. With one titanium collector, Liu [18] obtained 0.64 mW/m$^2$, 1.71 mW/m$^2$ with two and 2.92 mW/m$^2$ with three. The results from the Liu [18] study show an exponential power increase with increasing electron collectors connected in parallel. If a series connection was done for example, the results would potentially be a multiple of 0.64 mW/m$^2$ i.e. producing 1.28 mW/m$^2$ with two titanium rods and 1.92 mW/m$^2$ with three rods. This hypothetical multiplication is done based on the power and voltage relationship (see Section 8.3.1.2).

The hypothetical scaling of the results from Liu [18] is also possible since the internal resistance of the parallel connection calculated based on the series internal resistances, as (shown in Table 19), closely correlated to the measured internal resistance. The single series internal resistance was used to calculate potential internal resistance in a parallel connection. Therefore, theory of parallel and series connections was closely followed and multiplying Liu [18] single rod results by factors of 2 and 3 as done in the previous paragraph to predict series connection power was seen acceptable. Given the results from this experiment and the results from Liu [18], parallel connection seems to be a better connection type compared to the series connection within a set-up. However, if two separate set-ups are connected in parallel or series, it is speculated that the power output would be the same based on theory.

The PPD in the ‘root only cathode’ system produced negative current across all three tests indicating the system needed more time to establish positive current readings in the cathode placed at the roots. The PPD recorded was greater than the PPD recorded in Surface + Root electrode.

The PPD for the Surface + Root system was again low because of the low voltage readings, i.e. the positive surface cathode cancelling out the negative root cathode readings. Interestingly, the PPD was accompanied with a negative current in Test 1 and Test 2. However, in Test 3, the PPD started producing positive voltage readings. If the system was run for longer, higher positive PPDs may have been observed.

#### 8.3.3.2 Internal resistance

The internal resistance increased across all systems. This was expected because using a batch system causes the anode to clog therefore increasing the internal resistance [18]. The Root only cathode system had the lowest internal resistance. This was as a results of reduced electrode distance between the anode and the cathode. Reduced electrode distance has shown to decrease internal resistance [21, 119].

The lowest internal resistance was followed by Surface + Root, which again was expected as one of the electrodes was at the root layer, therefore having a lower electrode distance and reducing the overall internal resistance. Parallel connection also exhibited low internal resistances. This is as a result of the internal resistances are summed reciprocally (see Equation 9), therefore reducing the overall internal resistance.

The dual series electron collectors produced the highest internal resistance. This was as a result of summing the internal resistances from each electrode. For example, in Test 1, single series internal resistance was 950 ohms, therefore, the dual resistance becomes 950 + 950 = 1900 ohms. The addition used is because of the theoretical operation of a series connection (see Section 8.3.1.2 and Equation 12).

### 8.3.4 Organic removal

The organic removal was characterised in terms of VSS, COD, TKN and TP removal. The waste classification in terms of the aforementioned characterisation was measured at the start and end of experiment. This was mainly because the solids in the WAS settle and any samples taken during the course of the experiment would produce incorrect results.

The tests were done using the standard lab procedures [103]. The results are summarised in Figure 65. The graphs present results both in absolute values and in removal percentages. All the systems were planted with *C. papyrus*, which, from previous experiments, had great removal efficiencies. Therefore, the difference in organic removal efficiencies observed were as a result of electrode lay out.

The COD and VSS removal efficiencies in the control system (42.6 ± 11.8% for VSS and 42.4 ± 11.9% for COD) and the multiple surface electrode system (50.3 ± 5.1% for VSS and 50.4 ± 5.4% for COD) were higher in comparison to the surface + root cathode (26.3 ± 8.4% for VSS and 33.9 ± 3.0% for COD) and root only cathode (27.2 ± 10.0% for VSS and 24.6 ± 15.8% for COD).

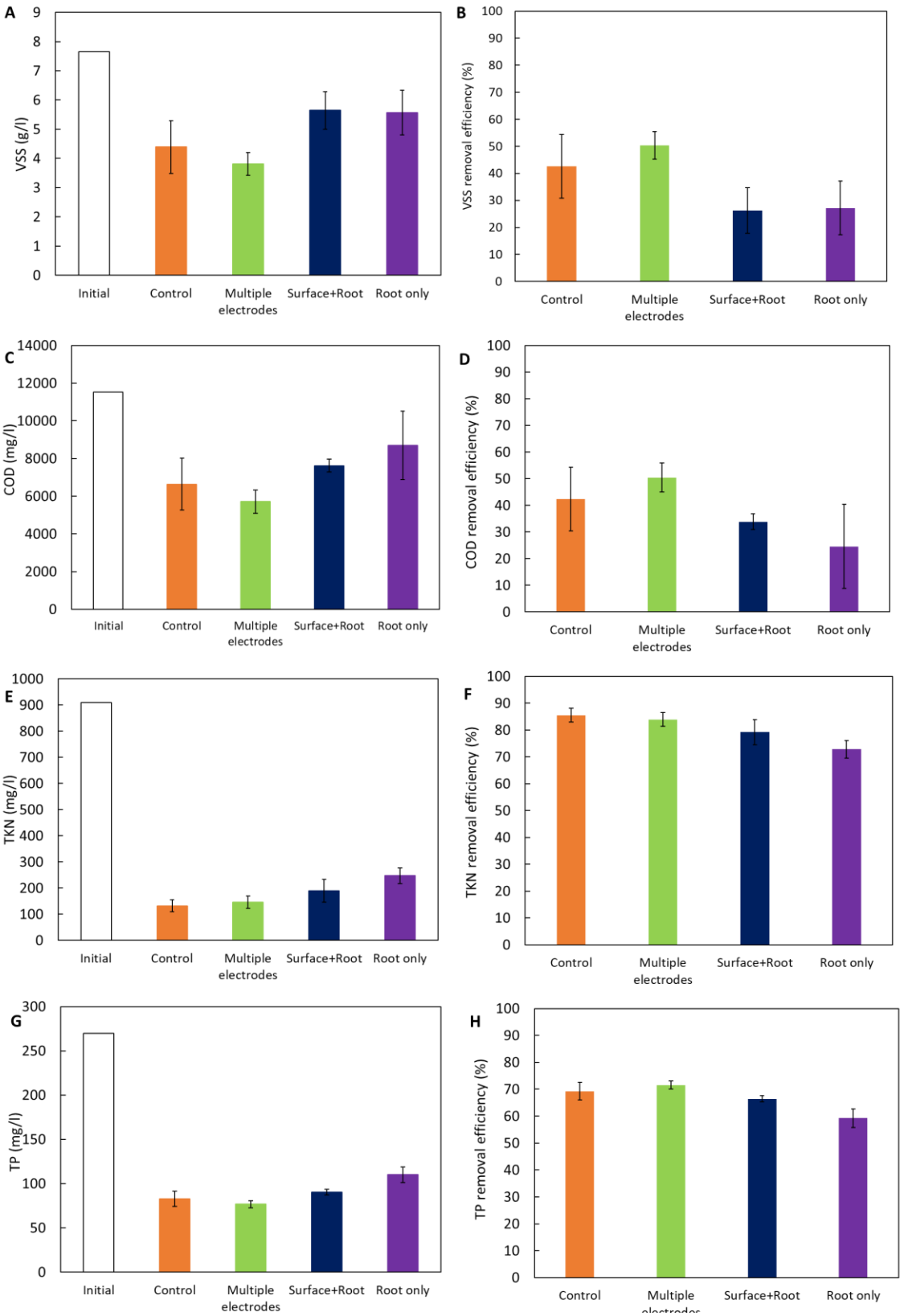



**Figure 65:** Graph showing VSS (A-B), COD (C-D), TKN (E-F) and TP (G-H) removal of different electrode configurations at the end of the experiment when WAS was used as substrate.

The control and multiple surface electrode produced the same VSS and COD removal given their overlapping standard deviations. Similarly, the surface + root and root only cathodes produced the same removal. This may be as a result of the presence of a platinum cathode at the roots.

Platinum is a strong active catalyst that is used to enhance the transfer of electrons to oxygen [94]. The cathodes used in this research were coated with 0.4 mg/cm$^2$ of platinum to enhance the oxygen reduction reaction at the cathode and therefore produce more power. This means that any oxygen released by the plant roots when the cathode is placed in that area would be used for electricity generation. COD removal, as shown in previous experiments and in literature [20, 83], is enhanced by plant roots because of increased microbial activity. The microbial activity in return is increased because of the exudates and oxygen released. Therefore, the presence of platinum cathode likely led to utilising oxygen released from plants for power generation instead of growth of microbes which in turn reduced COD removal in the system.

The same effect was observed in TKN removal. Since all the released oxygen may have been used for power generation lower TKN removal efficiencies were observed in root cathodes.

TP removal was lowest in the root only cathode i.e. 59.2 ± 3.4% followed by surface + root i.e. 66.6 ± 1.2% when compared to 71.5 ± 1.5% in multiple electrodes and 69.3 ± 3.3% in control. Reduced microbial activity also inhibits plant growth [33]. Therefore, slightly lower TP removal efficiencies in root cathodes was also observed as TP is removed by OrgP broken down to OP and taken up by plant.

### 8.3.5 Conclusion

The results obtained in this experiment indicated that multiple electrodes performed better than single electrodes in terms of power generation. Furthermore, within a set-up, parallel connection was seen as a better connection option than a series connection.

The presence of a cathode at the root performed poorly in both power generation and organic removal. The results show that is better to have surface electrodes instead of placing them at the roots for this design.

## 8.4 Optimisation experiment 3

Literature has shown that increasing electrode distance reduces peak power density but aids in COD and TN removal [119]. Therefore, this experiment was aimed at studying the voltage output when the electrode distance was varied. The experiment was started on the 3$^{rd}$ of January 2019 (day 0) and ended on the 14$^{th}$ March 2019 (day 70). This long experiment period also allowed for the investigation of how voltage is affected by time.

### 8.4.1 Experimental design

A triplicate of each system was set-up as described in Section 3.5.2. The difference being the difference in electrode distance. Also, all the systems did not contain a plant and therefore operated as SMFC.

#### 8.4.1.1 Experimental set-ups

Three electrode distances were tested in this experiment, namely:

1) 1xDist which refers 1 times the electrode distance of 13.5 cm as shown in Figure 17 B);
2) 0.5xDsit which refers to half of the electrode distance i.e. 6.75 cm; and
3) 1.5xDist which refers to 1.5 times the electrode distance i.e. 20.25 cm.

#### 8.4.1.2 Substrate classification

WAS was used as the substrate for this experiment. The WAS was sourced from an activated sludge system (ASS) running in the Water Quality Lab at the UCT. The ASS was operated on the UCT system and its aim was to grow a pure culture of PAOs. Since the ASS was lab scale, only 1.5 litres of WAS was collected every day for 12 days. The sludge collected per day was frozen. Once 18 litres was collected, the sludge was defrosted, and the experiment was started two days later.

The sludge was characterised based on its TSS, SS, ISS, COD, VFA, TKN, FSA, TP and OP. The results are summarised in Table 20.

**Table 20:** WAS characterisation.

| Characteristic | TSS | VSS | ISS | COD | TKN | FSA | TP | OP |
|---|---|---|---|---|---|---|---|---|
| mg/l | 7910 | 4260 | 3650 | 4621 | 339 | 56.4 | 947 | 323 |

### 8.4.2 Voltage results and discussion

The voltage readings across a 1000 ohms resistor recorded from day 5 to 70 for all three systems is provided in Figure 66. The systems were left as open circuit from day 0 to day 5 to allow them to stabilise. The voltage from day 42 to 46 and day 50 to 53 was not recorded due to voltage recording device malfunction.

The voltage readings across the 1.5xDist and 1xDist were seen to gradually drop with time. The 1.5xDist started with a voltage reading of approximately 0.4 V and this dropped to approximately 0.25 V by day 70. The 1xDist recorded approximately 0.2 V on day 10 which decreased to 0.12 V by day 70. The gradual drop in voltage readings was not observed in the 0.5xDist. It is speculated that not observing a voltage decrease in the 0.5xDist can be related to the addition of water to the

systems. As explained in Section 3.5, a constant water layer was maintained through the addition of water. With every addition, the water may have also played a part in unclogging the GAC as it was placed significantly closer to the surface relative to the deeply embedded anode in the 1xDist and 1.5x Dist.

From day 5 to day 70, the 1.5xDist recorded higher voltage readings compared to 1xDist. This system also produced higher voltage readings than the 0.5xDist from day 5 until day 22. However, after day 22, the voltage readings in the 0.5xDist were stabilised and approximately equal to 1.5xDist. With the decreasing voltage in the 1.5xDist, the 0.5xDist recorded higher voltage readings post day 46.

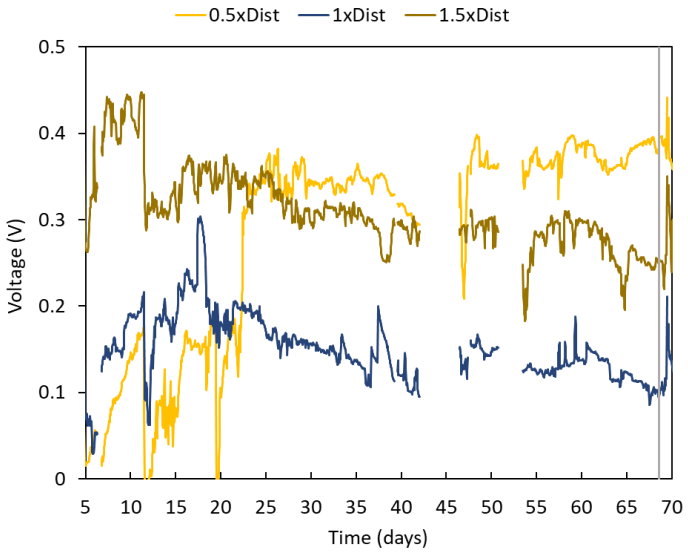



**Figure 66:** Long term voltage results recorded across three different electrode distances with the grey vertical line representing when the polarisation test day.

From literature, it is known that increasing the electrode distance increases the internal resistance, but at the same time it increases the oxygen gradient [16, 17, 21]. The 1.5xDist took advantage of the increased oxygen gradient to produce higher voltage readings relative to 1xDist while the 0.5xDist took advantage of lower internal resistances. The 1xDist however had a share of both advantages and disadvantages. Intuitively, the 1xDist should have produced the same voltage, but the results show otherwise. The1xDist produced the lowest voltage readings of the three systems. This suggests that the impact of the internal resistance and oxygen gradient is not equal with increasing electrode distance. To develop a relationship between the two, systems with more distance variations are required and as such further research is recommended.

### 8.4.3 Polarisation test results and discussions

The polarisation test to measure the PPD was done on day 68. The grey vertical line on Figure 66 indicates exactly when the test was done. On this day, the voltage measured across the 0.5xDist had surpassed the readings from the 1.5xDist. The impact of this can be seen in Figure 67 whereby the PPD is highest in 0.5xDist (664 ± 122 mW/m$^3$) followed by the 1.5xDist (453 ± 74 mW/m$^3$) and lowest in 1xDist (290 mW/m$^3$) (see t-test in Appendix I).

The highest PPD obtained from the lowest electrode distance has been observed in previous research. Sajana, et al. [119] observed a PPD increase from 3.1 mW/m$^2$ to 4.29 mW/m$^2$ as the electrode distance increased decreased from 100 cm to 50 cm. Cheng, et al. [94] saw an increase of 811 mW/m$^2$ to 1540 mW/m$^2$ as electrode distance was halved i.e. 2 cm to 1 cm. Both of these studies halved the electrode distance. Their results can be used to compare the results between the 0.5xDist and 1xDist or the 0.5xDist and 1.5xDist. However, they cannot be used to explain the drop in the PPD observed between the 1.5xDist and 1xDist even though the electrode distance decreased.

Jiang and Li [17] varied the electrode distance from 7 cm to 1 cm but interestingly, they obtained a maximum power density at 2 cm. This means that PPDs do not always increase with decreasing electrode distance as suggested by Sajana, et al. [119] and Cheng, et al. [94]. This means that the PPD for different distances is required to find the optimal distance.

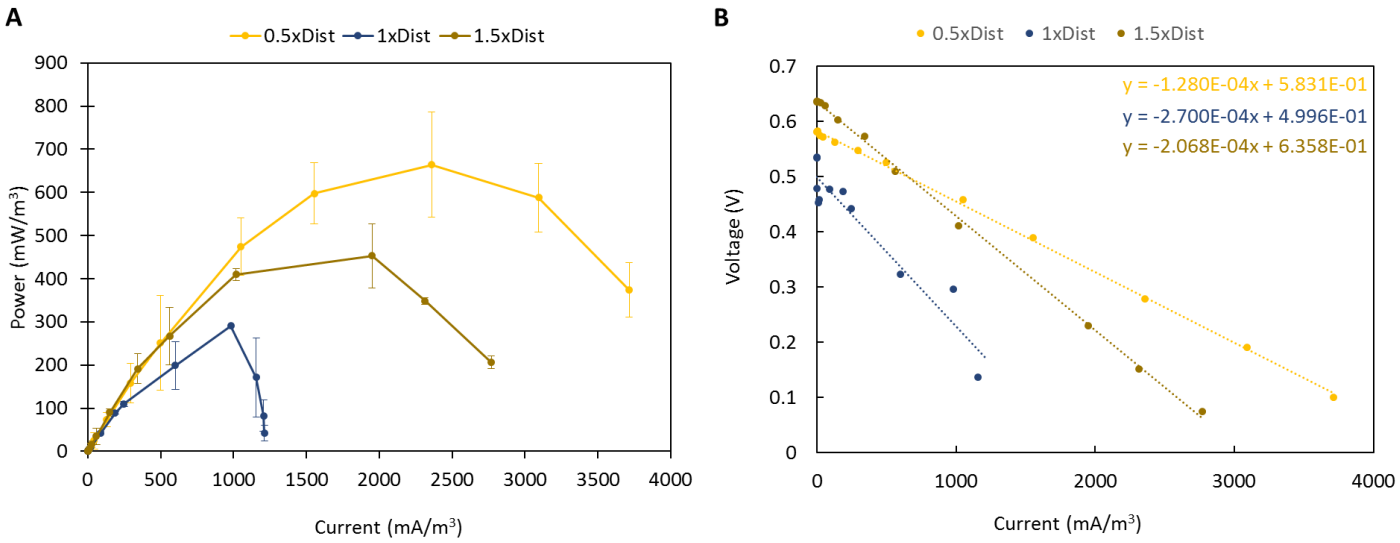



**Figure 67:** Polarisation test results on day 68 when using WAS as substrate. (A) shows PPDs for different electrode distances while (B) shows voltage versus current results. The error bar represents the standard deviation from the mean of the triplicate set-ps.

**Table 21:** Peak power density and internal resistance of the three electrode distances

| System | Peak power density ($mW/m^3$) | Internal resistance (Ω) | Open circuit voltage (V) |
|---|---|---|---|
| 0.5xDist | 664 ± 122 | 238 ± 2 | 0.58 ± 0.02 |
| 1xDist | 290 | 502 ± 15 | 0.54 ± 0.02 |
| 1.5xDist | 453 ± 74 | 384 ± 39 | 0.64 ± 0.06 |

Similar to the PPD, the internal resistance was lowest in 0.5xDist followed by the 1.5xDist and highest in 1xDist (see Table 21). From literature, the internal resistance always increases as the electrode distance increases [10, 13, 16, 21, 94, 119]. Even Jiang and Li [17], who varied electrode distances, observed an increase in internal resistance from 30 ohms to 75 ohms as the distance increased from 1 cm to 7.5 cm, even though the PPD was highest when the distance was 2 cm. The results obtained in this experiment agree with literature when comparing the 0.5xDist and 1.5xDist. However, when comparing the 1.5xDist to the 1xDist, the results do not agree with the literature. The test was redone after 5 days and the internal resistance measured 543 ± 26 ohms, which was still greater than that of 1.5xDist (i.e. 502 ± 15 ohms). This can either mean all the three set-ups for the 1xDist were done incorrectly and the results obtained are wrong or a more in-depth study of the internal resistance is required to ascertain why the results obtained do not conform with literature. Further research in which the anode and cathode polarisation tests are done separately to obtain internal resistances of each electrode is therefore recommended.

## 8.5 Comparison between MFC, SMFC and PMFC

Historically, MFCs were the first of the three systems to be developed. In the early years of MFC research, MFCs were designed to separate the electrodes using Nafion membranes, which allow only protons to pass through them [120]. Nafion membranes are commonly used in hydrogen fuel cells and cost approximately $1000 per $m^2$. Therefore, scaling up MFCs would require a large capital investment. With further advancement, researchers realised that other cations present at the cathode do not affect power output, the proton exchange membranes were replaced with the less expensive cation exchange membrane [121]. However, even with the cheaper membrane, it still costs $8 when using MFCs versus $0.1 when using ADs to treat a kg of wastewater COD [120].

Given the economic challenges, SMFCs and thereafter PMFCs were developed as alternate designs which do not make use of a membrane. They achieve this by embedding the anode deep in sediment and allowing the cathode to float above exposed to air. Power output based on both literature and experimental results from this study was compared along with other variables in a cell. The results are summarised in Table 22.

**Table 22:** Comparison between MFC, SMFC and PMFC based on experimental results from this study as well as various literature sources.

| System | Power | | Reactor volume (ml) | Substrate | | Mode of Operation | Electrodes | | Electrode distance (cm) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | Value (mW) | Based on | | Type | COD | | Anode | Cathode | | |
| MFC | 8 | /$m^2$ of anode | 25 | Starch plant | 400 | Continuous | Graphite felt | | 1 | [122] |
| | 12.2 | /$m^2$ of anode | 388 | Settled wastewater | 210 | Continuous | Graphite rod | 0.5 mg/cm2 Pt coated carbon sheet | N/A | [123] |
| | 1180 | /$m^2$ of anode | N/A | Acetate | 780 | Batch | Activated carbon | | 4 | [124] |
| | 12430 (day 15) - 3200 (day 185) | /$m^2$ of anode | 80 | AD effluent and PCE | 1933 | Batch | Carbon cloth | | N/A | [125] |
| | 393 | /$m^2$ of cathode | 20 | Domestic wastewater | 450 | Batch | Graphite brush | GAC | 1.5 | [120] |
| SMFC | 140 | /$m^2$ of anode | N/A | Dairy wastewater | N/A | Batch | Graphite rod | | 7 | [126] |
| | 2920 | /$m^2$ of anode | 24480 | Ocean sediment | N/A | Batch | Inverted GAC tube | 0.5 mg/cm2 Pt coated carbon cloth | N/A | [18] |
| | 45 | /$m^2$ of anode | 500 | Acetate and sand | 640 | Batch | Inverted GAC tube | 0.2 mg/cm2 Pt coated carbon sheet | 2 | Past experiments in our lab |
| | 290 | /$m^3$ of anode | 4000 | WAS | 4620 | Batch | GAC | 0.4 mg/cm2 Pt coated carbon sheet | 18 | This work |
| | 502 | /$m^3$ of anode | 4000 | WAS | 4620 | Batch | GAC | 0.4 mg/cm2 Pt coated carbon sheet | 9 | This work |

| System | Power | | Reactor volume (ml) | Substrate | | Mode of Operation | Electrodes | | Electrode distance | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | Value (mW) | Based on | | Type | COD | | Anode | Cathode | | |
| PMFC/ CW-MFC | 15.6 | /$m^2$ of anode | 270 x$10^3$ | Synthetic wastewater | 250 | Continuous | Graphite rod | | 25 | [19] |
| | 302 | /$m^3$ of anode | 35300 | Azo dye | 150 | Continuous | GAC | | 20 | [26] |
| | 7.47 | /$m^2$ of anode | 81000 | Synthetic wastewater | 344 | Continuous | Graphite | Magnesium | 25 | [20] |
| | 443 | /$m^3$ of anode | 4000 | WAS | 4620 | Batch | GAC | 0.4 mg/cm2 Pt coated carbon sheet | 18 | This work |
| | 510 | /$m^3$ of anode | 4000 | Thickened WAS | 175 x $10^3$ | Batch | GAC | 0.4 mg/cm2 Pt coated carbon sheet | 18 | This work |

From Table 22 it is apparent that MFCs generate significantly higher power outputs when compared to SMFCs and PMFCs. This higher output can also be attributed to very short electrode distances and small scale MFC set-ups. Increasing electrode distances proportionally increase internal resistance [16]. Furthermore, smaller reactor set-ups generate more power compared to scaled up systems [127].

Between the SMFCs and PMFCs, there is a large variability in the power output. What is more challenging is that power output values are quoted based on either a $m^2$ or a $m^3$ of anode area, but given the variability in the electrodes used, it becomes difficult to compare both systems. From experiments conducted in this study, PMFCs were shown to generate higher power outputs compared to SMFCs, but none of the PMFC experiments generated higher power output than 2920 $mW/m^2$ obtained by Liu [18]. However, lower power outputs are often seen when using wastewater compared to other substrates (e.g. ocean sediment in this case) used given wastewater's low electric conductivity [120].

When comparing the PMFC experimental results to literature values, it can be seen that the experiment produced higher PPDs than the literature values but at the same time, the substrate COD used was at least ten times greater making it difficult to establish if the experimental system was actually better.

Given the multitude of variables in these systems, as explained above, it becomes challenging to compare one system compared to another. A unified comparison method needs to be developed, potentially power generated per capital cost per kg of COD treated, but this requires further investigation.

# 9. Conclusions

With the growing energy demand and limited fossil fuels, it is of paramount importance to look for alternative sustainable energy sources. In addition, with a growing population, more wastewater which is rich in organic content is being generated. Some of this wastewater is currently treated in activated sludge systems which require significant amounts of energy. Some treatment works have anaerobic digestors which can produce methane for energy generation thus offsetting the energy demand of a WWTWs. However, conventional anaerobic digestors are not capable of ammonia removal. Alternatively, PMFCs can be used for direct electricity generation, bypassing methane production while also removing ammonia from the process. However, the power generated from PMFCs is significantly less in comparison and therefore more research is required.

This research designed a PMFC and operated using municipal wastewater sludge. As part of the research, three indigenous South African plant species were chosen and operated in the PMFC. The research also optimised the designed the PMFC.

## 9.1 Design and operation of the PMFC

The components chosen for the design of the PMFC were based on extensive literature review. The PMFC consisted of a 5 litre container with a diameter of 18.5 cm and a height of 21 cm. GAC of depth 2 cm was chosen as the anode to host bacteria and allow for the development of a biofilm. The GAC encased a nickel coated copper wire which was used as an electron acceptor. A carbon sheet of area 67.5 $cm^2$ coated with 0.4 $mg/cm^2$ platinum was chosen as the cathode.

The bucket was filled with substrate when a solid was used. When using a liquid substrate, it was filled with lightweight expanded clay aggregate plus sludge. Both were filled to a depth of 16 cm leaving 3 cm for gravel and water. Lightweight expanded clay aggregate was used in the case of liquid substrate to support plant growth. Three indigenous wetland plant species were tested, namely, *C. papyrus nanus, W. thyrsiflora* and *P. australis*. The plants were placed in the first half section of the container to encourage root growth.

The PMFC was operated as a batch system. The importance of having a ‘wet’ cell, the cathode contact area and the resistors connected was tested. When operating the PMFC, it was found that voltage readings approached zero as the cells dried out and stopped power production. It was also observed that when water was not added for nine days, some of the soil systems never recovered and produced negative voltages even in open circuit conditions. Negative voltages occur as a result of loss of bacterial activity. The days in which the water was not added caused the systems to dry out potentially leading to the death of bacteria. This problem was not present when using thickened sludge because of the high (84%) water content.

During the testing phase, the importance of the cathode having continuous contact with the sediment was noticed. Lifting of the cathode from the sediment reduced the overall contact surface area of the cathode and produced lower voltage readings. It was also noticed that

leaving the cathode on the sediment without an external force, in most cases, caused the cathode to lift from the surface. This problem was solved by placing pebbles on the cathode.

The operation also looked at the size of the external resistors connected. It was found that the choice of resistors connected plays an important role when comparing the performance of one PMFC system to another. When one of the systems was connected with a 100 ohm resistor, the voltage reading was 0.24 V which translated to 1070 mW/m$^3$. However, when connecting a 1000 ohms resistor, the voltage increased to 0.45 V, but the power dropped to 377 mW/m$^3$. It was also noted that when very low external resistors were connected, voltage readings approached zero making it difficult to compare one system's performance to another. Therefore, this gave rise to three concepts: (1) the power should only be discussed after performing a polarisation test in which the peak power density of a system can be measured, (2) voltage between systems should only be compared when the same external resistors are connected and (3) the external resistor chosen should be high enough to measure comparable voltage readings.

## 9.2 Substrates tested

For this research, the performance of three sludge types from typical municipal WWTWs were tested, namely, thickened WAS, liquid WAS and PS. The thickened WAS and liquid WAS had similar properties with the only difference being the water content. Liquid WAS would essentially thicken as it dries on a sludge drying bed. In both substrates, the biodegradable organic content is derived from the death of OHOs and/or PAOs. In PS this is derived from readily available biodegradable particulate and soluble organics.

When comparing the power output from each sludge type, it was noticed that the highest peak power density was obtained when using thickened WAS with *W. thyrsiflora* i.e. 1036 ± 59 mW/m$^3$ followed by liquid WAS with *W. thyrsiflora* with 290 ± 21 mW/m$^3$ and the lowest in unplanted PS 119 ± 31 mW/m$^3$. However, *W. thyrsiflora* grown in thickened died during the course of the experiment. The *C. papyrus* survived and produced 510 ± 92 mW/m$^3$.

When the power outputs were evaluated based on the COD consumption for the duration of the experiment, i.e. the efficiency of converting COD to power, it was found that the WAS outperformed PS. Thickened WAS produced a conversion of 1330 mW/m$^3$ per gram of COD consumed, WAS with 508 mW/(m$^3$·gCOD), and PS with 124 mW/(m$^3$·gCOD). This conversion was chosen as the initial COD of thickened WAS was over 10 times higher than WAS and PS and also the duration of the experiments was different. Thickened WAS performed better than liquid WAS but if the PMFC technology was to be implemented in a drying bed, the liquid WAS would produce the same power once the sludge thickens. Furthermore, thickened WAS is not practical when considering the PMFC application in WWTWs.

Furthermore, it was found that the power output from a PMFC utilising PS was significantly lower (over 500 times) when compared to PS digestion in an AD making the PMFC use of PS not economically viable. The benefit of utilising the PS in the PMFC instead of an AD was

primarily as a result of TKN removal. The anaerobic digestion of WAS was not found viable based on literature.

Based on the discussions above, WAS was chosen over PS as the feed for PMFC. Furthermore, liquid WAS was chosen over thickened WAS for further experimentation as: (1) WWTWs are continuous systems and need a liquid instead of a solid, (2) if the technology was implemented on a drying bed, the liquid WAS would thicken producing the same conversion as thickened WAS and (3) if the technology was implemented as a continuous system, a liquid phase is required.

## 9.3 Plant species tested

Three indigenous South African plant species were tested in this research: *C. papyrus nanus*, *W. thyrsiflora* and *P. australis*. It was observed that planted PMFCs performed better than unplanted, i.e. SMFC ones in both power generation and organic removal. The exception was with PS substrate where PMFC and SMFC power densities were similar. The plant roots aided in increasing surface area, provided root exudates and released oxygen to increase microbial activity, aid in organic removal and also increase peak power densities.

When testing the performance of the three plant species across the three chosen substrates, the *W. thyrsiflora* was found to produce higher power densities compared to the other two investigated plant species. However, the *W. thyrsiflora* system did not survive the organic load in thickened WAS and died. Similarly, in PS and WAS, the plant roots died, and new roots grew out on the surface layer. This means that the *W. thyrsiflora* would not be practical if the PMFC was implemented in a drying bed. Also, if this technology was used in a continuous system, the plant may not have the opportunity to grow new roots because of constantly changing feed.

When comparing the organic removal abilities, the *C. papyrus* plant species showed the best FSA, OP, TKN, TP, COD and VSS removal efficiencies. Also, the plant grew a thick fibrous root system indicating that it was able to withstand the high organic loads. Therefore, *C. papyrus* was chosen as the most suitable plant of the three species investigated.

## 9.4 Optimisation of the PMFC

Having chosen liquid WAS as the substrate and *C. papyrus* plant, the research focused on optimising the PMFC design based on three parameters: (1) use of a separator, (2) using multiple electrodes, and (3) varying electrode distance.

The research found that using a separator between the electrodes achieved a higher peak power density when compared to a system with no separator i.e. 191 ± 16 $mW/m^3$ versus 141 ± 16 $mW/m^3$. It was also observed that a separator encouraged root growth on the top surface of the PMFC providing oxygen directly to the cathode and therefore achieving higher PPDs. The voltage produced when using a separator was similar to the voltage produced by *W. thyrsiflora*.

Therefore, using a separator to contain the roots in the anodic region was seen as a better option for power generation.

In the second optimisation experiment, it was found that placing cathodes at the surface produced higher power generation compared to placing them at the roots. It was also found that the root cathodes achieved lower organic removal efficiencies. The research found that using multiple cathodes and electron acceptors instead of using single ones, produced higher power densities even though the total electrode areas were kept constant. Furthermore, the research showed that within a set-up, parallel connection was more efficient than a series connection of the electrodes.

The third optimisation experiment where distance between electrodes was varied, it was found that the highest peak power density was achieved when the electrode distance was half of the original design, followed closely by the system where electrode distance was increased by 50% compared to the original design. Interestingly, the lowest PPD was achieved with the original electrode design. Also, the internal resistance was not found to decrease with decreasing electrode distance as literature suggests. The original design achieved higher internal resistances when compared to the system with a 50% increased electrode distance.

# 10.Recommendations and future work

To potentially improve the power production, the following recommendations are made:

1. One of the major struggles in this research was the settling of solids in sludge. The settling allowed for FSA and OP measurements since these are soluble, but, the VSS, COD, TKN, and TP were not possible during the course of the experiment and only an initial and a final test was possible. If continuous measurements are taken, the kinetics of the system can be measured which can directly link to the microbial study of the system. Blending of the sludge was also tested and the sludge continued settling. A stirrer may be used to obtain a better sample of solids, but this would disrupt the system, unless the GAC is encased. This can also be solved by moving to a continuous system.

2. With regards to the plant species, a single root bulb was used per set-up. However, the plant mass of the *P. australis* used was significantly lower than the *C. papyrus* and *W. thyrsiflora*. It is speculated that the results may have been different if a greater plant mass was used. However, the follow-up problem would be how to ensure that the mass used is the same for all experiments since different plant species have different root and shoot masses. Also, what aspect of the plant mass should be considered, the roots only or both the roots and shoots? This is important as some species have more prominent aerenchyma than others, reducing their shoot mass. Therefore, better evaluation criteria between plant mass needs to be decided in order to compare one plant species to another.

3. This research only tested three plant species, more species should be tested, preferably wetland plants with a fibrous root system and the ability to release high dissolved oxygen concentrations. This may be related to the aerenchyma in the shoot. Also, a study of the oxygen release at the anodic region needs to be conducted to potentially answer the high internal resistances obtained when *C. papyrus* was used.

4. This research used only one GAC granules size. It has been shown in previous studies that changing particle size affects power production. Further research on the different sizes versus power production should be undertaken to obtain the most efficient GAC size. This will allow an empirical relationship to be developed. This can potentially be taken a step further by varying the thickness of the GAC layer to vary power which can be added to the empirical relationship.

5. A microbial study of the inoculum in the PMFC is strongly recommended. This research used the same mixed culture inoculum. Within a mixed culture, some bacteria are exoelectrogenic while others are not. Literature has shown that using a pure culture of exoelectrogenic bacteria, such as *Shewanella,* produces lower power output compared to a mixed culture. This means that within the mixed culture, there may be an optimal exoelectrogenic bacteria to non exoelectrogenic bacteria ratio. The microbial study could potentially be taken further by linking the microbial activity to the COD utilisation to better understand the results obtained in this research and other research findings. The microbial study can potentially be linked to the internal resistance as well since the bacteria are

responsible for depositing electrons on the anode. This will allow a better understanding of how the internal resistance of a system can be decreased.

6. In this research, it was shown that using PS in PMFC is not viable when comparing its power output to that of an AD. However, an AD does not treat the FSA and OP released as the organics used. A small content amount of biodegradable COD is also present in the AD waste along with the FSA and OP. This COD is derived from: (1) a fraction of the biodegradable COD that was not used in the AD and (2) acidogenic bacteria. Therefore, instead of running the PMFC on PS, it can potentially be run on the waste from an AD. The PMFC can then be used as a side stream treatment to consume the COD and remove the FSA and OP from the system.

7. In this research, a basic (stability classification only) evaluation of sludge use for fertiliser after it was used in a PMFC was done. This needs to be expanded considering the microbial and pollutant classification. Thereafter, the N:P:K ratio should be calculated. This means that the biodegradable fraction of the sludge should also be found for all the feeds tested. Once this ratio is obtained, a thorough economic evaluation is required to understand if the sludge is a viable fertiliser.

8. This research also tried to understand the variation of power output as the electrode spacing varied to try and understand the impact on power with scaled up systems. With the electrodes being close to one another, the anode is less anaerobic, but the internal resistance is lower. The internal resistance results were unexpected as the resistance did not decrease with decreasing electrode spacing as shown in literature. Therefore, it is recommended that a thorough study in which all the factors that contribute to PMFC internal resistance are tested. Also, in order better understand the impact on power in scaled up systems, the cross-sectional area may also be an important aspect to consider. The future goal should be to develop an empirical relationship between electrodes spacing, area and other PMFC components to predict power generation.

9. Since this research only focused on a batch test, future work should focus on continuous systems as these would be more applicable to large WWTWs which do not use drying beds. It is recommended that a study to link flow rate to organic removal efficiency and power generation be undertaken to develop a relationship between the three. The optimal flow rate can be used to estimate the PMFC area required. This will allow an economic evaluation between WAS treated in a PMFC or an aerobic digestor or compacted and sent to a landfill site be determined.

10. It is recommended to quantify how the platinum density varies during the course of the experiment to potentially link decreasing voltage results as time passes to cathode platinum density. This will also aid in an economic evaluation since platinum is an expensive metal.

# References


[1] United Nations, "The 2015 Revision of the UN's World Population Projections," *Population and Development Review*, vol. 41, no. 3, pp. 557-561, 2015, doi: 10.1111/j.1728-4457.2015.00082.x.

[2] M. Roser and E. Ortiz-Ospina, "World Population Growth", 2013 [Online]. Accessed on: 15th March 2019, Available: https://ourworldindata.org/world-population-growth

[3] F. Wagner, "The complex story of energy transition —an introduction", *EPJ Web of Conferences*, vol. 189, 2018.

[4] Canadian Energy Research Institute, *World Energy: The Past and Possible Futures*, Canada: Canadian Energy Research Institute, 2008. [Online]. Available: http://teachnuclear.ca/wp-content/uploads/2013/06/CNA_CERI07_EN.pdf. Accessed on: 15th March 2019.

[5] Lohuizen, "Drowning in Garbage," in *The Washington Post*, ed, 2017.

[6] J. Stromberg, "When will we hit peak garbage?," ed: Smithsonian, 2013.

[7] M. Xie, H. K. Shon, S. R. Gray, and M. Elimelech, "Membrane-based processes for wastewater nutrient recovery: Technology, challenges, and future direction," *Water Research*, vol. 89, pp. 210-221, 2016, doi: https://doi.org/10.1016/j.watres.2015.11.045.

[8] D. Cordell, J.-O. Drangert, and S. White, "The story of phosphorus: Global food security and food for thought," *Global Environmental Change*, vol. 19, no. 2, pp. 292-305, 2009, doi: https://doi.org/10.1016/j.gloenvcha.2008.10.009.

[9] D. Cordell, A. Rosemarin, J. J. Schröder, and A. L. Smit, "Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options," *Chemosphere*, vol. 84, no. 6, pp. 747-758, 2011, doi: https://doi.org/10.1016/j.chemosphere.2011.02.032.

[10] C. Abourached, K. L. Lesnik, and H. Liu, "Enhanced power generation and energy conversion of sewage sludge by CEA–microbial fuel cells," *Bioresource Technology*, vol. 166, pp. 229-234, 2014, doi: https://doi.org/10.1016/j.biortech.2014.05.027.

[11] J. P. Chouler, A. C. George, J. P. Petra, T. Kathrin, L. Maria-Magdalena, and M. Loannis Di Lorenzo, "Towards effective small scale microbial fuel cells for energy generation from urine," *Electrochimica Acta*, vol. 192, pp. 89-98, 2016, doi: https://doi.org/10.1016/j.electacta.2016.01.112.

[12] Z. Z. Ismail and A. J. Jaeel, "Sustainable power generation in continuous flow microbial fuel cell treating actual wastewater: influence of biocatalyst type on electricity production," *ScientificWorldJournal*, vol. 2013, p. 713515, 2013, doi: 10.1155/2013/713515.

[13] B. Xu, Z. Ge, and Z. He, "Sediment microbial fuel cells for wastewater treatment: challenges and opportunities," *Environmental Science: Water Research & Technology*, vol. 1, no. 3, pp. 279-284, 2015, doi: 10.1039/C5EW00020C.

[14] Z. Du, H. Li, and T. Gu, "A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy," *Biotechnology Advances*, vol. 25, no. 5, pp. 464-482, 2007, doi: https://doi.org/10.1016/j.biotechadv.2007.05.004.

[15] F. Kracke, I. Vassilev, and J. O. Krömer, "Microbial electron transport and energy conservation – the foundation for optimizing bioelectrochemical systems," *Frontiers in Microbiology*, vol. 6, p. 575, 2011.

[16] J. An, H. Moon, and I. Seop Chang, *Multiphase Electrode Microbial Fuel Cell System that Simultaneously Converts Organics Coexisting in Water and Sediment phases into Electricity*. 2010, pp. 7145-50.

[17] D. Jiang and B. Li, "Granular activated carbon single-chamber microbial fuel cells (GAC-SCMFCs): A design suitable for large-scale wastewater treatment processes," *Biochemical Engineering Journal*, vol. 47, no. 1, pp. 31-37, 2009, doi: https://doi.org/10.1016/j.bej.2009.06.013.

[18] B. Liu, Williams, Isaiah, Li, Yan, Wang, Lei, Bagtzoglou, Amvrossios, McCutcheon, Jeffrey, and Li, Baikun, "Towards high power output of scaled-up benthic microbial fuel cells (BMFCs) using multiple electron collectors," *Biosensors and Bioelectronics*, vol. 79, pp. 435-441, 2016, doi: https://doi.org/10.1016/j.bios.2015.12.077.

[19] J. Villaseñor, P. Capilla, M. A. Rodrigo, P. Cañizares, and F. J. Fernández, "Operation of a horizontal subsurface flow constructed wetland – Microbial fuel cell treating wastewater under different organic loading rates," *Water Research*, vol. 47, no. 17, pp. 6731-6738, 2013, doi: https://doi.org/10.1016/j.watres.2013.09.005.

[20] Ç. Saz, C. Türe, O. C. Türker, and A. Yakar, "Effect of vegetation type on treatment performance and bioelectric production of constructed wetland modules combined with microbial fuel cell (CW-MFC) treating synthetic wastewater," *Environmental Science and Pollution Research*, vol. 25, no. 9, pp. 8777-8792, 2018, doi: 10.1007/s11356-018-1208-y.

[21] L. Doherty, Zhao, Yaqian, Zhao, Xiaohong, Hu, Yuansheng, Hao, Xiaodi, Xu, Lei, Liu, Ranbin, "A review of a recently emerged technology: Constructed wetland – Microbial fuel cells," *Water Research*, vol. 85, pp. 38-45, 2015, doi: https://doi.org/10.1016/j.watres.2015.08.016.

[22] S. Liu, H. Song, S. Wei, F. Yang, and X. Li, "Bio-cathode materials evaluation and configuration optimization for power output of vertical subsurface flow constructed wetland — Microbial fuel cell systems," *Bioresource Technology*, vol. 166, pp. 575-583, 2014, doi: https://doi.org/10.1016/j.biortech.2014.05.104.

[23] R. Regmi, R. Nitisoravut, S. Charoenroongtavee, W. Yimkhaophong, and O. Phanthurat, *Earthen Pot-Plant Microbial Fuel Cell Powered by Vetiver for Bioelectricity Production and Wastewater Treatment*. 2018, p. 1700193.

[24] V. Gagnon, F. Chazarenc, Y. Comeau, and J. Brisson, "Influence of macrophyte species on microbial density and activity in constructed wetlands," vol. 56, no. 3, pp. 249-254, 2007, doi: 10.2166/wst.2007.510.

[25] J. S. Wang, Xinshan Wang, Yuhui Bai, Junhong Bai, Heng Yan, Dengming Cao, Yin Li, Yihao Yu, Zhilei, Dong, Guoqiang, "Bioelectricity generation, contaminant removal and bacterial community distribution as affected by substrate material size and aquatic macrophyte in constructed wetland-microbial fuel cell," *Bioresource Technology*, vol. 245, pp. 372-378, 2017, doi: https://doi.org/10.1016/j.biortech.2017.08.191.

[26] Z. Fang, H.-L. Song, N. Cang, and X.-N. Li, "Performance of microbial fuel cell coupled constructed wetland system for decolorization of azo dye and bioelectricity generation," *Bioresource Technology*, vol. 144, pp. 165-171, 2013, doi: https://doi.org/10.1016/j.biortech.2013.06.073.

[27] Z. Chen, Y.-c. Huang, J.-h. Liang, F. Zhao, and Y.-g. Zhu, "A novel sediment microbial fuel cell with a biocathode in the rice rhizosphere," *Bioresource Technology*, vol. 108, pp. 55-59, 2012, doi: https://doi.org/10.1016/j.biortech.2011.10.040.

[28] M. A. Moqsud, J. Yoshitake, Q. S. Bushra, M. Hyodo, K. Omine, and D. Strik, "Compost in plant microbial fuel cell for bioelectricity generation," *Waste Management*, vol. 36, pp. 63-69, 2015, doi: https://doi.org/10.1016/j.wasman.2014.11.004.

[29] N. Tapia, C. Rojas, C. Bonilla, and I. Vargas, "Evaluation of Sedum as driver for plant microbial fuel cells in a semi-arid green roof ecosystem," *Ecological Engineering*, vol. 108, p. 203, 2017.

[30] N. Kaku, N. Yonezawa, Y. Kodama, and K. Watanabe, "Plant/microbe cooperation for electricity generation in a rice paddy field," *Applied Microbiology and Biotechnology*, vol. 79, no. 1, pp. 43-49, 2008, doi: 10.1007/s00253-008-1410-9.

[31] L. Schamphelaire, L. van den Bossche, H. S. Dang, M. Hofte, N. Boon, K. Rabaey and W. Verstraete, "Microbial Fuel Cells Generating Electricity from Rhizodeposits of Rice Plants," *Environmental Science & Technology*, vol. 42, no. 8, pp. 3053-3058, 2008, doi: 10.1021/es071938w.

[32] G. Yvon-Durocher, A. P. Allen, D. Bastviken, R. Conrad, C. Gudasz, A. StPierre, N. Thanh-Duc and P. A. del Giorgio, "Methane fluxes show consistent temperature dependence across microbial to ecosystem scales," *Nature*, vol. 507, p. 488, 03/19/online 2014, doi: 10.1038/nature13164 https://www.nature.com/articles/nature13164#supplementary-information.

[33] Y. Waisel, A. Eshel, and U. Kavkafi, *Plant Roots The Hidden Half*. New York: Marcel Dekker, 1991.

[34] R. Nitisoravut and R. Regmi, "Plant microbial fuel cells: A promising biosystems engineering," *Renewable and Sustainable Energy Reviews*, vol. 76, pp. 81-89, 2017, doi: https://doi.org/10.1016/j.rser.2017.03.064.

[35] D. P. B. T. B. Strik, H. V. M. Hamelers, J. F. H. Snel, and C. J. N. Buisman, "Green electricity production with living plants and bacteria in a fuel cell," *International Journal of Energy Research*, vol. 32, no. 9, pp. 870-876, 2008, doi: 10.1002/er.1397.

[36] R. A. Timmers, D. P. Strik, H. V. Hamelers, and C. J. Buisman, "Long-term performance of a plant microbial fuel cell with *Spartina anglica*," *Appl Microbiol Biotechnol*, vol. 86, no. 3, pp. 973-81, Apr 2010, doi: 10.1007/s00253-010-2440-7.

[37] J. Zhang, H. Wu, Z. Hu, S. Liang, and J. Fan, "Examination of oxygen release from plants in constructed wetlands in different stages of wetland plant life cycle," *Environmental Science and Pollution Research*, vol. 21, no. 16, pp. 9709-9716, 2014, doi: 10.1007/s11356-014-2905-9.

[38] W. M. Kemp and L. Murray, "Oxygen release from roots of the submersed macrophyte Potamogeton perfoliatus L.: Regulating factors and ecological implications," *Aquatic Botany*, vol. 26, pp. 271-283, 1986, doi: https://doi.org/10.1016/0304-3770(86)90027-6.

[39] K. Sand-Jensen, C. Prahl, and H. Stokholm, "Oxygen Release from Roots of Submerged Aquatic Macrophytes," *Oikos*, vol. 38, no. 3, pp. 349-354, 1982, doi: 10.2307/3544675.

[40] R. F. Sage, "A portrait of the C4 photosynthetic family on the 50th anniversary of its discovery: species number, evolutionary lineages, and Hall of Fame," *J Exp Bot*, vol. 68, no. 2, pp. 4039-4056, Jan 2017, doi: 10.1093/jxb/erx005.

[41] A. Kouzuma, N. Kaku, and K. Watanabe, "Microbial electricity generation in rice paddy fields: recent advances and perspectives in rhizosphere microbial fuel cells," *Applied Microbiology and Biotechnology*, journal article vol. 98, no. 23, pp. 9521-9526, December 01 2014, doi: 10.1007/s00253-014-6138-0.

[42] V. Lakay, "An analysis of the performance of Constructed Wetlands in the treatment of domestic wasewater in SA," Master of Science, Environmental and Geographical Science, University of Cape Town, Cape Town, 2012.

[43] J. Bever, M. Chevalier, J. Colvin, T. Davis, R. Fallin, G. Hartman, E. Hoffmaster, R. Hoffmaster, R. Leiva, S. Marcum, P. Mock, T Murdock, D. Pope, L. Runion, B. Stanley, S. Townsend, J. Vaughan, T. Young and D. Yow, "The comparison between natural and mitigated wetland plant communities in Maryland," 2006.

[44] A. Gerber, C. J. Cilliers, C. Van Ginkel, and R. Glen, *Easy identification of Aquatic Plants*. Pretoria, South Africa: Department of Water Affairs and Forestry, 2004.

[45] C. E. van Ginkel, R. P. Glen, K.D. Gordon-Gray, C.J. Cilliers, M. Muasya and P.P. van Deventer, Easy identification of some South African wetland plants, *Water Research Comission Report number: TT 479/10,* 2011.

[46] S. P. Nobel, *Biophysical Plant Physiology and Ecology*. United States of America: W. H. Freeman and Company, 1983.

[47] P. H. Nye and P. B. Tinker, *Solute movement in the soil-root system*. Australia: Blackwell Scientific Publications, 1977.

[48] C. du Poldt, R. Edwards, L. Garber, B. Isaacs, L. Lapp, T. Murphy, G. Rider, M. Sayers, F. Suffian, C. Takita and H. Webster, A Handbook of constructed wetlands, *USDA-Natural Resources Conservation Services and US Environmental Protection Agency.* 1994.

[49] L. Perry, "pH for the garden," University of Vermont Extension, 2003.

[50] Divison of Agriculture and Natural Resources "Salinity Management." *University of California*, n.d. [Online]. Accessed: 14th October 2018. Available: https://ucanr.edu/sites/Salinity/Salinity_Management/Salinity_Basics/Salinity_measurement_and_unit_conversions/#

[51] D. Pant, G. Van Bogaert, L. Diels, and K. Vanbroekhoven, "A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production," *Bioresource Technology*, vol. 101, no. 6, pp. 1533-1543, 2010, doi: https://doi.org/10.1016/j.biortech.2009.10.017.

[52] Y. Sharma and B. Li, "The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs)," *Bioresource Technology*, vol. 101, no. 6, pp. 1844-1850, 2010, doi: https://doi.org/10.1016/j.biortech.2009.10.040.

[53] G. Ekama, *Organic Removal by Activated Sludge Part 2*, Water Research Group, Department of Civil Engineering, University of Cape Town, South Africa, 2013.

[54] D. J. Noziac and S. D. Freese, "Process Design Manual For Small Wastewater Works," *Water Research Comission,* 2009.

[55] E. Tilley, L. Ulrich, C. Luethi, P. Reymond, and C. Zurbruegg, "Planted Drying beds," *Eawag (Swiss federal institute of Aquatic Science and Technology)*, 2014.

[56] I. M. Kengne and E. Tilley, "Planted drying beds," Eawag Ed., 2014, ch. 8, pp. 155-176.

[57] P. H. Dodane, M. Mbeguere, I. M. Kengne, and L. Strande-Gaulka, "Planted Drying Beds for Faecal Sludge Treatment: Lessons Learned Through Scaling Up in Dakar, Senegal," *Excreta and Waste Management*, 2011. [Online]. Available: https://www.eawag.ch/fileadmin/Domain1/Abteilungen/sandec/publikationen/EWM/Treatment_Technologies/Planted_drying_beds_Dakar.pdf.

[58] S. B. Joceline, M. Koné, O. Yacouba, and Y. H. Arsène, "Planted Sludge Drying Beds in Treatment of Faecal Sludge from Ouagadougou: Case of Two Local Plant Species," *Journal of Water Resource and Protection*, vol. 08, no. 07, pp. 697-705, 2016, doi: 10.4236/jwarp.2016.87057.

[59] A. Lienard, P. Duchene, and D. Gorini, "A study of activated sludge dewatering in experimental reed-planted or unplanted sludge drying beds," presented at the 4th IAWQ Int. Specialist. Wetland systems for water pollution, Guanhzhou, China, 1994.

[60] S. Leila, M. Mhamed, H. Hermann, K. Mykola, W. Oliver, M. Christin, O. Elena and B. Nadia., "Fertilization value of municipal sewage sludge for Eucalyptus camaldulensis plants," *Biotechnology Reports*, vol. 13, pp. 8-12, 2017, doi: https://doi.org/10.1016/j.btre.2016.12.001.

[61] W. Fang, Y. Wei, and J. Liu, "Comparative characterization of sewage sludge compost and soil: Heavy metal leaching characteristics," *Journal of Hazardous Materials*, vol. 310, pp. 1-10, 2016, doi: https://doi.org/10.1016/j.jhazmat.2016.02.025.

[62] H. G. Snyman and J. E. Herselman, Guidelines for the Utilisation and Disposal of Wastewater Sludge, Volume 2: Requirements for the agricultrul use of wastewater sludge, *Water Research Commission report: TT262/06.* 2006.
[63] E. Vinje, "Organic Fertilizer Benefits," in *Planet Natural Research Centre* vol. 2019, ed. USA: Planet Natural Research Centre, 2014.
[64] D. Xue and X. Huang, "The impact of sewage sludge compost on tree peony growth and soil microbiological, and biochemical properties," *Chemosphere,* vol. 93, no. 4, pp. 583-589, 2013, doi: https://doi.org/10.1016/j.chemosphere.2013.05.065.
[65] UNEP and M. University, "Sludge treatment, resue and disposal," 2000. [Online]. Available: http://www.unep.or.jp/ietc/Publications/Freshwater/SB_summary/index.asp.
[66] H. K. Kim, T. I. Jang, S. M. Kim, and S. W. Park, "Impact of domestic wastewater irrigation on heavy metal contamination in soil and vegetables," *Environmental Earth Sciences,* vol. 73, no. 5, pp. 2377-2383, 2015, doi: 10.1007/s12665-014-3581-2.
[67] H. G. Snyman, J. E. Herselman and G. Kasselman, A metal content survey of South African sewage sludge and an evaluation of analytical methods for their determination in sludge. *Water Research Commission report:1283/04.* 2004.
[68] W. Fang, R. C. Delapp, D. S. Kosson, H. A. van der Sloot, and J. Liu, "Release of heavy metals during long-term land application of sewage sludge compost: Percolation leaching tests with repeated additions of compost," *Chemosphere,* vol. 169, pp. 271-280, 2017, doi: https://doi.org/10.1016/j.chemosphere.2016.11.086.
[69] (2006). *TT 261/06, Guidelines for the Utilisation and Disposal of Wastewater Sludge Volume 1: Selection of Management options*.
[70] K. K. Shamuyarira and J. R. Gumbo, "Assessment of Heavy Metals in Municipal Sewage Sludge: A Case Study of Limpopo Province, South Africa," *International Journal of Environmental Research and Public Health,* vol. 11, no. 3, pp. 2569-2579, 2003.
[71] Y. Zou, L. Zhang, L. Wang, S. Zhang, and X. Yu, "Effects of Aeration, Vegetation, and Iron Input on Total P Removal in a Lacustrine Wetland Receiving Agricultural Drainage," *Water,* vol. 10, no. 1, 2018, doi: 10.3390/w10010061.
[72] R. B. Wood and C. F. McAtamney, "Constructed wetlands for waste water treatment: the use of laterite in the bed medium in phosphorus and heavy metal removal," *Hydrobiologia,* vol. 340, no. 1, pp. 323-331, 1996/12/01 1996, doi: 10.1007/BF00012776.
[73] K. R. Reddy, *Biogeochemistry of Wetlands: Cycling of Iron and Manganese*, USA: University of Florida, 2008.
[74] L. C. Batty and P. L. Younger, "Effects of External Iron Concentration upon Seedling Growth and Uptake of Fe and Phosphate by the Common Reed, *Phragmites australis*," *Annals of Botany,* vol. 92, no. 6, pp. 801-806, 2003, doi: 10.1093/aob/mcg205.
[75] G. U. Chibuike and S. C. Obiora, "Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods," *Applied and Environmental Soil Science,* vol. 2014, p. 12, 2014, Art no. 752708, doi: 10.1155/2014/752708.
[76] A. J. M. Baker, "Accumulators and excluders -strategies in the response of plants to heavy metals," *Journal of Plant Nutrition,* vol. 3, no. 1-4, pp. 643-654, 1981, doi: 10.1080/01904168109362867.
[77] P. Zhuang, Q. W. Yang, H. B. Wang, and W. S. Shu, "Phytoextraction of Heavy Metals by Eight Plant Species in the Field," *Water, Air, and Soil Pollution,* vol. 184, no. 1, pp. 235-242, 2007, doi: 10.1007/s11270-007-9412-2.
[78] S. Saraswat and J. Rai, *Phytoextraction potential of six plant species grown in multimetal contaminated soil*. 2009, pp. 1-11.

[79] B. V. Tangahu, S. R. Sheikh Abdullah, H. Basri, M. Idris, N. Anuar, and M. Mukhlisin, "A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation," *International Journal of Chemical Engineering*, vol. 2011, p. 31, 2011, Art no. 939161, doi: 10.1155/2011/939161.

[80] G. Liu, M. D. Yates, S. Cheng, D. F. Call, D. Sun, and B. E. Logan, "Examination of microbial fuel cell start-up times with domestic wastewater and additional amendments," *Bioresource Technology*, vol. 102, no. 15, pp. 7301-7306, 2011, doi: 10.1016/j.biortech.2011.04.087.

[81] R. A. Timmers, D. P. B. T. B. Strik, C. Arampatzoglou, C. J. N. Buisman, and H. V. M. Hamelers, "Rhizosphere anode model explains high oxygen levels during operation of a Glyceria maxima PMFC," *Bioresource Technology*, vol. 108, pp. 60-67, 2012, doi: https://doi.org/10.1016/j.biortech.2011.10.088.

[82] P. Aelterman, K. Rabaey, H. T. Pham, N. Boon, and W. Verstraete, "Continuous Electricity Generation at High Voltages and Currents Using Stacked Microbial Fuel Cells," *Environmental Science & Technology*, vol. 40, no. 10, pp. 3388-3394, 2006, doi: 10.1021/es0525511.

[83] S. Liu, H. Song, X. Li, and F. Yang, "Power Generation Enhancement by Utilizing Plant Photosynthate in Microbial Fuel Cell Coupled Constructed Wetland System," *International Journal of Photoenergy*, vol. 2013, p. 10, 2013, Art no. 172010, doi: 10.1155/2013/172010.

[84] S. E. Oh and B. E. Logan, "Voltage reversal during microbial fuel cell stack operation," *Journal of Power Sources*, vol. 167, no. 1, pp. 11-17, 2007, doi: https://doi.org/10.1016/j.jpowsour.2007.02.016.

[85] C. Santoro, C. Arbizzani, B. Erable, and I. Ieropoulos, "Microbial fuel cells: From fundamentals to applications. A review," *Journal of Power Sources*, vol. 356, pp. 225-244, 2017, doi: https://doi.org/10.1016/j.jpowsour.2017.03.109.

[86] S. Dentel, B. Strogen, and P. Chiu, *Direct generation of electricity from sludges and other liquid wastes*. 2004, pp. 161-8.

[87] G. Zhu, Y. Yi, B. Xie, and H. Liu, "The Development of Electrode Materials for Microbial Fuel Cells " *School of Biological Science and Medical Engineering*, pp. 1085-1089, 2016, doi: 10.12783/dtetr/iceta2016/7137.

[88] M. Zhou, M. Chi, J. Luo, H. He, and T. Jin, "An overview of electrode materials in microbial fuel cells," *Journal of Power Sources*, vol. 196, no. 10, pp. 4427-4435, 2011, doi: https://doi.org/10.1016/j.jpowsour.2011.01.012.

[89] Y. Hindatu, M. S. M. Annuar, and A. M. Gumel, "Mini-review: Anode modification for improved performance of microbial fuel cell," *Renewable and Sustainable Energy Reviews*, vol. 73, pp. 236-248, 2017, doi: https://doi.org/10.1016/j.rser.2017.01.138.

[90] S. K. Chaudhuri and D. R. Lovley, "Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells," *Nature Biotechnology*, vol. 21, p. 1229, 2003, doi: 10.1038/nbt867.

[91] Y. Ahn and B. E. Logan, "Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures," *Bioresource Technology*, vol. 101, no. 2, pp. 469-475, 2010, doi: https://doi.org/10.1016/j.biortech.2009.07.039.

[92] B. Liu, I. WIlliams, Y. Li, L. Wang, A. Bagtzlou, J. McCutcheon and B. Li, "Towards high power output of scaled-up benthic microbial fuel cells (BMFCs) using multiple electron collectors," *Biosensors and Bioelectronics*, vol. 79, pp. 435-441, 2016/05/15/ 2016, doi: https://doi.org/10.1016/j.bios.2015.12.077.

[93] S. W. Hong, I. S. Chang, Y. S. Choi, and T. H. Chung, "Experimental evaluation of influential factors for electricity harvesting from sediment using microbial fuel cell,"

*Bioresource Technology*, vol. 100, no. 12, pp. 3029-3035, 2009, doi: https://doi.org/10.1016/j.biortech.2009.01.030.

[94] S. Cheng, H. Liu, and B. Logan, *Power Densities Using Different Cathode Catalysts (Pt and CoTMPP) and Polymer Binders (Nafion and PTFE) in Single Chamber Microbial Fuel Cells*. 2006, pp. 364-9.

[95] B. E. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W, Verstratete and K. Rabaey, "Microbial Fuel Cells: Methodology and Technology," *Environmental Science & Technology*, vol. 40, no. 17, pp. 5181-5192, 2006, doi: 10.1021/es0605016.

[96] R. A. Timmers, D. P. B. T. B. Strik, H. V. M. Hamelers, and C. J. N. Buisman, "Characterization of the internal resistance of a plant microbial fuel cell," *Electrochimica Acta*, vol. 72, pp. 165-171, 2012, doi: https://doi.org/10.1016/j.electacta.2012.04.023.

[97] L. P. Fan and J. J. Li, "Overviews on internal resistance and its detection of microbial fuel cells," *International Journal of CIrcuits, Systems and Signal Processing*, vol. 10, pp. 316-320, 2016.

[98] C. Archer, "Cyperus Prolifer Lam.." [Online]. Available: http://pza.sanbi.org/cyperus-prolifer

[99] A. Notten, "Wachendorfia thyrsiflora." [Online]. Available: http://pza.sanbi.org/wachendorfia-thyrsiflora

[100] P. W. Seeds, "*Wachendorfia thyrsiflora*," *W. thyrsiflora*, n.d.

[101] 123RF, "Stock Photo - Phragmites australis, Common reed Typical habitat of Little Bittern," 17097954, Ed., ed.

[102] J. K. Jang, J. Choi, Y. Sun Ryou, S. Hyoun Lee, and E. Lee, *Effect of Ammonium and Nitrate on Current Generation Using Dual-Cathode Microbial Fuel Cells*. 2012, pp. 270-3.

[103] G. Ekama, D. Ikumi, N. Thela, and H. Mafungwa, *Standard Lab Operating Procedure: Water Quality Lab*, South Africa: University of Cape Town, n.d.

[104] M. C. Wentzel, Ekama, Goerge A., "Principles in the design of single-sludge activated-sludge systems for biological removal of carbon, nitrogen and phosphorus," *Water Environental Research*, vol. 69, no. 7, pp. 1222-1231, 1980.

[105] V. J. Watson and B. E. Logan, "Power production in MFCs inoculated with Shewanella oneidensis MR-1 or mixed cultures," (in eng), *Biotechnol Bioeng*, vol. 105, no. 3, pp. 489-98, Feb 15 2010, doi: 10.1002/bit.22556.

[106] A. González del Campo, P. Cañizares, J. Lobato, M. Rodrigo, and F. J. Fernandez Morales, "Effects of External Resistance on Microbial Fuel Cell's Performance," in *Environment, Energy and Climate Change II: Energies from New Resources and the Climate Change*, Eds. Cham: Springer International Publishing, 2016, pp. 175-197.

[107] W. Borken, E. A. Davidson, K. Savage, E. T. Sundquist, and P. Steudler, "Effect of summer throughfall exclusion, summer drought, and winter snow cover on methane fluxes in a temperate forest soil," *Soil Biology and Biochemistry*, vol. 38, no. 6, pp. 1388-1395, 2006, doi: https://doi.org/10.1016/j.soilbio.2005.10.011.

[108] G. Ekama, *A mass balanced steady state model for anaerobic digestion*: Water Research Group, University of Cape Town, 2018.

[109] Y. Zhou, D. Xu, E. Xiao, D. Xu, P. Xu, X. Zhang, Q. Zhou, F. He and Z. Wu, "Relationship between electrogenic performance and physiological change of four wetland plants in constructed wetland-microbial fuel cells during non-growing seasons," *Journal of Environmental Sciences*, vol. 70, pp. 54-62, 2018, doi: https://doi.org/10.1016/j.jes.2017.11.008.

[110] G. Ekama, *Organic material removal by activated sludge Part 1*, South Africa: The Water Research Group, 2017, p. 131. [Online]. Available: https://vula.uct.ac.za/access/content/group/fff7fa84-bd57-41b2-afe5-236ad82bbe3f/4042FPowerPointNotes/3CIV4042F-OrganicRemovalPart1_.pdf. Accessed on: 5th March 2019.

[111] S. Masley, "What is the best organic NPK fertilizer?", 2013, [Online]. Available: https://www.grow-it-organically.com/npk-fertilizer.html

[112] S. Hosier and L. Bradley, "Guide to Symptoms of Plant Nutrition Deficiencies," *The University of Arizona Cooperative Extention*.

[113] G. Ekama, "Optimizing water and resource recovery facilities (WRRF) for energy generation without compromising effluent quality," presented at the The IWA 2017 Conference on Sustainable Wastewater Treatment and Resource Recovery: Research, Planning, Design and Operation, France, 2017.

[114] S. Nielsen, "Sludge treatment and drying reed bed systems," *Ecohydrology & Hydrobiology*, vol. 7, pp. 223 - 234, 2007.

[115] A. K. Yadav, P. Dash, A. Mohanty, R. Abbassi, and B. K. Mishra, "Performance assessment of innovative constructed wetland-microbial fuel cell for electricity production and dye removal," *Ecological Engineering*, vol. 47, pp. 126-131, 2012, doi: https://doi.org/10.1016/j.ecoleng.2012.06.029.

[116] A. Oodally, M. Gulamhussein, and D. G. Randall, "Investigating the performance of constructed wetland microbial fuel cells using three indigenous South African wetland plants," Bachelors in Science, Civil Engineering, University of Cape Town, South Africa, 2018.

[117] K. Gibbs, "Series and Parallel components", n.d. [Online]. Accessed: 14th November 2018, Available: http://www.schoolphysics.co.uk/age14-16/Electricity%20and%20magnetism/Current%20electricity/text/Series_and_parallel_components/index.html

[118] K. Wetser, K. Dieleman, C. Buisman, and D. Strik, "Electricity from wetlands: Tubular plant microbial fuels with silicone gas-diffusion biocathodes," *Applied Energy*, vol. 185, pp. 642-649, 2017, doi: https://doi.org/10.1016/j.apenergy.2016.10.122.

[119] T. Sajana, M. Ghangrekar, and A. Mitra, "Effect of pH and distance between electrodes on the performance of a sediment microbial fuel cell," *Water Science and Technology*, vol. 68, no. 3, pp. 537-543, 2013, doi: 10.2166/wst.2013.271.

[120] Z. A. Stoll, Z. Ma, C. B. Trivedi, J. R. Spear, and P. Xu, "Sacrificing power for more cost-effective treatment: A techno-economic approach for engineering microbial fuel cells," *Chemosphere*, vol. 161, pp. 10-18, 2016, doi: https://doi.org/10.1016/j.chemosphere.2016.06.072.

[121] R. A. Rozendal, H. V. M. Hamelers, and C. J. N. Buisman, "Effects of membrane cation transport on pH and microbial fuel cell performance," *Environmental science & technology*, vol. 40, no. 17, p. 5206, 2006, doi: 10.1021/es060387r.

[122] G. C. Gil, I. S. Chang, B. H. Kim, M. Kim, J. K. Jang, H. S. Park and H. J. Kim "Operational parameters affecting the performannce of a mediator-less microbial fuel cell," *Biosensors and Bioelectronics*, vol. 18, no. 4, pp. 327-334, 2003, doi: https://doi.org/10.1016/S0956-5663(02)00110-0.

[123] H. Liu, R. Ramnarayanan, and B. E. Logan, "Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell," *Environmental Science & Technology*, vol. 38, no. 7, pp. 2281-2285, 2004, doi: 10.1021/es034923g.

[124] P. Zhang, J. Liu, Y. Qu, J. Zhang, Y. Zhong, and Y. Feng, "Enhanced performance of microbial fuel cell with a bacteria/multi-walled carbon nanotube hybrid biofilm,"

*Journal of Power Sources*, vol. 361, pp. 318-325, 2017, doi: https://doi.org/10.1016/j.jpowsour.2017.06.069.

[125] L. Zhang, G. Fu, and Z. Zhang, "Electricity generation and microbial community in long-running microbial fuel cell for high-salinity mustard tuber wastewater treatment," *Bioelectrochemistry*, vol. 126, pp. 20-28, 2019, doi: https://doi.org/10.1016/j.bioelechem.2018.11.002.

[126] R. Saravanan, A. Arun, S. Venkatamohan, Jegadeesan, T. Kandavelu, and Veeramanikandan, "Membraneless dairy wastewater-sediment interface for bioelectricity generation employing sediment microbial fuel cell (SMFC)," *African Journal Of Microbiology Research*, vol. 4, no. 24, pp. 2640-2646, 2010.

[127] Z. Ge, J. Li, L. Xiao, Y. Tong, and Z. He, "Recovery of Electrical Energy in Microbial Fuel Cells," *Environmental Science & Technology Letters*, vol. 1, no. 2, pp. 137-141, 2014, doi: 10.1021/ez4000324.

[128] J. Stewart, *Calculus : concepts and contexts*, 4th ed., Metric international ed. ed. Belmont, CA: Brooks/Cole, Cengage Learning, 2010.

[129] G. Ekama. CIV5050Z - Ingtergrated plant wide design of wastewater treatment plants [Online] Available: https://vula.uct.ac.za/access/content/group/e244c138-3adb-4dee-ac5d-34c1efb8eac5/CIV5050Z2018DesignProjectBrief.pdf

[130] P. M. Maistry, A. M. Muasya, A. J. Valentine, and S. B. M. Chimphango, "Increasing nitrogen supply stimulates phosphorus acquisition mechanisms in the fynbos species *Aspalathus linearis"*, *Functional Plant Biology*, vol. 42, no. 1, pp. 52-62, 2014. [Online]. Available: https://doi.org/10.1071/FP14100.

# Appendix A: Polarisation test

The polarisation test was done to obtain the peak power density and internal resistance of each PMFC system during the course of the experiments. Since each system had three replicate set-ups, the results were averaged, and a standard deviation was calculated.

The polarisation test was done by connecting different external resistors (see Table 23) and measuring the voltage across the PMFC after three-minute intervals. This allowed the voltage reading to stabilise. The power in $mW/m^3$ and current in $mA/m^3$ were calculated using was calculate using Equation 3.

**Table 23:** Polarisation test results of PMFC system with *W. thyrsiflora* plant and WAS as substrate.

| Resistor (Ω) | Voltage (V) | Power ($mW/m^3$) | Current ($mA/m^3$) |
|---|---|---|---|
| 1000000 | 0.54 | 0.58 | 1.01 |
| 560000 | 0.57 | 1.11 | 1.88 |
| 100000 | 0.57 | 6.22 | 10.55 |
| 56000 | 0.56 | 10.82 | 18.61 |
| 33000 | 0.54 | 17.11 | 30.60 |
| 10000 | 0.52 | 52.04 | 97.20 |
| 4700 | 0.49 | 95.71 | 192.62 |
| 1000 | 0.37 | 255.81 | 687.76 |
| 560 | 0.30 | 290.83 | 982.20 |
| 220 | 0.16 | 228.71 | 1390.52 |
| 100 | 0.09 | 140.95 | 1618.74 |
| 50 | 0.05 | 75.85 | 1678.75 |

The slope of the voltage current graph gave internal resistance. The slope was found by using a trendline function on excel. The slope was corrected for the $m^3$ conversion (see Equation 4). Complete set of data for all experiments can be sourced from (https://drive.google.com/drive/folders/1ejWY5P7vwlwnifM74SE1dv1Ekfd-O8ku?usp=sharing).

$$\text{slope from excel} = -2.834\text{E} - 04$$

$$\text{R}_{\text{int}} = -2.834\text{E} - 04 \times -1 \times 1860 \times 1000 = 527\ \Omega$$

# Appendix B : Complete WAS voltage profiles

The voltage results from day 0 to day 35 that were not shown in **Figure 36** are presented here.

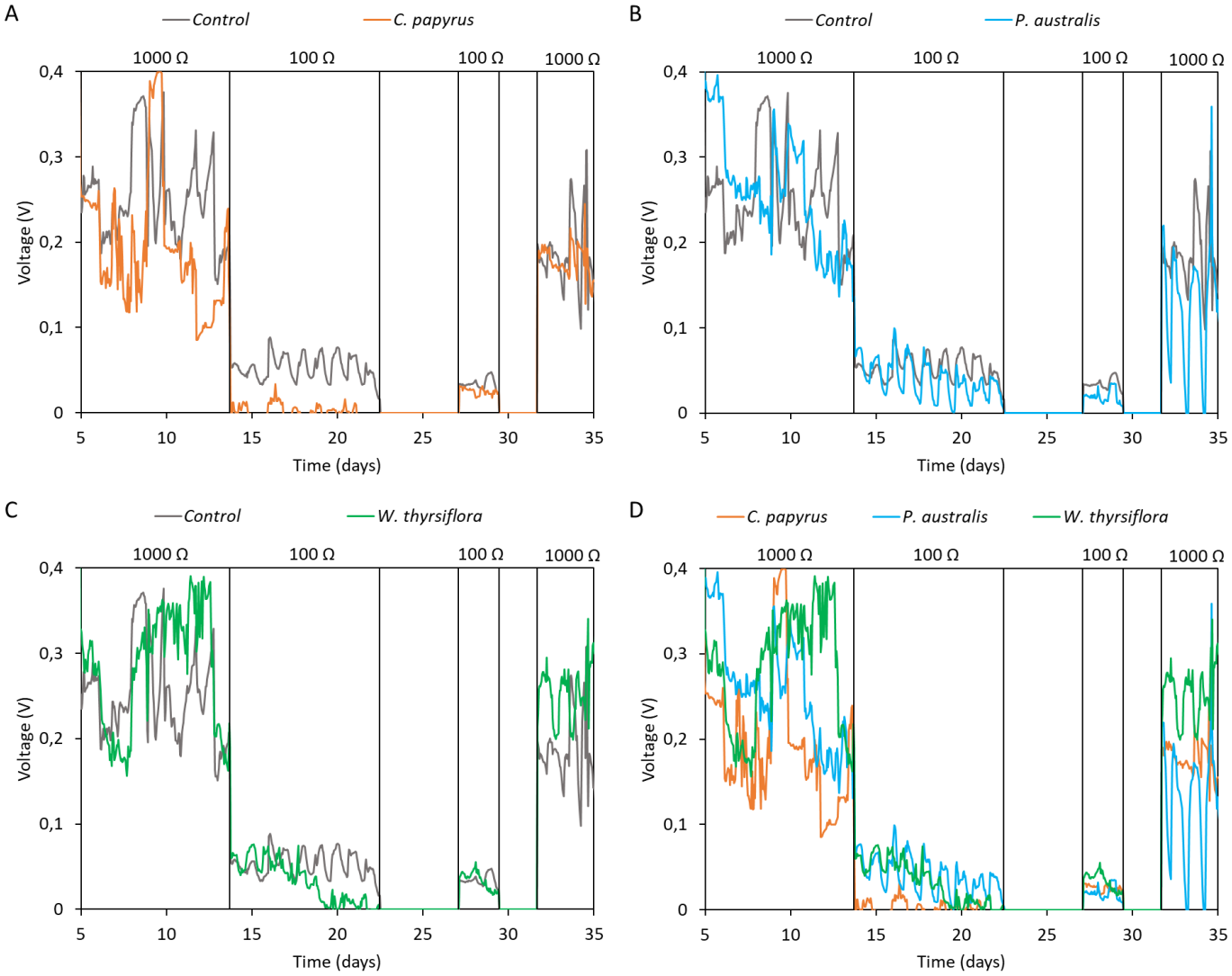



**Figure 68:** Voltage recorded across a 100 ohms resistor for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) in comparison to the control and a comparison of all plant species (D) investigated when using WAS. Vertical line indicates polarisation test day.

# Appendix C: Ammonia and OP profiles for all experiments

## C1: WAS experiment

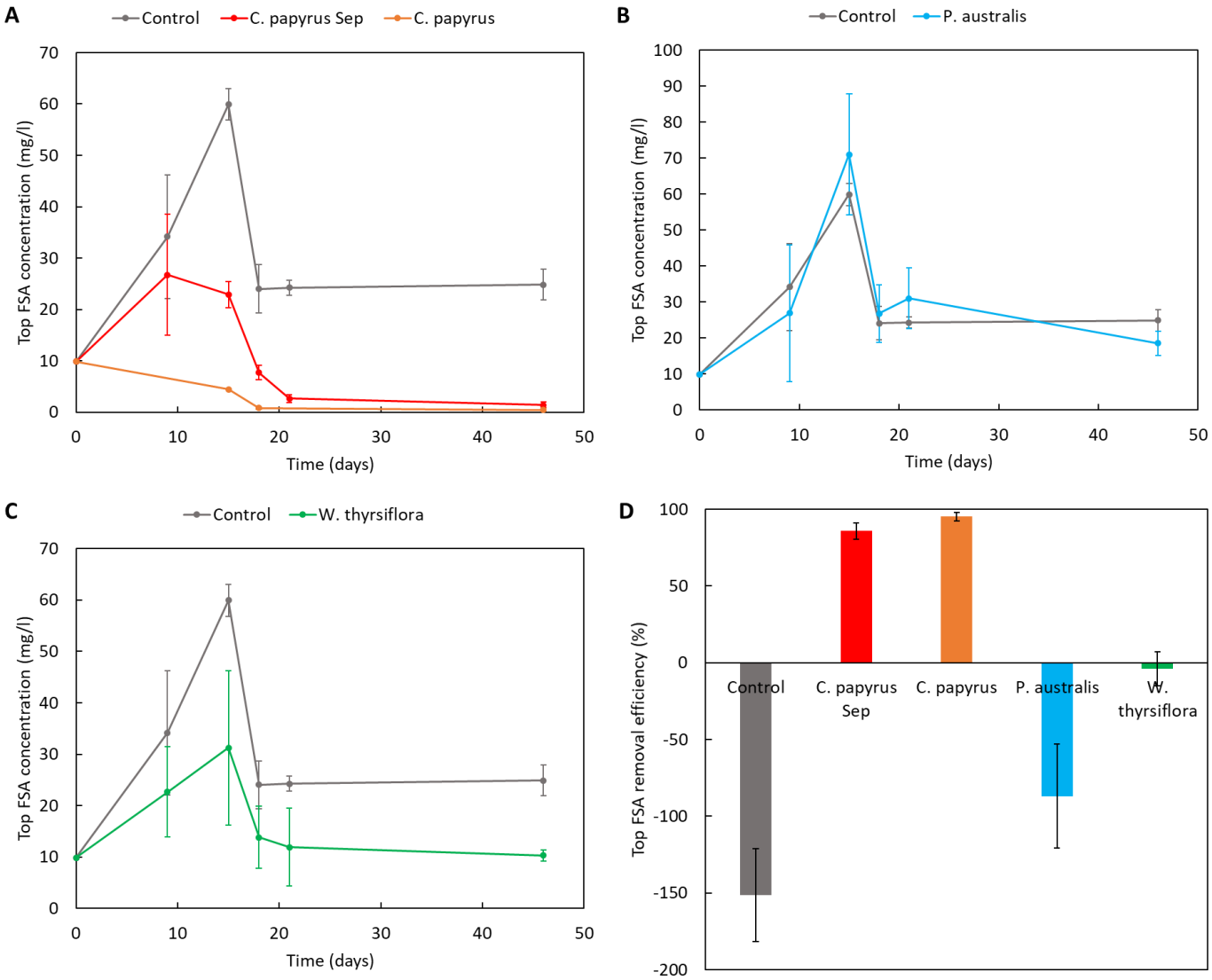



**Figure 69:** FSA release with time measured at the top sampling tube is shown for *C. papyrus* ES and *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using WAS.

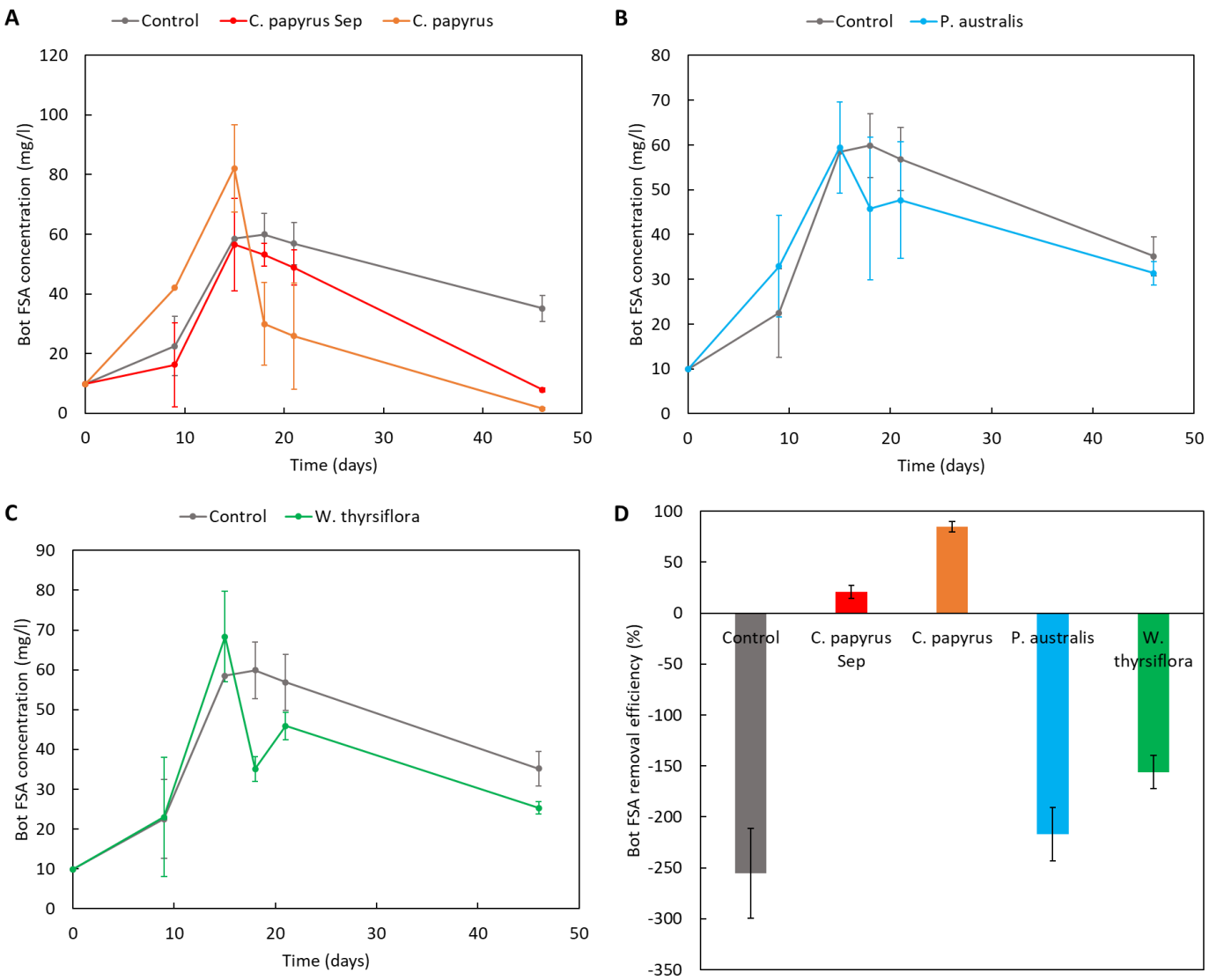



**Figure 70:** FSA release with time measured at the bottom sampling tube is shown for *C. papyrus* ES and *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using WAS.

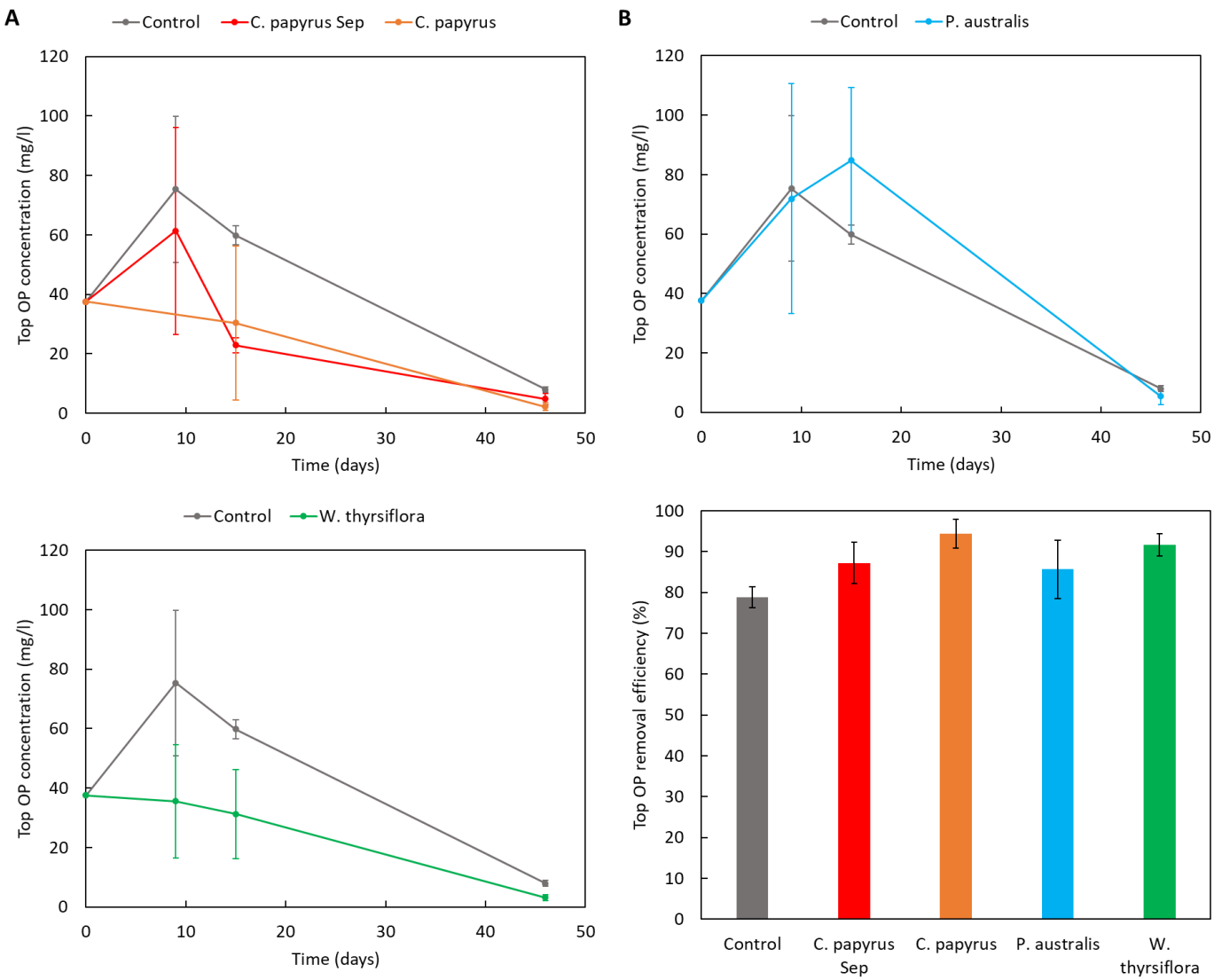



**Figure 71:** OP release with time measured at the top sampling tube is shown for *C. papyrus* ES and *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using WAS.

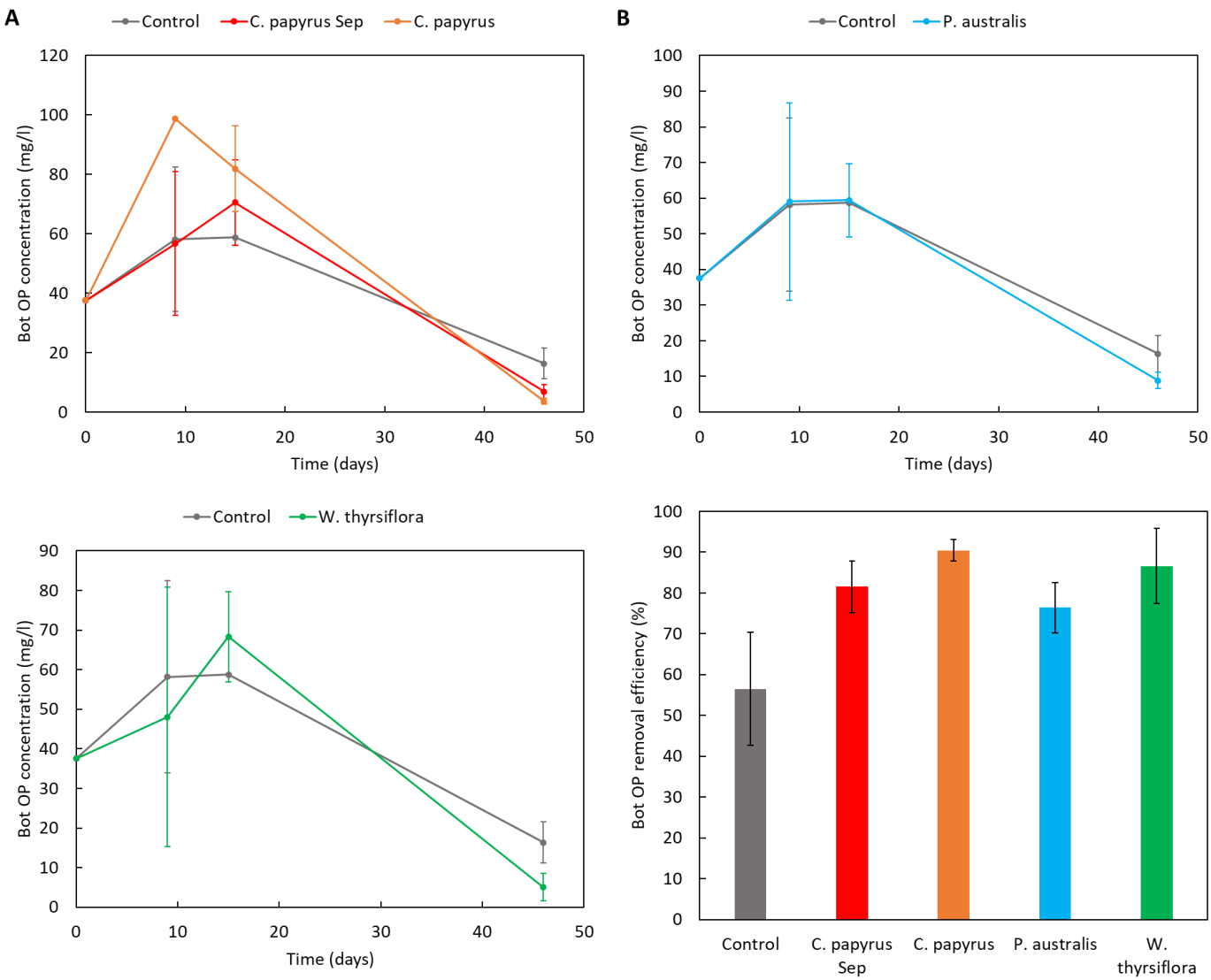



**Figure 72:** OP release with time measured at the bottom sampling tube is shown for *C. papyrus* ES and *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using WAS.

## C2: PS experiment

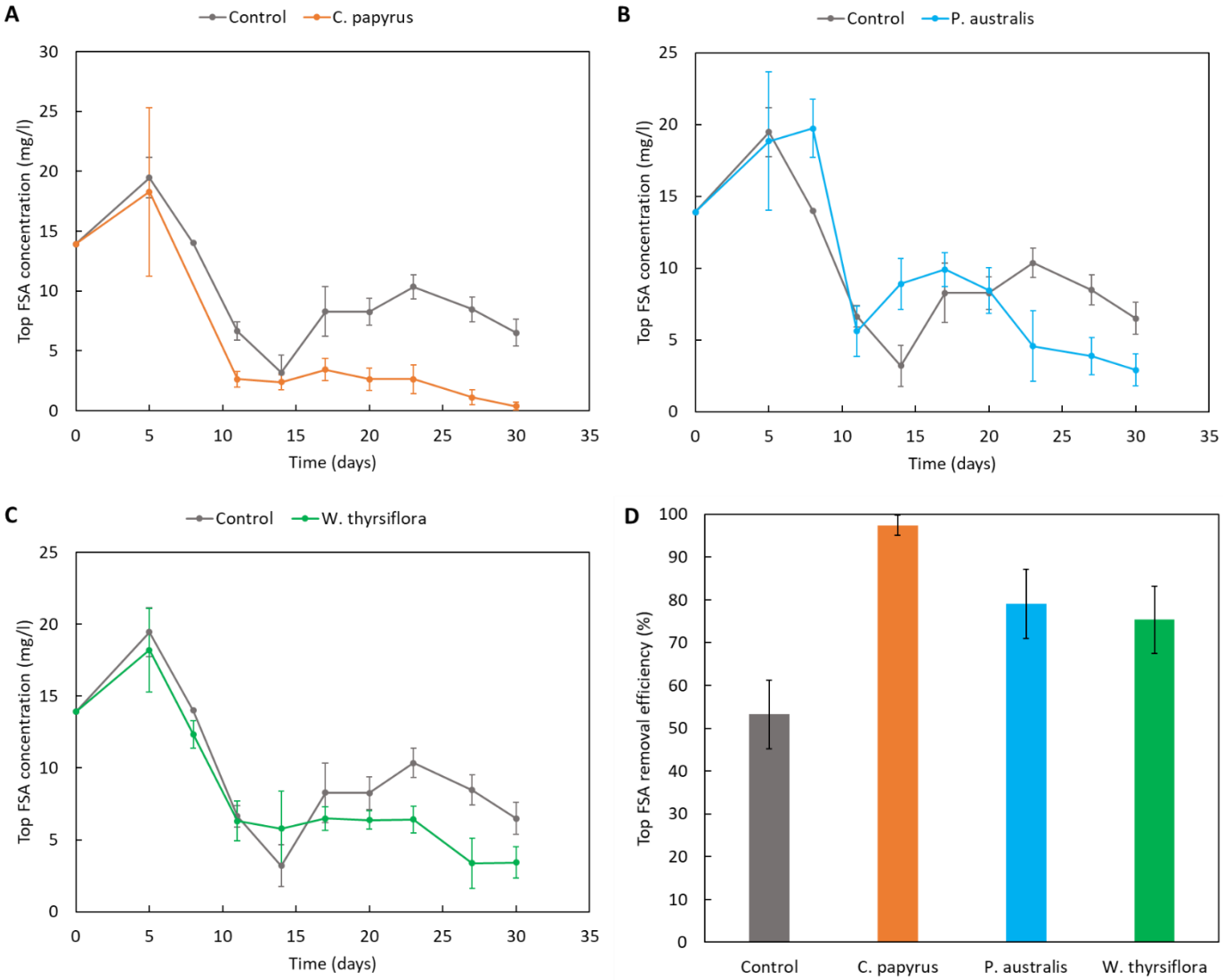



**Figure 73:** FSA release with time measured at the top sampling tube is shown for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using PS.

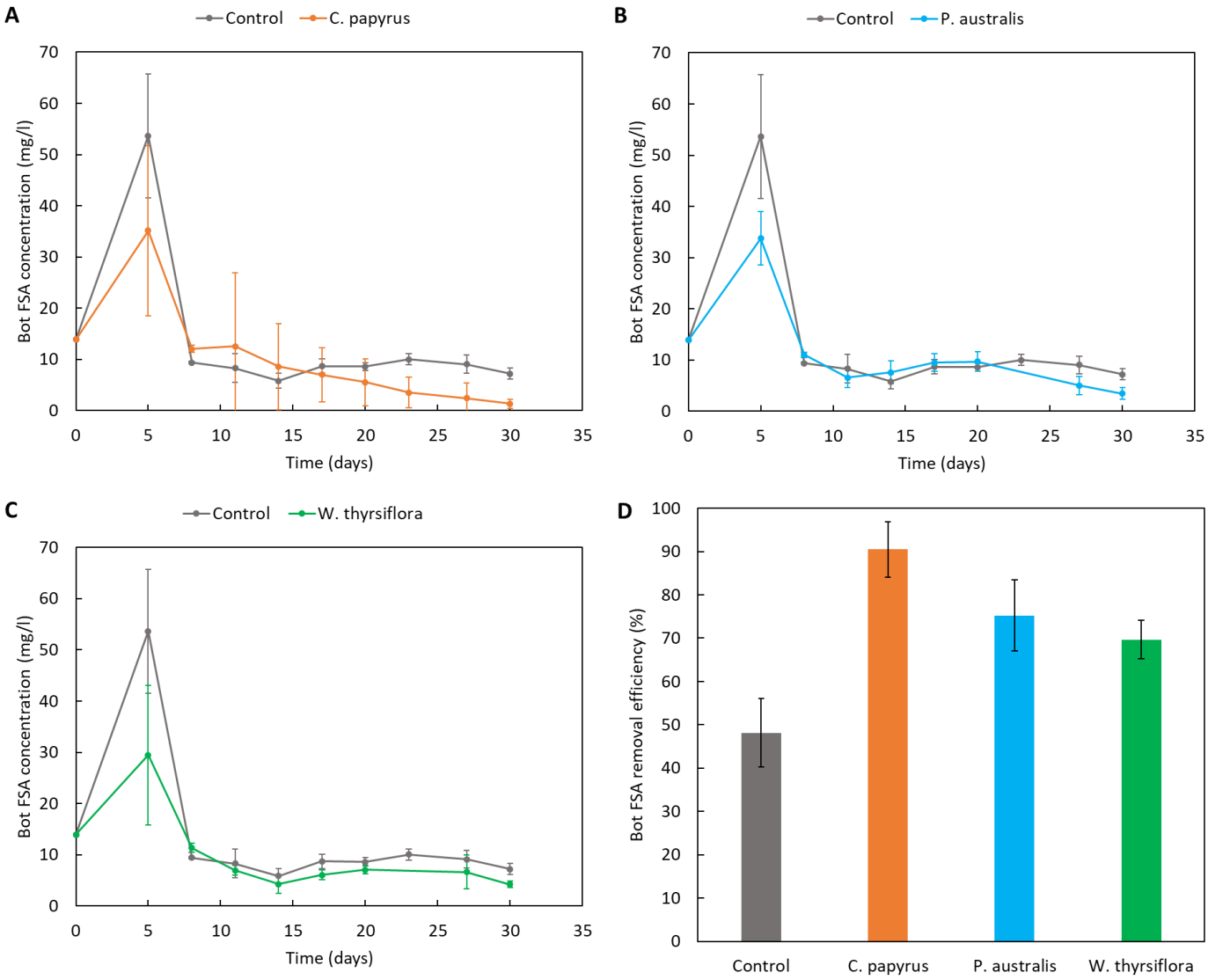



**Figure 74:** FSA release with time measured at the bottom sampling tube is shown for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using PS.

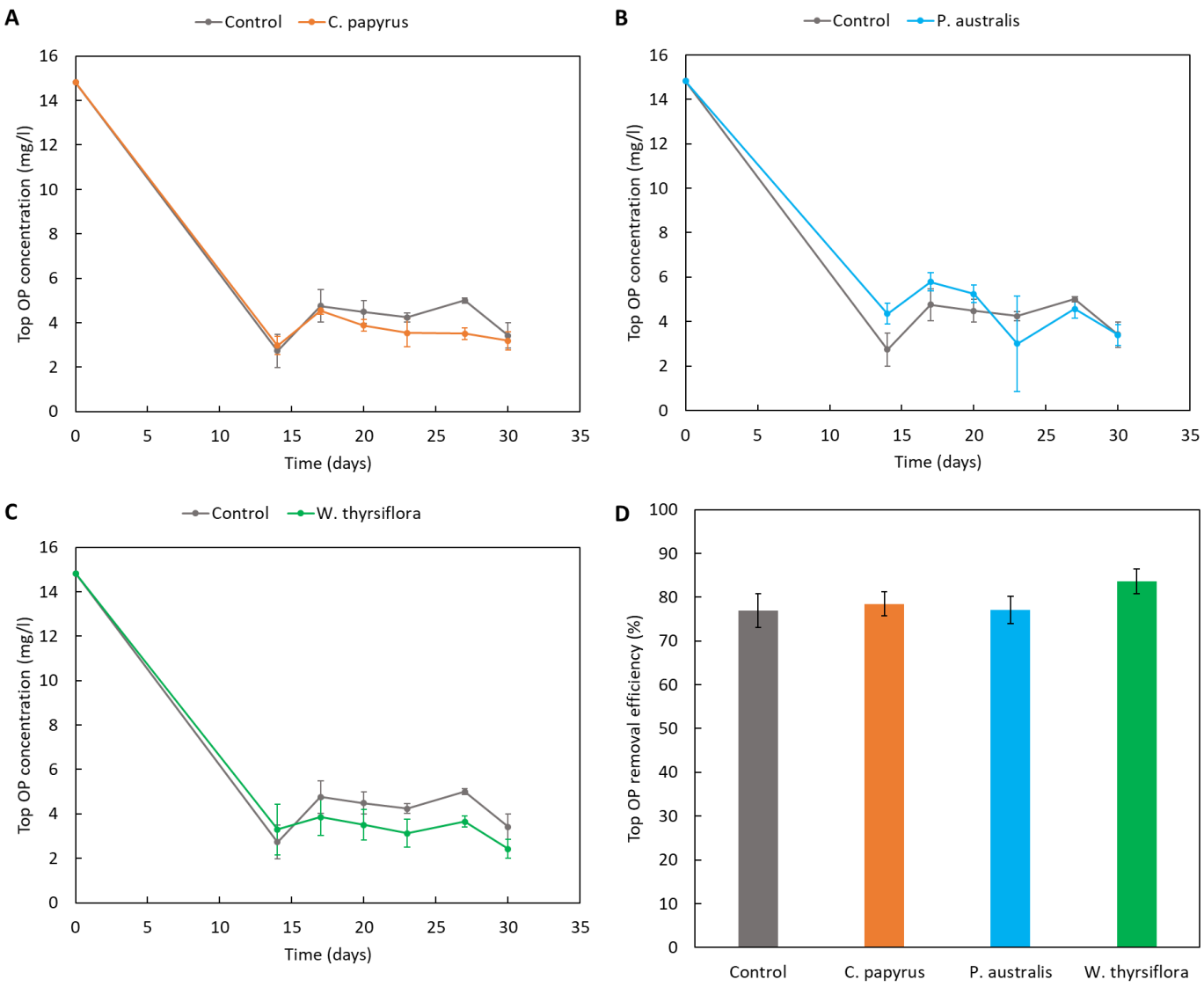



**Figure 75:** OP release with time measured at the top sampling tube is shown for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using PS.

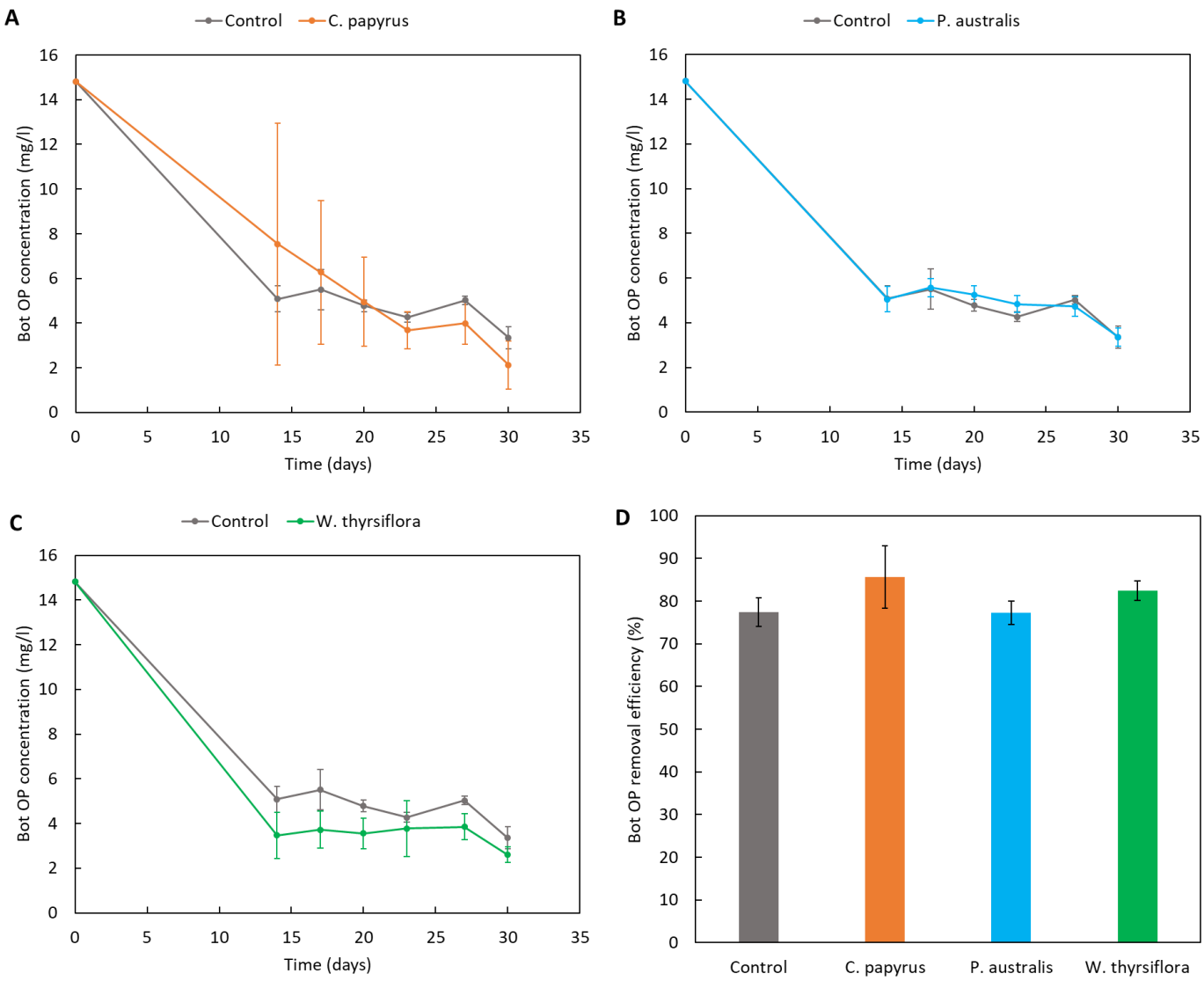



**Figure 76:** OP release with time measured at the bottom sampling tube is shown for *C. papyrus* (A), *P. australis* (B), *W. thyrsiflora* (C) and the overall removal efficiencies (D) when using PS.

## C3: Optimisation experiment 2

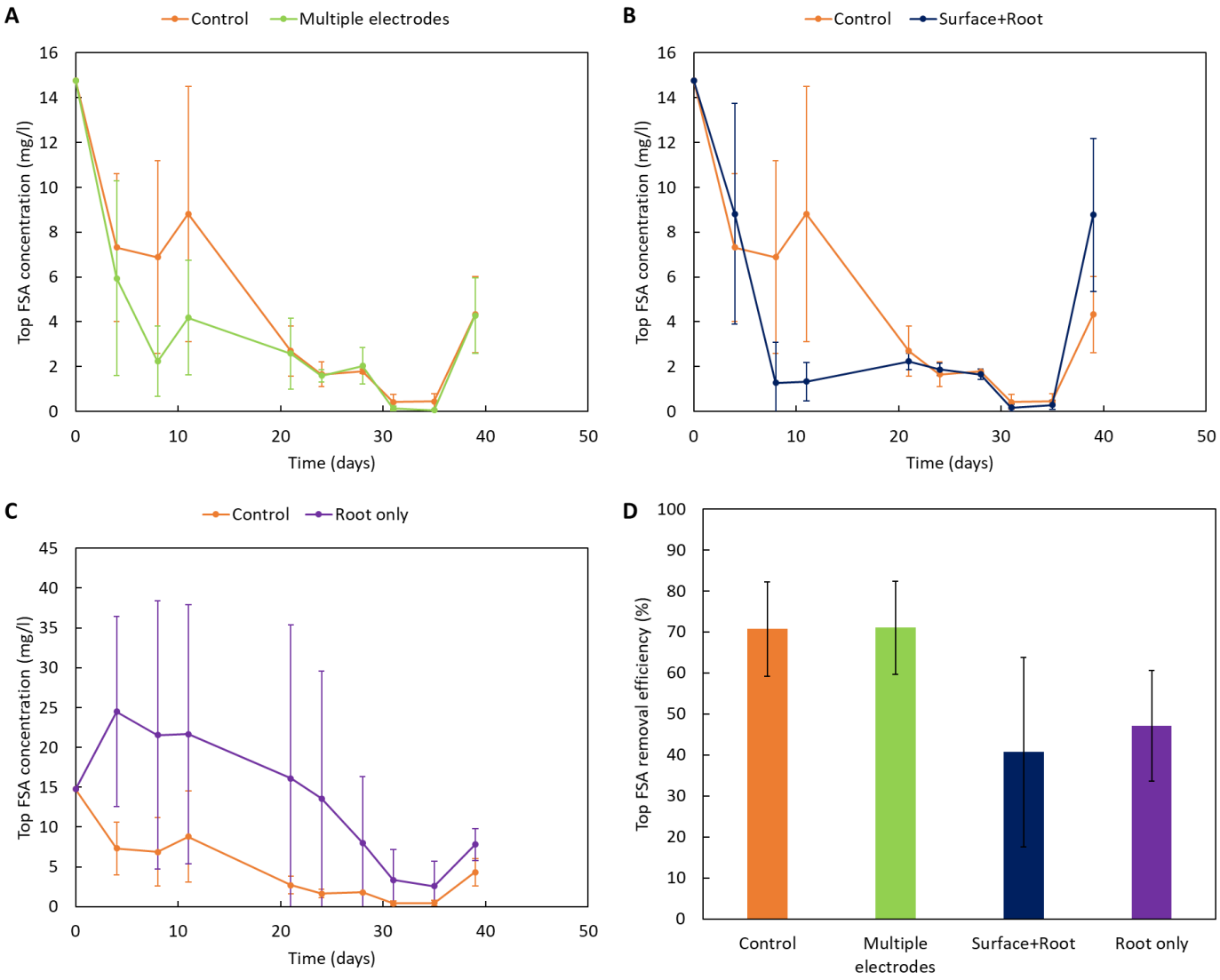



**Figure 77:** FSA release with time measured at the top sampling tube is shown when using multiple electrodes (A), a surface and root electrode (B), an electrode at roots (C) and the overall removal efficiencies (D) when using WAS.

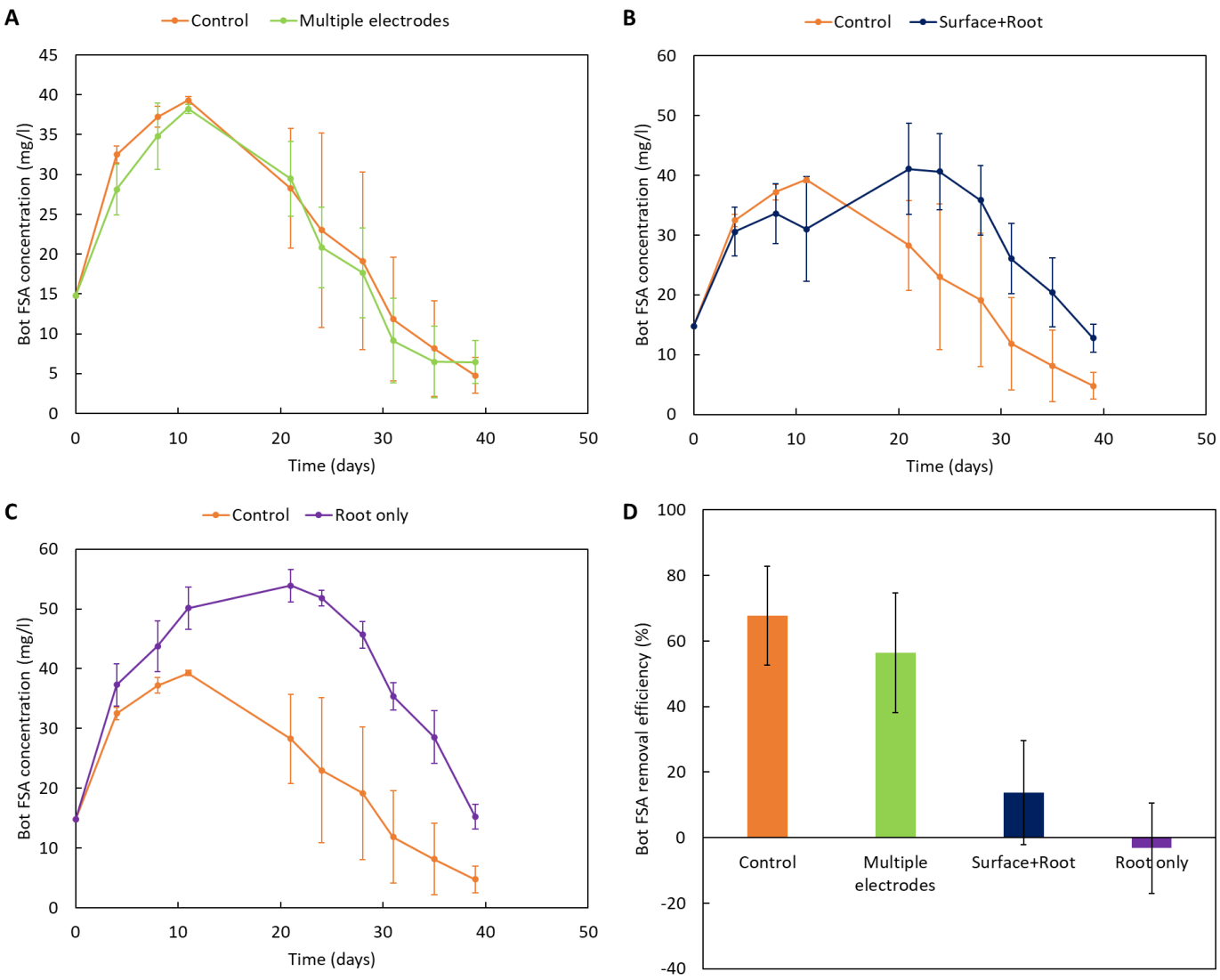



**Figure 78:** FSA release with time measured at the bottom sampling tube is shown when using multiple electrodes (A), a surface and root electrode (B), an electrode at roots (C) and the overall removal efficiencies (D) when using WAS.

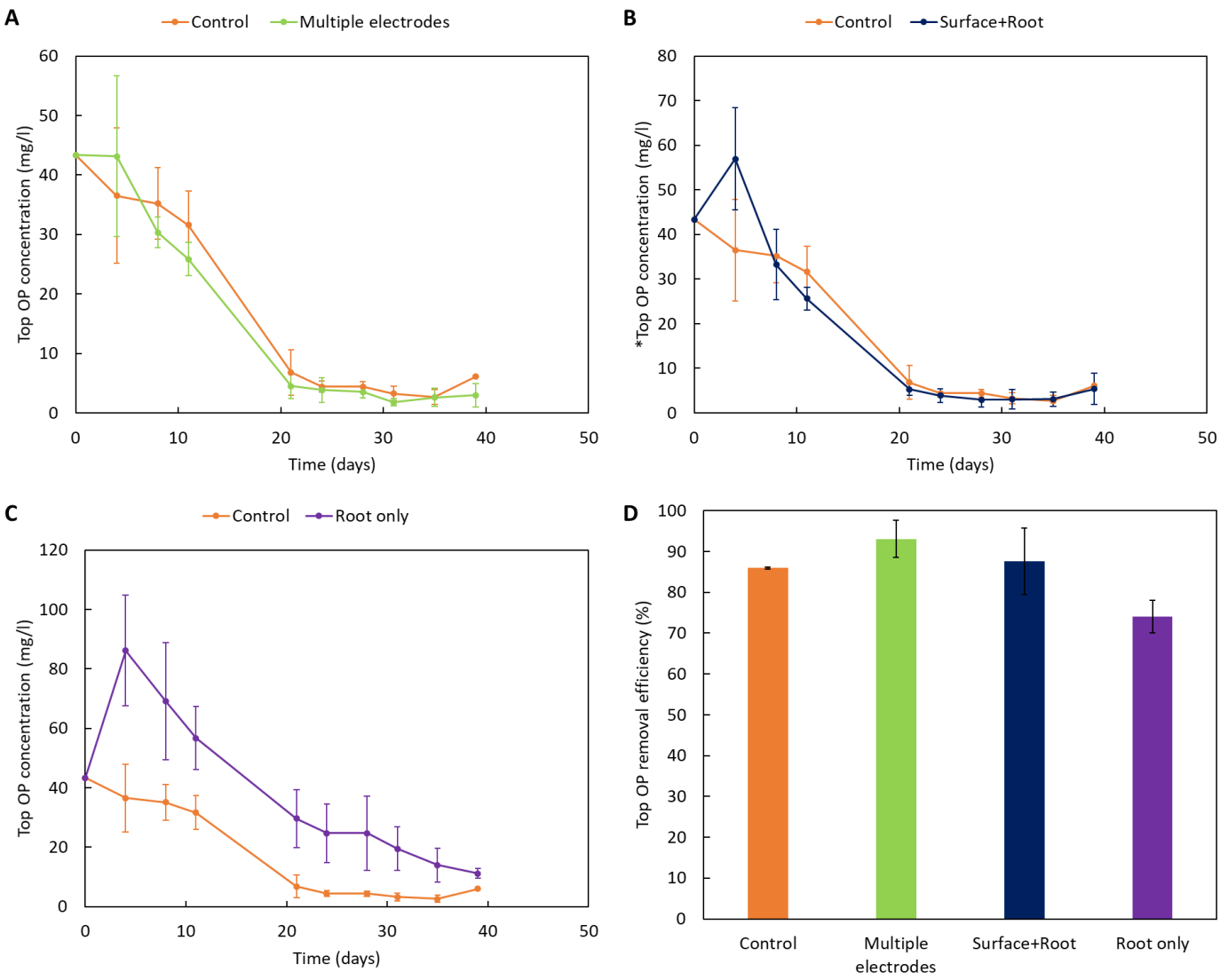



**Figure 79:** OP release with time measured at the top sampling tube is shown when using multiple electrodes (A), a surface and root electrode (B), an electrode at roots (C) and the overall removal efficiencies (D) when using WAS.

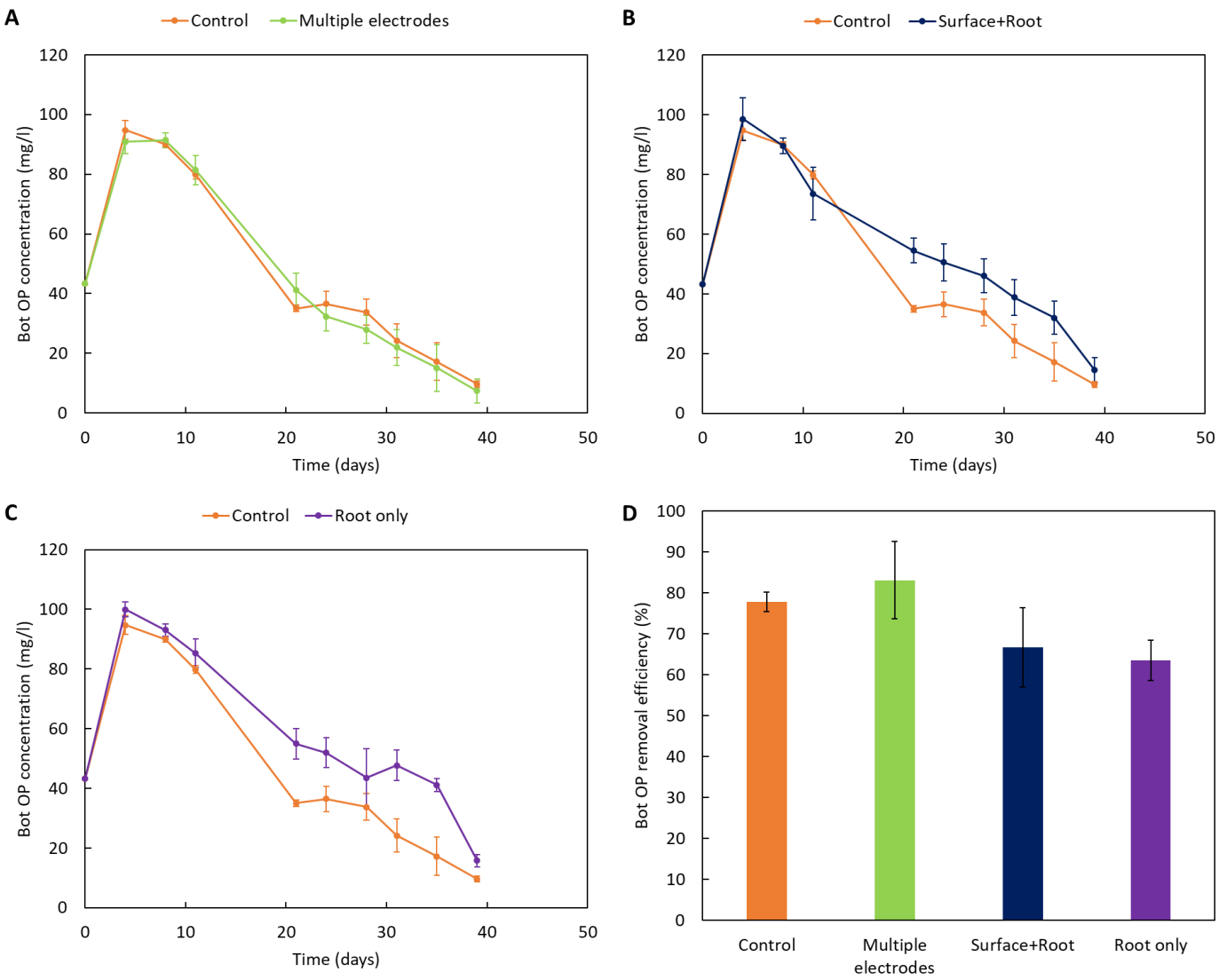



**Figure 80:** OP release with time measured at the bottom sampling tube is shown when using multiple electrodes (A), a surface and root electrode (B), an electrode at roots (C) and the overall removal efficiencies (D) when using WAS.

# Appendix D: Biodegradable content of WAS

The biodegradable fraction of WAS collected from Zandvliet WWTW for the second optimisation experiment was experimentally determined. A two-litre bottle was filled with one and a half litres of WAS and aerated. A magnetic stirrer was used to ensure to ensure aeration and sample collection homogeneity (see Figure 81). To counter the effects of evaporation, the level of WAS was kept at a constant 1 litre mark by addition of distilled water.

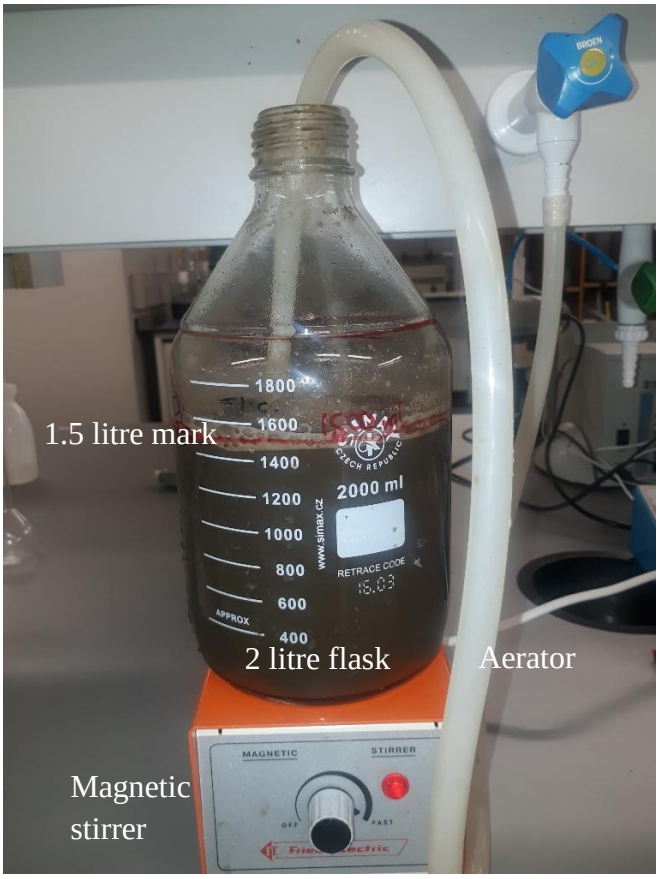



**Figure 81:** Experimental set-up to determine the biodegradable fraction of WAS.

The COD was measured at regular intervals to obtain a COD versus time profile (see Figure 82). The end point COD denoted by the constant COD values with time gave the unbiodegradable COD of the WAS. The biodegradable contact was a difference between the initial and final COD and consequently, the biodegradable fraction was the biodegradable COD/initial COD. The final COD i.e. the unbiodegradable COD measured was 6480 mgCOD/l. Therefore, the biodegradable COD was 5040 mgCOD/l and the biodegradable fraction was 0.562.

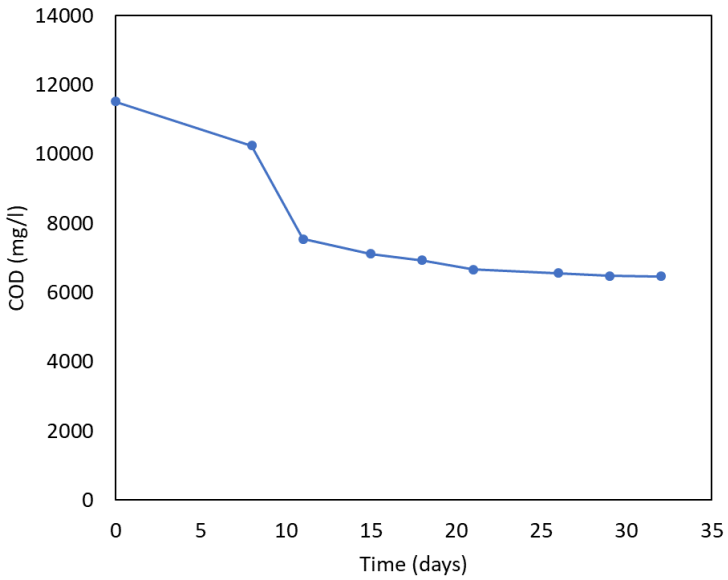
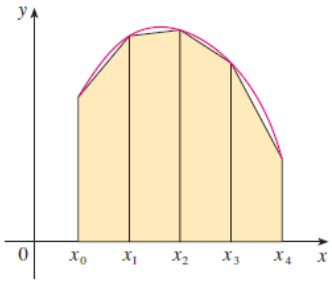



**Figure 82:** COD measured with time when aerating WAS.

# Appendix E : Power output per COD utilised

The power output per COD utilised was calculated using the Trapezoidal Rule. Since the voltage across an external resistance was measured (power) at regular intervals providing a power (y-axis) versus time (x-axis) profile, the Trapezoidal Rule was used to sum the total power produced over the duration of the experiment (see Figure 83).




**Trapezoidal Rule**

$$\int_a^b f(x)\,dx \approx T_n = \frac{\Delta x}{2}[f(x_0) + 2f(x_1) + 2f(x_2) + \cdots + 2f(x_{n-1}) + f(x_n)]$$

where $\Delta x = (b - a)/n$ and $x_i = a + i\,\Delta x$.

**Figure 83:** Trapezoidal Rule schematic and equation [128].

The total power produced over the duration of the experiment was then divided by COD consumed over the course of the experiment (initial – final) giving power per gram of COD used. This allowed the time duration and initial COD differences between the experiments to be negated. In instances where the cell was left as an open circuit, the power produced was zero as open circuit conditions imply infinite resistance.

The Trapezoidal Rule was done on excel and can be found by following this google drive link (https://drive.google.com/drive/folders/1ejWY5P7vwlwnifM74SE1dv1Ekfd-O8ku?usp=sharing).

# Appendix F: PS AD versus its use in PMFC

The power in watt-hours (area under the graph when time is in hours and not days) for the PMFC was calculated using the Trapezoidal Rule as explained in Appendix E. This was divided by the COD consumed during the course of the experiment, 5.946 gCOD/l giving power per COD consumed output of 0.0016 Wh/gCOD.

When PS is consumed in an AD, the biodegradable COD is converted to methane ($CH_4$), biomass and unused biodegradable COD [108]. Most of the COD is converted to methane, but for the purpose of this calculation it was assumed that only 50% of the COD is converted to methane.

To get the power per COD consumed in Wh/gCOD, the following method was used [108, 129]:

- $CH_4 + 2H_2O \rightarrow CO_2 + 8(H^+ + e^-)$
- 1 mol of $e^-$ is equivalent to 8 gCOD
- 8 $e^-$ = 64 gCOD
- 1 gCOD = 1/64 mol of $CH_4$
- Converting to volume (24.1 l/mol at 20°C and 760 mmHg pressure) gives 24.1/64 l $CH_4$/gCOD
- 1 $m^3$ $CH_4$ = 10 kWh assuming 100% efficiency
- 1 $m^3$ $CH_4$ = 2.25 kWh accounting for 50% efficiency of biodegradable COD to methane and another 45% of converting methane to power
- 1 l $CH_4$ = 2.25 Wh
- 24.1/64 l $CH_4$ = 0.847 Wh

Therefore, every gram of COD consumed in an AD equals 0.847 Wh of power. This is significantly greater than 0.0016 Wh/gCOD the PMFC produces.

# Appendix G: Exudate test procedure

The exudates were collected based by combining methodologies from two research papers, namely, Tapia, et al. [29] and Maistry, et al. [130]. The procedure was as follows:

1) Wash plant roots thoroughly with tap water after which wash them with distilled water.
2) Dip roots in 30 mg/l chloramphenicol solution for two hours to kill bacteria that will feed on exudates if left alive.
3) Remove from solution and wash with distilled water thoroughly.
4) Take 15 ml vortex tubes and autoclave them to ensure they are sterile.
5) Fill the vortex tubes with 15 ml of 200 µmol/l of $CaCl_2$.
6) Excise the root and place inside the vortex tube and close it.
7) Cover the vortex tube with aluminium foil and leave for 48 hours.
8) After 48 hours, filter the solution. Test the exudate rich filtrate.
9) Dry the roots to get a dry mass.

In this research, the filtrate was tested for COD only, however, future studies should focus on using a HPLC to obtain each organic compounds of the exudates.

# Appendix H: Agricultural use of sludge guidelines

The following are snips taken from the agriculture use of sludge guidelines book [62]. The snips are of the sludge classification pages. For more information, the reader is urged to read through the complete guidelines

## PART 3:

## CLASSIFICATION OF SLUDGE INTENDED FOR AGRICULTURAL USE

All sludge producers currently using or intending to use sludge in agricultural practices must confirm the classification of the sludge, even if a preliminary classification was done as stipulated in Volume 1 of the Guidelines.

### CLASSIFICATION SYSTEM

The South African Wastewater Sludge Classification System must be applied to classify the sludge intended for agricultural use:

**TABLE 2: CLASSIFICATION SYSTEM FOR SLUDGE**

| | | | |
|---|---|---|---|
| Microbial class | A | B | C |
| Stability class | 1 | 2 | 3 |
| Pollution class | a | b | c |

The characterisation and classification should be repeated if any major sludge production or processing changes occur that could affect the classification. This could include:

- When major extensions are implemented at the wastewater treatment plant.
- When major operational changes are made at the wastewater treatment plant.
- When the raw influent quality to the wastewater treatment plant changes in such a way that the sludge quality could be affected. In other words, when any major new wastewater contributor starts/ceases to discharge to the plant.

The sampling procedure (number of samples, sampling frequency and sample location) for the classification of sludge is the same as for the monitoring of the sludge. This is discussed in part 5, Section 6 "Restriction/Requirement: Monitoring Programme". The laboratory analyses and methods required for sludge classification are detailed in Appendix 2.

### Microbiological classification

The results of the microbiological analyses of the sludge samples can be used to determine the Microbiological class (Table 3).

**TABLE 3: COMPLIANCE AND CLASSIFICATION CRITERIA: MICROBIOLOGICAL CLASS**

| Microbiological class | Unrestricted use quality | | General use quality | | Limited use quality |
|---|---|---|---|---|---|
| | A | | B | | C |
| | Target value | Maximum permissible value | Target value | Maximum permissible value | |
| Faecal coliform ($CFU/g_{dry}$) | < 1 000 (5 log reduction) | 10 000 (4 log reduction) | $< 1x10^6$ (2 log reduction) | $1x10^7$ (1 log reduction) | $> 1x10^7$ (no reduction) |
| Helminth ova (Viable $ova/g_{dry}$) | < 0.25 (or one ova/4g) | 1 | < 1 | 4 | > 4 |
| **Compliance requirements** | | | | | |
| Requirements for classification purposes (Minimum 3 samples) | All the samples submitted for classification purposes must comply with these requirements | Not applicable | Two of the three samples submitted for classification purposes must comply with these requirements | The sample that failed may not exceed the Minimum Permissible Value | Not applicable |
| Requirements for monitoring purposes | 90% compliance | The 10% (maximum) of samples that exceed the Target Value, may not exceed the Maximum Permissible Value | 90% compliance | The 10% (maximum) of samples that exceed the Target Value, may not exceed the Maximum Permissible Value | Not applicable |

**Note:** Table 3 requires a 90% compliance for the monitoring programme. Some plants such as those producing < 1 tdry sludge/day are required to collect only three samples once a year. These plants will therefore only be able to prove 90% compliance after a few years. Larger plants will be able to prove compliance on an annual basis.

**Note to plants that produce Microbiological class A sludge:** The product produced by these facilities **could** be distributed to the public without any restrictions. If this is the case, each batch (or other reasonable sampling interval) leaving the plant must be sampled immediately after the process and the Microbiological class confirmed. Products that do not meet the specification will need to be re-processed.

## Stability classification

The Stability class (the potential to generate odours and attract vectors) was specifically introduced in the Sludge Guidelines as this is typically the issues that influence public perception.

The Stability class can be determined analytically and/or by complying with a vector attraction reduction requirement. A sludge producer is required to prove compliance to at least one of the vector attraction reduction options at any stage during operation. The different vector attraction reduction options are listed in Table 4 and described in detail in Appendix 3.

The achievement of a Stability class is especially important during the operational stages of a wastewater treatment plant. It is more important to consistently comply with a vector attraction reduction option, than the actual initial Stability classification.

Confirm the Stability class of the sludge by selecting at least one of the vector attraction reduction options in Table 4. Note that only Stability classes 1 and 2 are suitable for agricultural use of sludge.

**TABLE 4: DETERMINING THE STABILITY CLASS**

| Stability class | 1 | 2 | 3 |
|---|---|---|---|
| | Plan/design to comply with one of the options listed below on a 90 percentile basis. | Plan/design to comply with one of the options listed below on a 75 percentile basis. | No stabilisation or vector attraction reduction options required. |
| **Vector attraction reduction options (Applicable to Stability class 1 and 2 only)** | | | |
| Option 1 | Reduce the mass of volatile solids by a minimum of 38 percent | | |
| Option 2 | Demonstrate vector attraction reduction with additional anaerobic digestion in a bench-scale unit | | |
| Option 3 | Demonstrate vector attraction reduction with additional aerobic digestion in a bench-scale unit | | |
| Option 4 | Meet a specific oxygen uptake rate for aerobically treated sludge | | |
| Option 5 | Use aerobic processes at a temperature greater than 40°C (average temperature 45°C) for 14 days or longer (eg during sludge composting) | | |
| Option 6 | Add alkaline material to raise the pH under specific conditions | | |
| Option 7 | Reduce moisture content of sludge that do not contain unstabilised solids (from treatment processes other than primary treatment) to at least 75 percent solids | | |
| Option 8 | Reduce moisture content of sludge with unstabilised solids to at least 90 percent solids | | |
| Option 9 | Inject sludge beneath the soil surface within a specified time, depending on the level of pathogen treatment | | |
| Option 10 | Incorporate sludge applied to or placed on the surface of the land within specified time periods after application to or placement on the surface of the land | | |

## Pollutant classification

The results of the sludge analyses can be employed to determine the Pollutant class (Table 5).

**TABLE 5: DETERMINING THE POLLUTANT CLASS**

| *Aqua regia* extractable metals (mg/kg) | Pollutant class | | |
|---|---|---|---|
| | a | b | c |
| Arsenic (As) | <40 | 40 - 75 | >75 |
| Cadmium (Cd) | <40 | 40 - 85 | >85 |
| Chromium (Cr) | <1 200 | 1 200 - 3 000 | >3 000 |
| Copper (Cu) | <1 500 | 1 500 - 4 300 | >4 300 |
| Lead (Pb) | <300 | 300 - 840 | >840 |
| Mercury (Hg) | <15 | 15 - 55 | >55 |
| Nickel (Ni) | <420 | 420 | >420 |
| Zinc (Zn) | <2 800 | 2 800 - 7 500 | >7 500 |
| Note : A 90% compliance is required to comply with the requirements of a pollutant class. The compliance will therefore only be evident once 10 sample results are available. | | | |

**Note:** Table 5 requires the analyses of eight (8) potentially toxic metals and elements. These were specifically chosen as they are typically the elements that might be of concern. However, the sludge produced at a specific wastewater treatment plant could be compromised by other elements due to unique circumstances. A full elemental analysis including a number of other trace metals and elements is required for the preliminary classification as detailed in Volume 1. The results of those analyses need to be consulted to determine if any other element is of concern. In cases where additional element(s) were identified, these also need to be included in the analyses for classification and monitoring purposes.

# Appendix I: Two sample t-test for peak power densities

The two sample t-test conducted for the peak power densities were done based on a 5% significance level. In cases where the 5% significance level was not significant enough, a 10% and up to a maximum of 20% was tested.

The test was conducted using the equation:

$$t = \frac{\overline{X}_A - \overline{X}_B}{\sqrt{\frac{{S_A}^2}{n_A} + \frac{{S_B}^2}{n_B}}} \qquad \text{(Equation 14)}$$

where:
$\overline{X}_A$ is mean of system A
$\overline{X}_B$ is mean of system B
$S_A$ is standard deviation of system A
$S_B$ is standard deviation of system B
$n_A$ is number of set-ups in system A
$n_B$ is number of set-ups in system B

Since the standard deviations and means were known, the t value was calculated and compared to the t value of 2 degrees of freedom (since there were three set-ups per system) and 5% significance, 2.92. If the calculated t-value was greater than 2.92, the test of one system's PPD being greater than another system's PPD was true. This means for example it is stated that the PPD of *W. thyrsiflora* > *C. papyrus*, and it is true to 5% significance, then there is a 95% chance that the PPD of *W. thyrsiflora* is greater than that of *C. papyrus* taking their standard deviations into account.

In instances where the calculated t-value was less than 2.92, there was no difference between the PPDs of two systems to 5% significance. In these cases, a 10% significance was tested thereafter a 20% significance. In instances where even a 20% significance (i.e. 80% chance of one system's PPD being greater than another system's PPD) was not sufficient, it was concluded that the two systems being compared had the same PPD.

**Table 24:** Two sample t-test for PPDs of all experiments conducted.

| Experiment | t-test | t value | Significance level |
|---|---|---|---|
| **Thickened WAS: PPD check** | C. papyrus < W. thyrsiflora | 7.163 | TRUE 5% |
| | Control < C. papyrus | 1.466 | TRUE 20% |
| | Control < W. thyrsiflora | 11.04 | TRUE 5% |
| **Liquid WAS: PPD check** | C. papyrus < W. thyrsiflora | 9.841 | TRUE 5% |
| | P. australis < W. thyrsiflora | 8.720 | TRUE 5% |
| | C. papyrus < P. australis | 0.271 | NOT TRUE TO 20% |
| | Control < C. papyrus | 6.893 | TRUE 5% |
| | C. papyrus < W. thyrsiflora | 17.29 | TRUE 5% |
| | Control < P. australis | 5.972 | TRUE 5% |
| **PS: Test 2 PPD check** | C. papyrus < W. thyrsiflora | 10.33 | TRUE 5% |
| | P. australis < W. thyrsiflora | - | - |
| | C. papyrus < P. australis | - | - |
| | C. papyrus < Control | 1.311 | TRUE 20% |
| | C. papyrus < W. thyrsiflora | 0.826 | NOT TRUE TO 20% |
| **Optimisation 1** | C. papyrus < *C. papyrus* ES | 3.827 | TRUE 5% |
| **Optimisation 2: Test 1 PPD check** | Single series < Parallel | 25.65 | TRUE 5% |
| | Dual series < Parallel | 6.391 | TRUE 5% |
| | Control < Parallel | 29.24 | TRUE 5% |
| | Single series < Control | 0.529 | NOT TRUE TO 20% |
| | Control < Dual series | 5.599 | TRUE 5% |
| | Surface+Root < Single Series | 11.94 | TRUE 5% |
| | Root only < Single Series | 9.360 | TRUE 5% |
| | Surface+Root < Root only | 4.648 | TRUE 5% |
| **Optimisation 3: PPD check** | 1xDist < 1.5xDist | 3.815 | TRUE 5% |
| | 1xDist < 0.5xDist | 8.754 | TRUE 5% |
| | 0.5xDist < 1.5xDist | 2.561 | TRUE 10% |
| **Optimisation 3: Internal resistance check** | 1.5xDist < 1xDist | 4.891 | TRUE 5% |
| | 0.5xDist < 1xDist | 30.22 | TRUE 5% |
| | 0.5xDist < 1.5xDist | 6.476 | TRUE 5% |