PERFORMANCE OF A MODIFIED TRICKLING FILTER PACKED WITH ORGANIC SUBSTRATES FOR TREATMENT OF AQUACULTURE WASTEWATER

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Performance of a Modified Trickling Filter Packed With Organic Substrates for Treatment of Aquaculture Wastewater

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other university

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DEDICATION

I give thanks to the Almighty God for His grace during my study period. I dedicate this work to my loving family: My husband Vincent and my dear children Aideen and Adriel, my mother Nancy and my late father John. My dear sisters Winnie, Elfridah, Claire and Beatrice and my brothers Moses and the late Eng. Nimrod. You all believed in me and supported me. May God richly and abundantly bless you.

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ABBREVIATION AND ACRONYMS

AEZ Agro Ecological Zones

BOD Biological Oxygen Demand

C:N Carbon Nitrogen Ratio

COD Chemical Oxygen Demand

EMCA Environmental Management and Coordination Act

GDP Gross Domestic Product

GOK Government of Kenya

HRT Hydraulic Retention Time

JKUAT Jomo Kenyatta University of Agriculture and Technology

KALRO Kenya Agriculture and Livestock Research Organization

NEMA National Environmental Management Authority

MBR Membrane Bio Reactor

SS Suspended Solids

WRM Water Resource Management

WRUA Water Resource Users Association

ABSTRACT

Globally, environmental sustainability is the most critical aspect of humanity. In order to achieve a sustainable environment, technologies of waste and wastewater management have been improving over the years. The development of wastewater treatment infrastructure lags behind the rates of urbanization. There is a need to come up with innovative and effective approaches of wastewater treatment to complement the existing wastewater treatment systems. Aquaculture wastewater is characterized by high nutrients and organic load. The organic compounds are broken down to simpler structures of carbon (IV) oxide and water at the secondary stage of treatment. It is therefore important to polish the water to remove contaminants so as to avoid pollution and eutrophication of receiving water bodies. The main objective of the study was to evaluate the performance of a bioreactor trickling filters packed with different substrates in polishing aquaculture wastewater effluents using locally available materials (woodchips, sugarcane bagasse and maize cobs) as substrate. These organic materials are suitable for bioreactors as they act as substrate for the microorganisms. The wastewater was obtained from the outlet of a fish pond. The bioreactor variables studied include; substrate column height, hydraulic retention time, particle sizes. The most suitable operating conditions was determined for all the three substrates. Wastewater was analyzed before and after treatment. The raw wastewater compositions were; nitrates, 208±1.24 mg/l 24.5±0.4mg/l nitrites and 20.66±0.31mg/l Phosphates. Other parameters Such as temperature and pH were within normal range of 19^{o}c and 6 respectively. These values are higher acceptable discharge standards set by GOK indicating need for polishing before discharge. The model wastewater bioreactor had a capacity of 12 liters. Each bioreactor unit was packed with different particle sizes of woodchips, maize cobs and sugarcane bagasse at various column heights; 14cm, 18cm and 22cm. The hydraulic loading rate and thus hydraulic retention time were varied at intervals of 12hour, 24hours, 48 hours and 60 hours. Samples were then collected and analyzed for nitrates, nitrites and phosphates using a UV VIS spectrophotometry. Data was analyzed to determine the efficiency of each substrate in the removal of contaminants. An analysis of the performance of each substrate at varied particle size and contact time was done. Wood chips was the most efficient substrate in the removal of contaminants at 22cm substrate column height, the smaller particles of woodchip (30-38mm) was the most efficient with 94%, efficiency in the removal of nitrates. Maize cobs was slightly more efficient compared to sugarcane bagasse in the removal of phosphates because of its adoptive properties. The optimal HRTs were as follows: For the woodchip at 22cm substrate column height 45 hours is required and in the 18cm substrate column height 72 hours: For the maize cobs and sugarcane at 22cm height of substrate 65 hours and 67 hours were the optimal HRTs. It is recommended that policy makers adopt this finding in developing regulations of unconventional water treatment in the country.

CHAPTER ONE
INTRODUCTION

1.1 Background Information to the study

Globally, environmental sustainability is the most critical aspect of humanity. In order to maintain a sustainable environment, technologies of waste and wastewater management have been improving over the years. Water is a basic necessity for all living organisms and makes up about 80% of living organisms. Generally, the volume of water in the world remains constant throughout and just circulates as it moves from one point to another and as it changes state in a cycle known as the hydrological cycle. For this reason, it is important to take care of the water resources in order to ensure sustainability (Muraguri, 2013). The demand for fresh water supply is high leading to stiff competition for its use between the differ-rent sectors mainly the domestic, industrial and agricultural sectors. Therefore, there is an urgent need for the treatment, recycle and reuse of wastewater to compliment the available water sources (Saddoud *et al.,* 2006).

The management of domestic wastewater in Kenya relies on the infrastructure created during the post-colonial period and it does not meet the increasing rate of wastewater generation due to increasing population and ballooning of the urban and peril- urban centers (Lowe, 1994). The nature of economic activities such as fish farming and crop production along the banks of water resources leads to pollution caused by water containing high levels of organic load, nitrogen pollutants and other ions. The contaminants find their way into the water bodies and leads to degradation of water bodies such as the invasion of water hyacinth, eutrophication and decrease in the population of fish (Opal, 2016). As a result the receiving water body losses its fundamental utility values and aesthetics. On the other hand, leaching of ions into the ground water also leads to contamination of the ground water sources.

Aquaculture sector has been growing and is currently contributing to more than 40% of the global fish production. With the increase in fish production there is an increase in wastewater generation characterized with high levels of organic load and nitrogen based contaminants which leads to pollution of the receiving water bodies. Nitrogen based contaminants are responsible for the eutrophication in water bodies which results in oxygen depletion leading to loss of biodiversity in the aquatic ecosystem. Other forms of nitrogen are greenhouse gases such as nitrous oxide and may lead to acid rain.

With advances in technology several treatment options such as the membrane bioreactors, rotating bio contactors, among others have been developed for the treatment of wastewater. However, these techniques requires expert knowledge for their operation which cannot be managed by local small scale fish farmers. This therefore suggests that such techniques may not be suitable for local fish farmers. Trickling filters are treatment systems that use bio agents attached to a media to remove contaminants in wastewater treatment (US-EPA, 2000). As the water flows through the filtration system, a film of bacteria attaches itself on the substrate and starts feeding on the substrate and the organic content in the water. Trickling filters have been used in the treatment of wastewater from different sources using different substrates and has been reported as an efficient system for secondary treatment of wastewater (Wamberee, 2014). In addition to the use of trickling filters a design that will ensure minimal cost of operation hence is an important method to consider while choosing and installing a wastewater treatment system. Its advantage is that it requires low energy gravity flow systems instead of using pumps and use of locally available material as filters substrate materials for the removal of pollutants.

1.2 Statement of the Problem

Water pollution is the main cause of poor water quality. Contaminants from socio-economic activities taking place around the catchment area are the main sources of pollutants. Agriculture is ranked as one the leading activities that consumes a lot of fresh

water (Fraiture *et al.,* 2007). Aquaculture is one of the production systems that contribute to degradation of water resources due to disposal of untreated effluent into the receiving water bodies (Brazil, 2001). Due to the global increase in population growth, there is a corresponding increase in the demand for food which includes crop production and fish/crustaceans farming. Therefore, there is an estimated increase in fish farming as a complimenting source of fish, whose demand is continuously rising.

Agricultural wastewater from farms and aquaculture ponds around the water bodies are the main sources of excess nitrogen and phosphorus which leads to eutrophication and overgrowth of the water hyacinth in some water bodies. Consequently, depletion of dissolved oxygen in the water bodies is reported to occur. The increase in organic content reduces the utility potential of the water there by destroying the ecosystem leading to loss of bio diversity. In addition to this, the economic potential of such water resources is reported to decline (Makisha and Nesterenko, 2018).

Biological treatment systems remove organic carbon from the wastewater. However, the removal of ions requires additional treatment to meet the requirements allowable for discharge (Yamashita and Yamamoto, 2014). With nitrogen species and phosphorus ions being the limiting factor in wastewater disposal or reuse, it is important to have highly efficient processes to achieve low concentrations of these contaminants. The goal of the study was to determine the most efficient substrate in a modified trickling filter for the removal of nitrates, nitrites and phosphates in aquaculture wastewater.

1.3 Null Hypothesis

This study compares the different aspects of a substrate in a trickling filter bioreactor with its efficiency in the removal of nitrates, nitrites and phosphates. The null hypothesis were as follows:

There is no significant difference in the efficiency of woodchips, maize cobs and sugarcane bagasse as organic substrates in the removal of nitrates, nitrites and phosphates from aqua cultural wastewater

There is no significant difference in the efficiency of bioreactors packed with substrate materials at varied column height and varied particle size.

1.4 1.4 Objectives

1.4.1 Main Objectives

The main objective was to evaluate the treatment performance of a modified trickling filter packed with different organic substrates in polishing aquaculture wastewater.

1.4.2 Specific Objectives

The Specific objectives were to:

1. Evaluate the performance of different substrates (wood chips, maize cobs and sugarcane bagasse) in removing nitrates, nitrites and phosphates under varying operational parameters.
2. Determine the optimal hydraulic retention times (HRTs) of the trickling filters packed with wood chips, maize cobs and sugarcane bagasse in polishing aquaculture wastewater.
3. Evaluate the adsorption behavior of substrates wood chips, maize cobs and sugarcane bagasse in the removal of phosphates under different adsorption kinetics.

1.5 Research Questions

a) Which is the most suitable substrates (wood chips, maize cobs and sugarcane bagasse) in removing nitrates, nitrites and phosphates by varying the operational parameters?

b) How does hydraulic retention times (HRTs) of the trickling filters packed with wood chips, maize cobs and sugarcane bagasse affect the treatment efficiency of wastewater effluent?
c) What is the adsorption behavior of woodchip, maize cobs and sugarcane bagasse in the removal of phosphates under different adsorption kinetics?

1.6 Justification of the Study

Globally, the volume of wastewater production is high and agriculture is one of the leading activity that produces highly contaminated wastewater (Alexandratos and Bruinsma, 2012). The most common method of agricultural wastewater treatment is activated sludge method. However, this method leads to the residual slurry high in nutrient such as nitrates and phosphates (Kizito *et al.,* 2016). Therefore, there is a need for complementary polishing system for the wastewater produced before discharging it into the environment to avoid pollution of the resources. The major challenge in aquatic wastewater treatment is high levels of organic and nitrogen based contaminants (Feng *et al.,* 2016). There is need to reduce the nitrogen in the water before discharge. Consequently, treating the water using biological processes and further polishing it using bio filters in trickling tanks makes the water suitable for discharge to water bodies.

Different subtrates have been used in polishing the wastewater including activated carbon and wood in various forms, however, activated carbon is very expensive to prepare and requires expert knowledge to handle (Kaetzl *et al.,* 2018). Hence there was a need to identify a cheaper, environmental friendly and easily available substrate for use in a bioreactor trickling filter. Maize cobs and sugarcane bagasse were easily available in the country hence reduces the operating cost when used as substrates.

1.7 Scope and Limitations of the Study

This research was based on the concept of a polishing system for aquaculture wastewater using bioreactors packed with different substrate materials. It aimed at evaluating the

treatment performance of bioreactors packed with different substrates/media (wood chips, maize cobs and sugarcane bagasse) in polishing aquaculture wastewater in a batch flow. The substrates used were pre dried and were not subjected to any form of chemical pretreatment or preservation. The chemical composition and permeability of the substrates were not analyzed. The study only assessed the removal of nitrates, nitrites and phosphates from fisheries wastewater using a UV-VIS spectrometer (model 1800 simdzu).

The limitation of the study was the substrates were not subjected to any form of pretreatment or preservation process. This would have changed the efficiency values.

1.8 Conceptual Framework

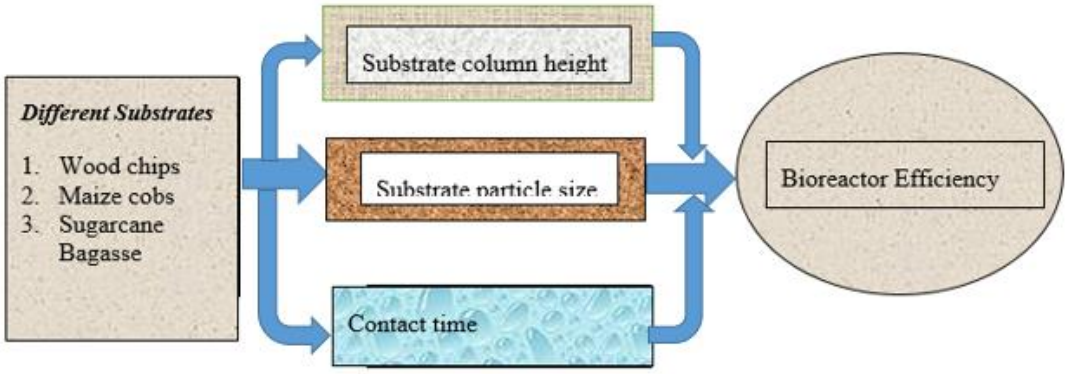

Figure 1.1: Conceptual framework

CHAPTER TWO
LITERATURE REVIEW

2.1 Wastewater Composition and Quality

Wastewater is referred to water that has undergone a certain process and cannot be used for the same process without undergoing a certain treatment. The composition of the water depends on the process the water has gone through. Therefore, the treatment process depends on the contaminants present in the water. The largest volume of wastewater production is from domestic and industrial processes. Domestic wastewater is characterized by high levels of BOD_5 and COD, high levels of nitrogen due to yellow water from the toilets, and other contaminants found in soaps and detergents. In domestic wastewater, nitrogen exists in the form of organic and inorganic nitrogen. Phosphorus in wastewater is in the form of ortho-phosphate and organically bound phosphate, which controls the efficiency of wastewater treatment system (Yamashita and Yamamoto, 2014).

According to Bryan (2017), any quantity of nitrogen greater than 3mg/L and any traceable quantity of phosphorus greater than 0.025 mg/L indicates the possibility of pollution. Inorganic nitrogen is in the form of ammonia and nitrates which exerts high oxygen demand reducing the concentration of dissolved oxygen (Jenkins and Sanders, 2012). When nitrogen in any form is released into the environment it leads to eutrophication which has a major impact on the aquatic ecosystem. It leads to depletion of dissolved oxygen leading to the destruction of the aquatic ecosystem and loss of bio diversity. Nitrogen in wastewater could also generate a potent greenhouse gas nitrous oxide (Gold *et al.,* 2012). The total nitrogen in water should be managed to a level of less than 100 mg/l before discharge into the environment, which is the minimum acceptable level by the Environmental Protection Agency EPA (Yildirim & Topkaya, 2012). The water quality regulation (2015) of EMCA requires any wastewater to be treated before discharge. The acceptable total nitrogen concentration at 100 mg/l and nitrates at 10 mg/l. The widely

accepted method of nitrogen removal is by use of nitrifying and denitrifying bacteria (Greenan *et al.*, 2006) and in most treatment processes the removal of phosphate is through adsorption of complexes or precipitation process (Jenkins and Sanders, 2012). Therefore a bioreactor is suitable in the removal of nitrogen based contaminants by use of denitrifying bacteria and adsorption of phosphates simultaneously.

2.2 Wastewater from Aquaculture

Wastewater from fishponds and fisheries processing units is characterized by very high levels of organic loads and mineral content. There are two categories of aquaculture waste: solid waste which includes the feeds and fecal matter and wastewater containing dissolved contaminates excreted by the fish and excess fertilizer (Crispp and Bergheim, 2000). Aquaculture wastewater is characterised by high levels of nitrogen greater than 200 mg/l, phosphurus greater than 100 mg/l with BOD and COD above 200 mg/l. theirefore, it requires treatment before reuse or discharge (Feng, *et al.,* 2016) and (Mburu *et al.,* 2019). Nitrogen in wastewater is present in the form of nitrogen gas, nitrates, nitrites and ammonia. These four forms of nitrogen are directly interchangeable and require biological processes for their conversion (Kasima, 2014). Phosphate in aquaculture wastewater is present in the form of phosphates and it is broken down to simple phosphate ions which are removed by adsorption (Jiang and Graham, 1998).

The pollution caused by aquaculture wastewater has been a global issue and in 2004, the Environmental Protection agency EPA of the the United States developed effluent management standards. The main purpose for these standards is to ensure there is protection of the water resources. These contaminants are best removed from the water using biological processes (Azizi, Valipour, & Sithebe, 2013).

2.2.1 Environmental Impacts of Wastewater

The presences of contaminants in wastewater pose both public health and environmental concerns. When the nitrates and phosphates finds their way to the water resources, they leads to eutrophication which is the biggest concern causing pollution and degradation of the existing aquatic ecosystem and water resources. Aquatic species are also affected by contaminated environment leading to decrease in their population (Yildirim and Topkaya, 2012). Heavy metals could also bio accumulate within the cells of aquatic species which may lead to bio magnification across the food chain (Sorensen, 2018). Pollutants in water resources also reduces the utility of the water and destroy the aquatic ecosystem. Therefore, pollution control is a key element in ensuring that the ecosystem is sustainably managed.

2.2.2 Wastewater Treatment Processes

Water resources are scarce and there is competition for the limited supply available for agriculture, household and industrial use. For this reason clean water has been termed as the "oil of the 21st century" (Sutton and Schuman, 2006). Globally there is need to develop a wastewater treatment strategy that will incorporate water reuse for financial viability and resources sustainability. Several methods are available some are highly sophisticated and have high energy demand making the treatment systems expensive. Consequently, one of the biggest challenge in waste water treatment is the identification of a system that will produce effluent that meet the required levels and run at the least operation cost (Arif *et al.*, 2018).

The process of wastewater treatment has several stages depending on the source of the wastewater and the ultimate use of the treated water. Generally the treatment process is categorized into the following steps; preliminary treatment, primary treatment (physical),

secondary treatment (biological), tertiary treatment (chemical) and finally, adjacent treatment (sludge treatment and removal) (APHA, 2005). Preliminary treatment involves: screening, grit removal and equalization processes. These ensure the smooth operation of the entire system and prevent damage of equipment by coarse materials. The next step is primary treatment which includes the removal of much finer solids (suspended and filterable solids). Secondary treatment process is responsible for the removal of dissolved organic content in the water using biological processes. Finally tertiary treatment process involves mainly chemical processes including disinfection. However, chemical treatment of wastewater containing phosphorus can lead to precipitation due to formation of phosphate salts which will require additional treatment process (Yamashita and Yamamoto, 2014: Zhou *et al.,* 2019). The main objective in wastewater treatment is to correct the deficiency in the water quality to achieve a certain standard (Chapman *et al.,* 2012).

The removal mechanism of various components of wastewater depends on the design of the system and contaminants present. Organic matter responsible for high BOD and COD is removed via aerobic and anaerobic microbial degradation. Nitrogen species is removed via nitrifying microorganisms, the process known as nitrification. Nitrification depends on temperature, pH and carbon-nitrogen ratio (C: N). However, in the absence of oxygen, nitrates are broken down to molecular nitrogen or nitrogen gas by microorganisms in a process called denitrification. This process is dependent on environmental factors such as temperature and humidity. Phosphate and orthophosphates are removed by adsorption process. The standard rate of nitrification is the conversion of ammonia to nitrates which is given in Equation 2.1 (Brazil, 2001) as:

$$\boldsymbol{R_{nit} = k^{o}_{\max nit} f_{nit}(T) \frac{NH_4}{NH_4 + K_{mNH_4}} \frac{O_2}{O_2 + K_{mO_2}}} \quad \textbf{(2.1)}$$

Where:

$\boldsymbol{R}$ =the overall rate of ammonia oxidation to nitrate (in mole N.)

$\boldsymbol{K^{o}}$ = the maximum nitrification rate constant (3.10^{-10} mole N)

$\boldsymbol{F(T)}$ = the temperature dependence function

$\boldsymbol{K_{mNH}}$ = the half-saturation constant for NH (250.10 mole N)

$\boldsymbol{K_{mO}}$ =the half-saturation constant for O (15.10 mole O)

The microorganisms responsible for the nitrification process include: Nitrosonomous bacteria which catalyzes un-ionized ammonia to nitrate. The Nitrobacteria bacteria which oxidize nitrite to nitrate.

2.2.3 Wastewater Management in Kenya

The conventional secondary treatment of wastewater in Kenya involves the use of facultative lagoons and ponds. Local authorities (County Governments) are responsible for the monitoring and testing of treated wastewater before it is released into the environment. Industrial wastewater has to be treated onsite before it is allowed to flow to the existing sewer system or to the environment. Wastewater production has increased due to the increasing population and the increasing utility for the resource (Juma, 2014).

Several researches have been done to improve the efficiency and effectiveness of the existing wastewater treatment systems. The use of foreign investors in the management of the water resources has greatly improved the utilization of water in Kenya. The introduction of biological digester systems, trickling filter systems and recirculating bio contactors for domestic and industrial wastewater has managed to reduce wastewater generation and to allow the reuse and recycling water (Kasima, 2014).

2.3 Legal and Policy Frameworks for Wastewater Treatment in Kenya

The main source of law in Kenya is the constitution. It provides a legal platform for all activities. Legal regulations that are considered in water treatment in Kenya are listed in

the Kenyan constitution. These regulations includes the Environmental Management and Coordination Act (EMCA), Water Act and the Public health Act among others as discussed in the following section.

2.3.1 Kenya Constitution, 2010

The constitution of Kenya is a collection of Acts of parliament. This is the umbrella of the law in Kenya. It defines the structure of the government and the function of the bodies under the government. Before an act is established it is proposed and discussed at the parliament and once it has passed three hearings and accepted the president then signs it for approval. An act can be revised with time. The environmental management and coordination act and the water act was formulated under the constitution. (Sihanya, 2017)

2.3.2 Environmental Management and Coordination Act, EMCA (1999)

This is an act that relates to the management, use, and distribution of natural resources and the environment to prevent the destruction/ degradation/ pollution of these resources. It is the framework law on environmental management and conservation. This act provides the establishment of National Environmental Management Authority, National Environment council, National Environment Trust Fund, among many other bodies relating to the management and protection of the environment and natural resources. NEMA was established under this act and charged with the responsibility of implementing all policies relating to the environment. (Environmental Managment and Coordination Act, 1999)

2.3.2.1 EMCA Water Quality Regulation (2012)

This applies to water used for industrial purposes, agricultural purposes, recreational purposes fisheries and wildlife and any other purposes (Constitution of Kenya). It ensures quality standard and monitoring of sources of water to be used in different sectors. This regulation ensures the protection of the water resources by prohibiting discharge of

polluted effluent into the environment. It provides standards for discharge of effluent into the sewer and aquatic environment. NEMA issues licence to the individuals who discharge effluent into aquatic ecosystems, this ensures that the quality standard of the effluent does not degrade the water body. According to the Eighth schedule, no person should re use waste water for agriculture unless it has undergone treatment to meet the stipulated standards (EMCA, 1999). The quality standards for water to be discharged into the environment or to be used for irrigation or recreational purposes is given by Schedules 3, 8 and 9 of the Environmental Management and Coordination Act (EMCA, 1999) water quality regulation on the water reuse guidelines. The quality of domestic water as listed in schedule 3 is as summarized in Table 2.1 (EMCA, 2015). This is monitored by NEMA who ensures the quality of the effluent meets the requirements.

Table 2.1: Standard for source of domestic water in Kenya

Parameters	**Concentrations**
pH	6.5-8.5
Nitrogen	10 mg/l
Phosphorus	10 mg/l
Turbidity	4 NTU
Color	15 units
BOD_5	30 mg/L
COD	50 mg/L
Lead	0.5 mg/L
Oil and grease	Nil

Table 2.1 provides the minimum standards that should be met before abstracting water for domestic use, which is the quality requirements as outlined under (EMCA, 2015). The

quality standards for water to be discharged into the environment as given by the third schedule (EMCA, 2015) are listed in Table 2.2.

Table 2.2: Standards for effluent discharge into the environment according to EMCA

Parameters	**Concentrations**
Ph	5-9
Ammonia, Ammonium compounds, No_2 compounds and No_3 compounds.	<100 mg/l
Phosphorus and phosphate compounds	1 mg/l
Total Nitrogen	Guideline values
Total Phosphorus	Guideline values
E Coli	NIL
Color	15 H,U
Total dissolved solids	15 mg/l
Lead	0.01
Oil and grease	NIL

A polishing system before the water is discharged may help to attain a suitable quality standard for re-use or safe disposal. This study provides the required polishing procedure by comparing the performance of different carbon based substrate to come up with the most suitable substrate.

2.3.3 Water Act (2016)

This act is aimed at improving the management of water resources and providing proper distribution of water resources to their respective users. This act provides the regulation

and management of water and sewerage services by providing the quality requirements for disposal. It also provides institutional arrangements for bodies that deal with the management of water resources. This act also provides the policy relating to the activities of treatment of wastewater and reuse of treated wastewater. Different sources of wastewater have a specified quality of the effluent before it is released into the environment (Water Act, 2016).

2.4 Wastewater Treatment Technologies

2.4.1 Conventional Wastewater Treatment Technologies

The oldest secondary treatment of sewage and industrial wastewater in Kenya entails the use of facultative lagoons and ponds. In most of the urban cities these lagoons and ponds were constructed in the mid-1990s. However due to increased urbanization, the expansion of facultative pond require large tracts of land, which is not available. Other forms of domestic wastewater management in areas that are not connected to the municipal sewer system are engineered septic tanks, soak pits and pit latrines. The lagoons are mainly used for secondary/biological treatment of wastewater. The major challenge in the biological treatment of sewage wastewater in Kenya is the organic and chemical loading and the increasing volume exceeding the design capacity (Barongo *et al*., 2006). Therefore, there is need for the biological treatment to be highly effective in order to reduce the BOD and the COD of the effluent. Due to improved technologies and expansion of knowledge, the use of constructed wetlands for industrial waste water treatment has been adopted. Aerobic and anaerobic processes can be integrated within the wetland to improve its efficiency (Azizi, Valipour, & Sithebe, 2013).

Adsorption is a physical treatment process used in the removal of contaminants by absorbent materials. Most common adsorbent materials used is Bio char derived from various plant based biomass. Adsorption process is highly preferred to reverse osmosis because it is more cost effective to operate and requires less energy resource. Adsorbent materials are characterized with high porosity and is likely to form chemical bonds with

the solute in the wastewater. The adsorption equilibrium can be given as a function of the solute concentrations as described in Equation 2.2 (Mohamed, 2007).

$$\mathbf{q_e = V(C_o - C_e)/M} \quad (2.2)$$

Where:

$\mathbf{q_e}$= adsorption amount (mg/l)

V = volume (**l**)

$\mathbf{C_o}$= concentration of analyte in wastewater (mole/liter)

$\mathbf{C_e}$= concentration of the analyte post treatment (moles/liter)

2.4.2 Non-conventional Wastewater Treatment Technologies

There are several non-conventional treatment technologies which include rotating bio contactors, activated sludge and membrane filtration method. The rotating bio contactors is made up of a circular rod that is submerged in the waste water such that 70% of the rods (coated in bio film) are immersed in water. The rods rotate continually such that the whole surface area is continuously exposed to air. However, this method does not meet design expectations leading to, excess biomass accumulations, shaft breakage, high energy demand and undesirable biological growths (Jenkins and Sanders, 2012). In the recent past research has been carried out to develop systems that overcomes these challenges and improve efficiency by introducing preliminary and primary treatment before the rotating bio contactors.

Another method used in wastewater treatment is the activated sludge method which uses additional biomass in the reactor volume to increase the capacity of the system. This sludge is reintroduced into the system to increase the micro-organism. This method can be applied in any type of flow. This method of treatment is suitable for a wide variety of wastewaters. This is because it is flexible in the manipulation of the design to make it

efficient in the removal of any type of contaminant (Arif *et al.*, 2018). However, activated sludge method is limited due to its susceptibility to seasonal changes, high energy requirement and challenges of waste sludge disposal (Nazaroff and Alvarez, 2016).

The membrane filtration method has been applied in wastewater treatment in conjunction with other methods including activated sludge on a micro porous membrane that uses the principal of reverse osmosis (Arif *et al*., 2018). It is a system that will generate minimal volumes of sludge and produce high quality of effluent. Technologies of integrating membrane into aerobic and anaerobic activated sludge systems have been evolving since 1969. With increasing technological knowledge, the use of MBR has evolved. However, the use of MBR has not yet been established for large scale treatment of wastewater. Currently, studies are being carried out to develop MBR system to treat large scale production of waste using integrated membrane systems. The membrane bioreactor works by allowing specific molecules to pass through the membrane while microbial degradation of organic content occurs (Mburu et al., 2021). The membrane filtration method acts as activated sludge on a micro porous membrane. The functioning of an MBR relies on three different categories of operation: pore size filtration, molecular cut off and pressure (Abdel-Kader, 2007; Mburu *et al.*, 2019). Other MBR processes uses the principal of reverse osmosis. This system will generate minimal volumes of sludge and produce high quality of effluent. Although, it has not yet been embraced for large scale treatment of wastewater, MBR systems is likely to be the future of wastewater treatment systems. However, energy requirements for such systems and the need for expert knowledge in its operation and maintenance might translate to high operational costs.

2.5 Design Consideration of Trickling Filters

Trickling filters are wastewater treatment systems designed with a reactor tank packed with inert material where a film of aerobic and anaerobic microorganisms grow. The wastewater is applied at the top through a rotary arm. As the wastewater trickles downwards, it makes the biofilm wet and catalyzes bacterial growth. The oxygen from the

air spaces in the void is made available to aerobic bacteria grown in the biofilm by diffusion of oxygen through the biofilm. Organic matter from the wastewater is adsorbed on the biofilm layer and it is degraded by the aerobic bacteria present in the biofilm. As the thickness of the film layer increases the condition near the surface of the substrate becomes anaerobic because of limited oxygen supply. The microbes will then lose their ability to cling to the surface of the substrate and the layer sloughs off and is washed out along with flowing liquid (US-EPA, 2000; Wamberee, 2014).

Trickling filters have been used in processes of wastewater treatment for over a century, their design and operation has been well established in the practice of wastewater engineering (Metcalf & Eddy, 1991). It has been used in the removal of organic content and nitrogen based contaminants in wastewater. According to Wamberee (2014), trickling filters are compact systems for biological treatment of wastewater and are suitable for treatment of water where there is limited space. They are compact and effective in polishing the effluent. The most suitable media to be used in a trickling filter should be characterized with a high surface area to volume ratio.

Trickling filters are less complex and require little technical knowledge compared to other biological wastewater treatment processes such as rotating bio contactors and membrane bioreactors. They are resistant to shock loads, power failures and they have a small environmental foot print (Ali *et al.*, 2016). Trickling filters allow for simple design consideration and require low energy with limited repair and maintenance

The design of a trickling filter relies on the hydraulic system and the distribution system (Wamberee, 2014). The diameter of a mechanical trickling filter relies on the type of equipment used for spraying water. The use of trickling filters relies mostly on pretreated water for this reason there is need for continuous monitoring and repair to avoid clogging and accumulation of excess biomass in the system (US-EPA, 2000). Different studies have been carried out by different researchers with reference to the following design considerations: overcoming potential clogging of the media system as a result of

inadequate screening; minimizing excessive growth of micro-organisms, which could plug the media system or cause free-floating media to sink (Gupta *et al*., 2008); and to avoid inadequate mixing or short-circuiting, resulting in inefficient use of the media (Jenkins and Sanders, 2012).

According to Gregory (2011), the efficiency of a system depends on the design parameters. The designs of bioreactors rely on several aspects. There are different types of bioreactor systems with different concepts of design. A trickling filter design is based on the relationship of the degree of wastewater to be treated and the required filter volume. This research considered a modified form of the film bioreactors also known as solid state bioreactors. This is where the microorganisms are allowed to grow on a solid surface which also acts as a substrate.

The design of a treatment system is given by mathematical models to estimate the volume of waste water to be treated and size of the system (Metcalf and Eddy, 1991). The design consideration for this type of bio filtration trickling tank depends on the volume of wastewater to be treated, the specific substrate and microorganisms to be used. For aerobic bioreactor an aeration system has to be included in the system. The efficiency of the system relies on the functionalities of the microorganism, organic loading rate, hydraulic loading rate and ambient conditions. Therefore, the design parameters of a system must consider the limiting factors of microbial functions i.e. pH, Temperature, organic loading, hydraulic loading and availability of oxygen (US-EPA, 2000). There are different theories of design of a trickling filter depending on whether it is low rate loading or high rate loading trickling filter. A high rate trickling filters have a hydraulic loading rate of 10 to 40 m^3/m^2 and an organic loading rate of 0.3 to 1 kg BOD/m^2. On the other hand, a low rate filter has a hydraulic loading rate of 1 to 4 m^3/m^2 and an organic loading rate of 0.08 to 0.32 kg BOD/m^2. According to design criteria stated by Park *et al.*, (2015), the recommended specific surface area for organic component treatment is $100m^2/m^3$ and for

nitrogen based treatment is about $300m^2/m^3$ due to the slow growth of nitrifies microorganisms (US-EPA, 2000).

2.5.1.1 National Research Council of US

The National Research Council of US developed an efficiency equation which relies on the efficiency of removal of contaminant. It gives the efficiency as the function of the loaded volume and the BOD removal rate. This is the most commonly used design. This equation is used to calculate the efficiency as a function of the BOD value and the recirculating factor as shown by Equation 2.3. (Rossi *et al.,* 2015)

$$\boldsymbol{E = \frac{100}{1.44(\sqrt{x/RF}}} \tag{2.3}$$

Where:

E = the efficiency (%).

x = contaminant concentration (mg/l).

RF = the recirculating factor

2.5.1.2 Rankins Equation

This equation has been used widely for the design of single stage and second stage filters at different temperatures. However the contaminant removal efficiency in this method is slow since there is a continuous recirculating of the effluent within the system to a point where the BOD of the effluent is three times less than that of the influent. The efficiency of a single stage filter in rankings equations given by Equation 2.4 (APHA, 2005)

$$\boldsymbol{E = \frac{(contaminant + R)}{1.5 + R}100} \tag{2.4}$$

Where:

E = Efficiency (%.)

R = recirculating factor

The recirculation factor is given by the Equation 2.5 as:

$$\boldsymbol{R = \frac{Q_1 - Q}{Q}} \quad (2.5)$$

Where:

$\mathbf{Q_1}$ = the total flow ($m^{3/}h$.)

Q = the effluent flow in cubic meters (m^3h) (APHA, 2005)

2.5.2 Inorganic Trickling Filter Media

A bioreactor is defined as a system where the process of conversion of complex compounds takes place due to the action of micro-organisms. Substrate play an important role in microbial culture development. Non organic materials such as rocks, synthetic and plastics have been used as bio reactors media. However, to increase its efficiency a carbon based media is used in the bioreactors as a substrate material. This is because carbon based media provides the energy requirements for the bacteria growth which is important for the action of nitrifying and denitrifying microorganisms. In low oxygen concentration, the microorganisms use the nitrate to metabolize the carbon in the process converting it to atmospheric nitrogen. Several carbon based materials have been used in previous experiments with activated carbon being the most popular. This is because of its filtration and adsorptive properties (Daud *et al.,* 2014). Activated carbon is generated from lignite coal. The cost of manufacture of activated carbon is high since it includes a number of processes. Various carbon based materials are refined and processed to generate activated carbon. Activated carbon has high efficiency of adsorption of complexes and ions (Kanawade and Gaikwad, 2011). However, due to the long-term unsustainability of coal

resources, environmental concern and potentially increasing costs of production, there is a need to replace the activated carbon as a substrate material (Quresh *et al*., 2008).

Film membrane substrates generated in a laboratory are used as a substrate in membrane bioreactor. However, this simple form of carbon has to be replaced severally leading to high consumption of ethanol, making the process expensive. Also the introduction of simple carbon in the water leads to an increase in the organic load consequently reducing the efficiency of the treatment system On the other hand agricultural residue is cheap and easily accessible which lowers the cost and improves the function of the bioreactor (Gold *et al*., 2012). The reuse of agricultural biomass in wastewater treatment bioreactors also provides a solution to the management of these waste material which poses a potential risk to the environment if it is not well managed (Zhou *et al.,* 2019).

2.5.3 Organic Trickling Filter Media

Organic filter media refers to materials that have been derived from living organisms such as woodchips, rice husks, maize cobs and sugarcane bagasse. Several researchers have concluded that organic substrate is efficient and low cost for the biological treatment processing of domestic wastewater.

2.5.3.1 Wood Chips

Wood is obtained from the stem and branches of both soft and hard wood trees. Wood chips are obtained from processes that use timber as a raw material. Wood is mostly used in bioreactors because of its availability and longevity. Woodchips are characterized by decadal longevity due to its nature of slow decay (Schipper *et al.,* 2010). Due to this property woodchips are characterized as a slow release carbon media. The form of carbon dominant in wood chips is 22-28% lignin, 40-45% cellulose and 25-40% hemicellulose in the form of –Glucomannan, Glucuronoxylan and Other polysaccharides (Sjostrom, 1993). The length of a cellulose molecule is equivalent to 10,000 glucose units. These makes it have a long half-life therefore making wood a slow releaser of carbon. In the recent past,

developing countries have embraced wood chips as a substrate material in bio reactors. In previous experiments wood chips have been used as substrates in bio-reactors for treatment of point nitrogen pollution from agricultural sources (Lepine *et al*., 2016). One of the advantages of using woodchips is because of its ability of closing loops making it suitable for different processes of treatment. The waste woodchips from the bioreactor can also be used in farms to enrich the soil (Kaetzl *et al*., 2018).

2.5.3.2 Sugarcane Bagasse

Sugarcane bagasse is an industrial waste product that is produced after the extraction of juice for the production of sugar. The rate of production of sugarcane bagasse is high since one tone of sugarcane produces approximately 280 kg of bagasse (Arif *et al*., 2018). It has a 90.22% concentration of carbon in the form of cellulose, hemicellulose and lignin distributed as follows: 45-50% cellulose, 25-35% hemicellulose and 15-25% lignin (Daud *et al*., 2014). The carbon chains are broken down by decomposition. Sugarcane bagasse is suitable for bioreactors because of its complex carbon matrix and a slow rate of carbon release (Ingles *et al.*, 2009). It has been tested and used for treatment processes during the removal of dye in solutions (Kanawade and Gaikwad, 2011). Sugarcane bagasse has a high adsorption capacity due to its chemical nature and porous characteristic. The use of bagasse in industrial wastewater treatment also helps in managing industrial waste (Reza and Abedin , 2013).

2.5.3.3 Maize Cobs

Maize is a major crop in Africa and Asia and the staple food in Kenya. It serves as a major source of carbohydrates. A maize plant has the stalk also known as the stem, the flowers and the fruit. The maize grains are attached on a cob which is not edible. Maize cobs have been a source of fuel and fodder for livestock. Maize cobs have been reported to have exelent substrate properties. It is characterized by a high retention capacity and adsorption rate due to its complex lingo-cellulosic structure. It is made up of 35-40% cellulose, 40-

50% hemicellulose and 10-20% lignin. This variation occurs in different varieties of the crop (Pointne *et al*, 2014). Maize cob have a highly adsorbent surface due to its porous nature and wide surface area (Singh *et al.*, 2017). According to Khan (2018) maize cobs have high filamentous structures with inter cob voids which increases its surface area for contact making it suitable substrate in wastewater treatment. Due to its highly filamentous structure, it allows for rapid growth and attachment of microorganisms making it suitable as a substrate material for trickling filter (Ali *et al*., 2016). Maize cobs have been used in industrial wastewater treatment to remove heavy metals, paint and grease from the waste water (Mwangangi, 2015). Table 2.3 shows a summary of the characteristics of the proposed substrate

Table 2.3: Summary of substrate characteristics

	Wood chips	**Maize cobs**	**Sugarcane bagasse**
Density	0.578-0.878 g/cm^3	0.17-0.29 g/cm^3	0.120-0.15 g/cm^3
Porosity	0.17 ml/g	0.098 ml/g	0.094 ml/g
Carbon content	90.8%	88.8%	90.2%
Lignin	22- 30%	10-20%	15-25%
Cellulose	40-45%	35-40%	25-45%
Hemicellulose	20-40%	40-50%	45-50%

2.6 Hydraulic Retention Time in Bioreactors

The rate of flow of the water through the substrate is governed by the required Hydraulic Retention Time (HRT). The HRT also affects the efficiency of the microorganisms. A suitable HRT is one that allows maximum contact between micro-organisms and the water. When the HRT is too low it leads to overgrowth of micro-organisms and high reduction of substrate content. When the HRT is too high the efficiency of the treatment is poor and substrate can be washed out easily. Varying the hydraulic retention time helps in identifying the optimal contact time for removal of contaminants. This is important as it determines the most suitable contact time of the wastewater with the micro-organisms

(Merino-Solís *et al*., 2016). In various studies, the HRT is determined using a tracer experiment.

Tracer experiment is carried out using colored water. The time the water is released at the inlet is recorded and the time the entire volume is collected at the outlet is also recorded. This is done repeatedly by varying the quantity of flow in both the inlet and the outlet. The data obtained in this study is then used to generate a response curve. From the response curve optimum HRT for that particular substrate is obtained. The efficiency of the system depends on the hydraulic retention time and many researchers test the significance of a substrate in a microbiological reaction with reference to the retention/contact time (Cantrell *et al.,* 2008). This is because HRT is easily manipulated to achieve different outcomes. Varying the HRT affects the mean time of contact of the waste water and the substrates. The mean time of contact is a function of the hydraulic loading rate and the depth of the substrate as illustrated by Equation 2.6.

$$\boldsymbol{T = {C * D}/{Q^N}} \qquad (2.6)$$

Where:

T= Mean time (hours)

D= Depth of the substrate (m).

Q= Hydraulic loading rate (m^3/h)

C and N are constants.

2.7 Alternative Treatment Systems for the Removal of Nitrogen and Phosphorus

There are several alternative methods used in the removal of nitrogen. The most common is the use of methanol to remove nitrogen. Methanol is injected into a tank of wastewater; the mixture is then allowed to settle for a while. The nitrifying microorganisms use the carbon in methane as a substrate. This method is highly effective in denitrification but

leads to a high concentration of organic carbon in the effluent. This leads to post treatment operation which is expensive (Yamashita and Yamamoto, 2014). Another limiting factor of this method, is that other contaminates are not removed. Alternatively, nitrogen in the form of ammonia can be removed from a solution through a cation exchange absorption reaction. An oxidizing solution which is used in most cases is sodium hypochlorite with a concentration of 0.5% (Bisekwa, Njogu and Taye, 2021). This removal is not long term since it is loosely bound to the substance and breaks free easily (Vandith *et al*., 2018).

Activated sludge method uses additional biomass in the reactor volume to increase the capacity of the system. This sludge is reintroduced into the system to increase the micro-organism. It is effective in the removal of organic components and certain species of nitrogen. It is widely used because of its adoptability to any type of wastewater (Arif *et al*., 2018). This method can be applied in any type of flow. Although this method is efficient, it is only suitable for the removal of organic compounds and not denitrification and removal of phosphorus. The studies of activated sludge method started as early as 1900s the efficiencies has improved due to integration of the system into modified filters (Sorensen, 2018).

Open channel systems such as wetlands and ponds have a challenge of flooding during the rainy seasons reducing the efficiency of these system. This system also occupies a big part of land making it less suitable for already developed cities. Further, the treatment of wastewater in lagoons and ponds leads to the release of greenhouse gasses which have negative effects on the environment (Musa *et al*., 2018).

2.8 Optimization and Modeling

Optimization is an act of determining the best conditions or results under any given circumstances. It is a mathematical operation which can be achieved by graphical liner and quadratic functions. Studies have been carried out to calculate optimal conditions for varied experiments (Astolfi, 2006). Through research, different mathematical operation

and techniques have been developed to determine optimal/critical values in experimental design. Computer software have also been developed and used in the broad field of science and technology. Statistical models such as Analysis of Variance (ANOVA), regression and software have been developed and used widely (Nocedal and Wright, 2007).

Response Surface Methodology (RSM) is a computer software optimization technique that helps to identify the relationship between exploratory and response variables to determine the most suitable design conditions in a set of data that has several variables (Montegomery, 1997). RSM requires the use of mathematical optimization to determine the optimal operating conditions with reference to other variables included in the experiment. A mathematical equation is generated to show the relationship between the variables and the efficiency as illustrated by the Equation 2.7. (Mohammed *et al.* , 2015).

$$\boldsymbol{E = f(x_1 + x_2 + \cdots x_n) \pm \varepsilon} \qquad (2.7)$$

Where:

E = the efficiency variable. (%)

x = independent variables. (mg/l)

n = number of independent variables.

$\boldsymbol{\varepsilon}$ = statistical error.

From Equation 2.7, future predictions can be made when the independent variables are known and the statistical error is calculated or can be obtained graphically (Kakoi, 2018). RSM is a computer software and also requires software expert knowledge for its use.

Analysis of variance (ANOVA) has also been used widely to determine preferences and optimal condition for experimental results within different classes. It is used to identify the adequacy of statistical significance of different groups of data (Bui, 2018). Analysis

of variance can also be used in optimization especially when there is different sets of data and in an experimental design. When the statistical probability value (p value) is less than 0.05 then the difference is significant and the researcher should accept the null hypothesis. Other statistical operations such as T-test and Fischer test (F test) are used alongside ANOVA to determine its statistical significance. A comparison between F value and P values helps in making statistical conclusions. When p> F value equal to 0.05 shows that it is highly significant. When the p> F value is greater than 0.1 then the difference is not significant (Trinh and Kang, 2011).

Modeling of the efficiency of wastewater treatment systems in the removal of contaminants have been described by various scholars. There are several models used to describe nutrient removal from wastewater. Kinetics models have been used with reference to the rate of removal of contaminants in the wastewater. First order removal model is described as a first derivative function as shown in Equation 2.8_a and further simplified into 2.8_b when it is equated to Zero (Vandith *et al.*, 2018).

$$\frac{dS}{dt} = \frac{QS_o}{V} - \frac{QS}{V} - k_1 s \qquad (2.8_a)$$

$$\frac{S_o - s}{HRT} = k_1 s \qquad (2.8_b)$$

Where:

S= contaminant value (mg/l)

$\mathbf{S_o}$= initial contaminant value (mg/l)

$\mathbf{K_1}$= the constant given by the gradient of the curve.

The second order model is a modification of equation 2.8_a to be expressed as an integral function. This is more suitable for heterogeneous conditions such as bioreactor with varied layers of filter materials (Vandith *et al.*, 2018).

Modeling of the efficiency of nitrate breakdown can be described by both linear functions and nonlinear equation depending on the accuracy level required and the variables under consideration. According to Rossi et *al.* (2015), a denitrification model can be derived from the first order model as a differential function as in Equation 2.9.

$$\frac{d[NO_3^-]}{dt} = [NO_3^-]in\frac{F}{Vo} - \lfloor NO_3^- \rfloor out\frac{F}{Vo} - k_d^o \quad (2.9)$$

Where:

$[NO_3^-]in\frac{F}{Vo}$ = Nitrate in raw water (mg/l)

$\lfloor NO_3^- \rfloor out\frac{F}{Vo}$ = Nitrate in treated effluent (mg/l)

k_o= Denitrification reaction kinetic constant,

The maximum value of nitrate removed can therefore be calculated by equating Equation 2.9 to zero and simplified to obtain Equation 2.10 as:

$$[NO_3^-]ss = \lfloor NO_3^- \rfloor in - \frac{k_d^o}{k_o} \quad (2.10)$$

Where:

$[NO_3^-]ss$= Nitrate in effluent

$\lfloor NO_3^- \rfloor in$= Nitrate in raw water

k_d^o= kinetic constant of the zero order kinetics

k_o= Denitrification reaction constant,

This model is suitable in the zero and first order kinetics but relies on the HRT values such that any variation in the HRT affects model dynamics. The values of k_d and $\lfloor NO_3^- \rfloor in$ can be obtained from the graph of $[NO_3^-]ss$ vs $k_o$as the gradient and y intercept respectively.

Since the retention time is a variable in this study, this model may not yield the most accurate results because the efficiency is a function of the HRT.

Stover Kincannon model is suitable for biofilm reactors and biological filters. It expresses the substrate utilization rate/ breakdown rate as a function the loading rate by monomolecular kinetics (Vandith et al., 2018). This model is described by the series of Equations 2.11s and the efficiency of the system is derived from Equation 2.11a all through to Equation 2.12. When kinetic constants are introduced into Equation 2.11_a, it yields the function in equation 2.11_b

$$\frac{ds}{dt} = \frac{Q}{V}(S_o - S) \qquad (2.11a)$$

$$\frac{ds}{dt} = \frac{U_{max}\left(\frac{QS_o}{v}\right)}{K_B\left(\frac{QS_o}{v}\right)} \qquad (2.11_b)$$

When –ds/dt is taken for the function, and plotted against the inverse of the loading rate (V/Q_s) a straight line will be formed with an Intercept as $1/U_{max}$ and a gradient given by K_B/U_{max}. Therefore the function can be expressed as in Equation 2.11_c.

$$-\frac{ds}{dt} = \frac{k_b}{u_{max}}\left(\frac{V}{Q}\right) + \frac{1}{U_{max}} \qquad (2.11_c)$$

At steady state the Equation 2.11_c can be expressed by the function in Equation 2.11_d.

$$QS_O = QS + V\frac{ds}{dt} \qquad (2.11_d)$$

Substituting Equations $2.11_b$and 2.11_d it yields Equation 2.11_e as follows

$$QS_O = QS + V\left|\frac{U_{max}\left(\frac{QS_o}{v}\right)}{K_B\left(\frac{QS_o}{v}\right)}\right| \qquad (2.11_e)$$

This Equation can therefore be solved for effluent parameter concentration or efficiency of the removal of contaminants as shown in Equation 2.12_a and 2.12_b respectively obtained by substituting the kinetic constants U_{max} and K_B.

$$s = s_o - \frac{U_{max}S_o}{K_B + \left(QS_O/V\right)} \qquad (2.12_a)$$

$$E = \frac{S_o - S}{S_o} = \frac{U_{max}}{K_B + \left(QS_O/V\right)} \qquad (2.12_b)$$

In adsorption processes (the removal of phosphates), Langmuir and Freundlich adsorption isotherm models are commonly used to determine the efficiency of phosphate removal and give future predictions (Mohamed , 2007). The Langmuir model is described by the function in Equation 2.13 which is commonly used for homogeneous adsorbent while the Freundlinch model is used for heterogenous adsorbents and is expressed as a logarithimic function as shown in Equation 2.14.

$$\frac{C_e}{q_e} = \frac{1}{bx_m} + \frac{c_e}{x_m} \qquad (2.13)$$

$$\log q_e = \log k + \frac{1}{n\log c_e} \qquad (2.14)$$

Where:

Q_e= equilibrium adsorption per unit weight (mole/g)

C_e= concentration of the analyte post treatment (mole/g)

x_m and **b** are constants obtained from the gradient and y-intercept of the graph respectively. These constants are obtained by plotting $\frac{C_e}{q_e}$ against c_e. The value of x_m is the maximum adsorption capacity and b is the energy of adsorption constant.

The Langmuir isotherm can be expressed by a constant called the correlation/separation factor R_l which is expressed by the Equation 2.14 as derived by (Zhou *et al.,* 2019)

$$R_l = \frac{1}{1 + k_l C_o} \qquad (2.14)$$

Where K_l is the Langmuir constant and C_o the initial concentration of the analyte. When the R_l values are greater than or less than 1 ($R<1$), ($R>1$), the adsorption is unfavorable but when it is equal to 1 ($R\approx1$) the adsorption is favorable (Nimibofa, Ebeleji, and Wankasi, 2017).

Graphical and linear equations are the oldest and simplest method of optimization and model forecasting. It is because of its ability to quantify theoretical assumptions and forecast the parameters of the analyte (Nimibofa, Ebeleji, and Wankasi, 2017). This involves the process of developing an equation using the graph and using the equation to determine the values of the X variables at the suitable value of the Y variable and vice versa. Several researchers have developed equations for their research using mathematical and graphical equations including (Sweetapple & Fu , (2016); Dutta, (2007) and Majozi & Gouws, (2009). However, linear regresion is bound to cause several errors in the function therefore computers nonlinear modeling is preffered.

2.9 Analytical Techniques

Analytical techniques is defined as a method used to determine the concentration of chemical compounds. The complexity of an analytical technique depends on the parameter being tested and the desired accuracy. Due to constant change in technology different methods have been developed by scientists. The most suitable method to an individual

depends on their need for accuracy and precision, equipment and regents available and ions to be analyzed. Several methods vave been used including colorimetric methods, spetrometric methods and chromatographic methods. The choice of a suitable analytical technique relies on the desired results, equipment and reagents available (Daud *et al*., 2014)

2.9.1 Analytical Techniques of Phosphates

Phosphorus is present in water in the form of ortho phosphates or organically bound phosphates. The analysis of phosphates takes two forms: conversion into soluble ions then calculation of the moles and colorimetric determination of dissolved orthophosphates. Molybdate reagent or stannous chloride reagent is used in the Colorimetric analysis of phosphorus within the range of 1-20mg/l and 0.1 to 6 mg/l respectively. The use of Molybdate in Colorimetric analysis provides more accurate results while using the spectrometer hence it is highly recommended (Rodger, 2017).

2.9.2 Analytical Techniques for Nitrates and Nitrites

The analysis of Nitrogen based ions is complex due to the possibility of the presences of interfering complexes in the analyte solution. All forms of nitrogen are bio chemically inter-convertible and exist in the nitrogen cycle. The changes in its oxidation state is one of the properties used in analysis of nitrogen based compounds (PALIN, 1950). The level of nitrates and nitrites can be determined using the techniques listed in the following sub-sections.

2.9.2.1 Titrimetric Methods

Also known as titration is the most common laboratory method. A standard solution of the titrant is prepared with a known volume and concentration. This is then used to determine the analyte concentration. The volume of the titrant that reacts with the analyte is referred to as the titration volume. The titration volume is used to calculate the

concentration of the analyte. This method is best used for Acid-base reactions and REDOX reactions for non-Acid base reactions, a buffer solution is required to maintain the pH at neutral (Wisniak, 2014). A color indicators required in any titration process, this method requires very high level of accuracy and is not suitable for some ion analysis.

2.9.2.2 Colorimetric Methods

Colorimetric analysis is used in to determine the quantities of nitrates and nitrites within the range of 0.5 to 1000µg/l within the photometric measurement. However, this range can be adjusted with automation of equipment and calibration of wavelengths (US-EPA, 1979). This range maybe extended with the use of dilution. In the testing process, the sample is passed through a column of granulated copper and cadmium which reduces the nitrates to nitrites. Then reagents is then added to the filtrate to form a dye and the concentration of nitrates is the tested calorimetrically (O'Dell, 1993). In some cases Colorimetric is used hand in hand with other methods such as titration and spectrometry. This method is suitable for ions/ analyte that change color during a chemical reaction. This method is suitable for analysis of nitrites when used with spectrometry.

2.9.2.3 Spectrophotometric Methods

There are different methods of spectrometry including mass spectrometry which measures the mass to charge ratio of charged particles, flame spectrometry which is divided into: flame atomic emission spectrometry and flame atomic absorption spectrometry (Corey and Polefer, 2017). Electromagnetic spectrometry relies on the interaction of electromagnetic radiation as it passes through the analyte. The radiation can be emitted, absorbed or scattered. Electromagnetic waves have different radiation characterized by their wavelength. The varying wave length is the main property used in spectrometry as an analytical technique (Syed, 2007). The ultraviolet and visible light spectrum is the region commonly used in analytical techniques. Due to the simplicity and availability of equipment, this technique was used in the analysis of nitrates, nitrites and phosphates.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Design

The study design for this research was experimental design since it required laboratory analysis. Qualitative and quantitative analysis were carried with a view of determining the effect of different substrate on the treatment efficiency of bioreactors in the removal of nitrates, nitrites and phosphates in aquaculture wastewater. Experimental research design provided the techniques for quantitative data collections and analysis.

3.1.1 Experimental Design

A quantitative and analytical analysis was carried out to determine the efficiency of woodchips, Maize cobs and Sugarcane Bagasse in polishing aquatic wastewater. A data collection schedule was used to record the laboratory results of the parameters tested (nitrates, nitrites and phosphates) for all the three substrates.

The study was divided into four broad areas listed as:

i. Development of model modified trickling filter system which involved sizing of the bio filter bed.
ii. Evaluation of the different substrates at varied operating conditions in the removal of nitrates, nitrites and phosphates.
iii. Use of the design in testing efficiency of the different media (Woodchips, Sugarcane bagasse and Maize cobs) at different hydraulic retention time (12hours, 24hours, 48 hours and 60 hours).
iv. Fitting the results of phosphate in a model and determining the accuracy for adsorption of phosphate by the bioreactor.

3.2 Development of the trickling filter

The design and development consideration for this bioreactor relied on the strength of the influent and the desired quality of the effluent. This water polishing modified trickling filter required the effluent quality to meet the standard for effluent discharge set by NEMA as listed in Table 2.2. The design for sizing the bio filter bed was obtained by manipulating the operation variables. This operation variables includes the height of the substrate column, particle sizes of the substrate and varied hydraulic retention time.

3.2.1 Dimension of Reactor Tanks

The tanks were designed according to Hochheimer and Wheaton, (2000) theory that states "an optimal theoretical Hydraulic retention time for a wastewater treatment is 12hours". Working with this theory a system was designed with a hydraulic loading rate of 1liter per hour. Therefore the system handled a capacity of 12 liters of raw water in one day derived from the theory of theoretical hydraulic retention time given by (Hochheimer and Wheaton, 2000). Therefore, the influent retention tank was of a capacity of 15liters to allow for aeration before treatment so that it can allow growth of aerobic bacteria. The reactor tanks dimensions was calculated from Equation 3.1 given as:

$$\boldsymbol{V = A * H} \tag{3.1}$$

Where:

$$\boldsymbol{A = \pi r^2} \tag{3.2}$$

H = height (m)

r = radius (m)

The width of the reactor tanks was constant since there was no need for a mechanical equipment for spraying or pumping. Therefore, this allowed the manipulation of the parameters of the Equation 3.1. To allow for manipulation of the height of the substrate

column, the height of the reactor tank was the limiting factor. With a maximum substrate height of column at 22cm, and a known diameter the reactor tanks height was calculated by the Equations 3.2.

3.2.2 Pipe Dimensions

The rate of flow Q (liters/hour) given by Equation 3.3 was used to manipulate the desired pipe dimensions and the velocity of flow.

$$Q = AV \tag{3.3}$$

Where:

Q = is the discharge in m^3/ hour

A = the cross sectional surface area of the pipe/valve in M^2

V = is the velocity of flow in m/hour

3.2.3 Hydraulic Loading Rate

According to Dorothy (2014) and Musa *et al*. (2018) hydraulic loading rate is defined as the total flow applied per unit area of the trickling filter per day. The hydraulic loading rate is a function of the flow rate Q per day, and the cross-sectional area A as illustrated in Equation 3.4.

$$HLR = Q/A \tag{3.4}$$

Where:

Q= Discharge (m^3)

A= Area (m^2)

When the hydraulic loading rate is between 1-4$m^3/m^2/d$ the design is for a low rate filter. Any values greater than 10$m^3/m^2/d$ is a design for a high rate filter.

3.3 Treatment System Setup

The inlet was at the top of the reactor tank to allow the water to dissolve oxygen, and the outlet at the bottom to allow wastewater to flow downwards by gravity. The system was built and each bioreactor tank packed with different substrates to the required height and labeled as “A”, “B’ and “C” accordingly, where:

i. Trickling filter A – packed with wood chips.
ii. Trickling filter B – packed with maize cobs.
iii. Trickling filter C –packed with sugarcane bagasse.

The tanks were placed on a platform structure with the retention tank on a higher platform than the reactor tanks to allow the flow of raw wastewater into the reactor tank by gravity, illustrated in Plate 4.3 in the next chapter. Figure 3.1 shows the layout of the system

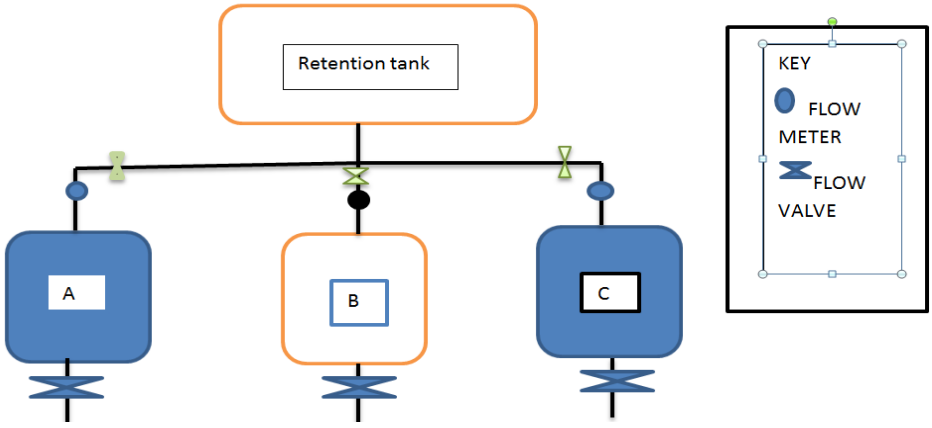

Figure 3.1: Layout Overview

The inlet of the reactor tanks was at the top of the tank as a network of perforated pipes with a diameter of ¾ inches to distribute the water evenly. Each reactor tank had an outlet at the bottom to allow the flow of water by gravity. Figure 3.2 shows an illustration of a single unit.

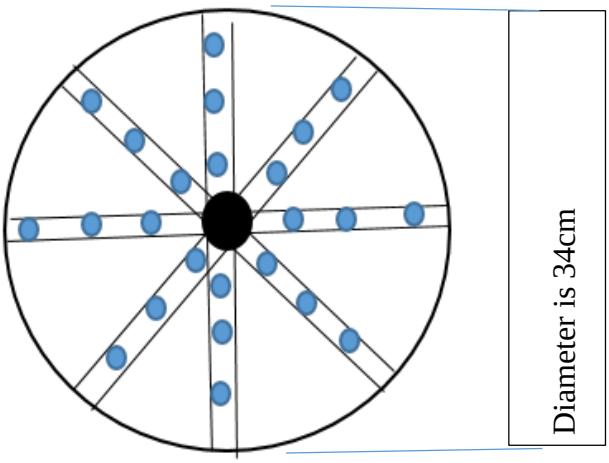

Figure 3.2: Network of inlet pipes overview

For the trickling filter the following items and parts were used:

i. Wood chips, maize cobs and sugarcane bagasse
ii. Pipes and joints
iii. valves
iv. Plumbing equipment

The tanks were packed with the substrates at different heights of 14cm, 18cm and 22 cm and were subjected to varying operational conditions such as: varying hydraulic retention time at an interval of 12, 24, 48 and 60 hours and substrate particle size in order to identify the most suitable condition for the removal of contaminants. The substrate sizes was distinguished by passing the substrates through a sieve. The particles that passed through the sieve were classified as group B consisting of smaller particles of size 30-38mm, while the retained particles were classified as group A consisting of larger particles of size 38-50mm.

The experiment was set as follows; at 14cm of substrate column height for group A, 38-50mm substrate particle size, the bioreactors were subjected to a contact time of 12, 24, 48 and 60 hours and samples were collected for all of the three substrates. Then, at 14cm

of substrate column height for group B the 30-38 substrate particle size, the bioreactors were subjected to a contact time of 12, 24, 48 and 60 hours and samples were collected for all of the three substrates. The same was repeated for the 18cm and 22cm substrate column height for both 38-50mm and 30-38mm substrate particle size. The results were automatically replicated by the spectrometer and the average values were used in the calculations.

3.4 Substrate Packing Materials

Three substrates were used in the study: woodchips, maize cobs and sugarcane bagasse. The substrates were used in their dried form. A comparison between the performances in pollutant removal of the substrate at different sizes was also studied.

The mass of the substrates was measured and recorded as shown in Table 3.1 as shown.

Table 3.1: Substrate mass

	Wood chips	Maize cobs	Sugarcane bagasse
14 cm	7.25 kg	3.28 kg	2.98 kg
18cm	9.45 kg	4.22 kg	3.69 kg
22cm	11.54 kg	5.15 kg	4.98 kg

3.5 Analysis of the Influent and Effluent

3.5.1 Sample Collection

Samples from each reactor were collected separately in labeled glass sampling bottles after every operating condition of water treatment. The collected samples were transported to the laboratory within the hour of collection and refrigerated. The samples were tested within 6 hours of collection to protect the integrity of the results. Three sample of the raw water was collected from the pond and tested before treatment so as to compare the performance of each bioreactor in order to determine the most efficient substrate and treatment condition.

3.5.2 Effluent Analysis

The samples were collected and analyzed after every operating condition which took place over a period of four months (July- October 2018). The operating conditions varied with reference to the following: varied particle sizes, column height and different hydraulic retention time at an interval of 12h, 24h, 48h and 60h. The samples collected were analyzed for the following parameters:

i. Nitrates
ii. Nitrites
iii. Phosphates

The samples were prepared using laboratory standard procedure described in part 3.5.3 to 3.5.6. It was analyzed in the water resource laboratory at Sino-Africa-Joint-Research-Center (SAJOREC), Jomo Kenyatta University of Agriculture and Technology (JKUAT) using an ultra violet and visible light spectrometer UV VIS (model 1800 simdzu) to test for the levels of nitrates, nitrites and Phosphates. The analysis of parameters was done using the procedure in the Standard Methods for the Examination of Water and Wastewater 1999 handbook by Rodger *et al*, (2017)

3.5.3 Nitrate Analysis

The spectrometer was calibrated using a standard nitrate solution. A stock nitrate solution was prepared using anhydrous potassium nitrate, 0.72g of KNO_3 was dissolved in 1000ml of distilled water. An intermediate solution was prepared from the stock solution by diluting 100ml of the stock solution in 1000ml distilled water. The intermediate solution was used to prepare standard solutions by diluting the following volumes to ratios 50ml: 0, 50:1, 50:2.5.50:4 and 50:5 ml. These solutions were used to calibrate the spectrometer. The measurements was read against the distilled water transmittance set at zero at a wavelength of 220nm. After the calibration the samples were then tested. 50ml of sample was measured and 1ml of HCL was added to it as a

reagent then this prepared sample was analyzed in the spectrometer and the results recorded.

3.5.4 Nitrite Analysis

The analysis of nitrite used two analytical techniques; Colorimetric analysis where a colored reagent was prepared and spectroscopy where the colored solution was analyzed. The color reagent was prepared by adding 100ml 85% Phosphoric acid to 800ml of distilled water. 10g of sulfonamide was added and dissolved completely then 1g of N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) was added to the solution. It was then diluted to 1000ml and the solution was stored at -4^0C. A standard nitrite solution was prepared by Dissolving 1.232 g of $NaNO_2$ in water and diluted to 1000 ml, this was preserved with 1 ml $CHCl_3$. Intermediate nitrite solutions were prepared from the stock solution as follows: 50.00 ml of standard 0.01M $KMnO_4$, 5 mL conc H_2SO_4, and 50.00 mL stock NO_2– solution were measured into a glass- flask. This was then diluted to 1000ml. Nitrite standard solution was then prepared from this by diluting the following volumes to the ratio 50ml: 0, 50:1, 50:2.5.50:4 and 50:5 ml this was used to calibrate the spectrometer for nitrite analysis.

Once the spectrometer was calibrated the samples were analyzed. 2ml of NED solution was added to 50ml of the sample and allowed to set for 20min to allow for color development before it was analyzed using the calibrated spectrometer.

3.5.5 Phosphate Analysis

The reagents were prepared according to Rodger *et al.,(*2017) as follows: 70 ml conc H_2SO_4 was diluted to 500 ml distilled water, 1.3715 g of Potassium antimonyl tartrate was dissolved in 400ml distilled water then diluted to 500ml, 1.76 g of ascorbic acid was dissolved in 100 mL distilled water and 20 g of Ammonium Molybdate was diluted in 500 mL distilled water and stored in a glass-stoppered bottle. The four reagents were then

mixed in the following proportion: 50 mL 5N H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium Molybdate solution, and 30 mL ascorbic acid solution.

The phosphate stock solution was prepared by dissolving in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 ml. Using the stock solution the standard solution was prepared by dissolving 50ml of the stock solution in 1000ml of distilled water to a ratio of 50ml: 0, 50:1, 50:2.5.50:4 and 50:5 ml. The samples were prepared by adding the mixed reagent and allowing them to set for 10min before analysis. The samples were then analyzed and the results recorded.

3.6 Hydraulic Retention Time

To achieve the variation in the HRT, the valves were adjusted to allow a certain amount of flow per hour using iteration of trial and error method as described by (Lepine *et al*., 2016). According to Hochheimer and Wheaton, (2000) theory that states "an optimal theoretical Hydraulic retention time for a wastewater treatment is 12hours". Therefore, the same approach was adopted and the bioreactor was subjected to different retention time at intervals of 12 hours for each operation (height of the column and substrate particle size) so as to determine the most suitable retention time. To achieve this, the loading flow rate and volume was varied, such that to increase the retention time small volumes were loaded at a low flow rate this was adopted from the research done by Lepine *et al.* (2016). To reduce the retention time large volumes of wastewater were loaded at a high flow rate. The optimal HRT was then determined using a model linear equation obtained from the plotted Concentration-Time graphs

Table 3.2: Loading Rate for each contact time

Sn	Time	Working (volume/time)	Loading rate(liters/hour)

1	12	12/12	1
2	24	12/24	0.5
3	48	12/48	0.25
4	60	12/60	0.2

The loading rate values on Table 4.1 were used to set the valves to the specific HRT desired.

3.7 Data Processing and Analysis

Once the samples were collected and tested, the data obtained was analyzed and presented using statistical techniques. The data was tabulated and generated into graphs

ANOVA was used to test if there is a significant difference in the efficiency of:

1. Bioreactors of the same substrate at different height of substrate at a constant time HRT.
2. Bioreactors of same substrates at different HRT at a constant height H.
3. Bioreactors of different substrates at constant height H and time HRT.

The efficiency of the trickling filters was calculated using model equations. The efficiency of nitrate removal was calculated in percentage form using the efficiency equation as described in Equation 3.5 that was derived in Equations 2.11.

$$\%\boldsymbol{E} = \frac{\boldsymbol{X_N - X}}{\boldsymbol{X}} * \mathbf{100} \tag{3.5}$$

Where:

$\boldsymbol{\%E}$ = percentage efficiency

$\mathbf{X_N}$ = least value of contaminant detected (mg/l)

X = value of contaminant in raw water (mg/l)

The efficiency of phosphate adsorption was fit into the Laguimur adsorption isotherm model as illustrated using Equation 3.6. This was derived from Equation 2.13.

$$\frac{c_e}{q_e} = \frac{c_e}{x_m} + \frac{1}{bx_m} \qquad (3.6)$$

Where:

q_e= adsorption amount per unit weight (mole/g)

C_e= concentration of the analyte post treatment (mole/g)

x_m = Langmuir constant related to adsorption capacity. Obtained graphically.

3.8 Evaluation of Optimal Conditions

The aim of this research was to evaluate the different variables in the design of a bioreactor including the substrate column height, the contact time and the substrate particle sizes. Therefore, the optimal hydraulic retention time of a substrate was a function of the column height and substrate particle size. With two sets of groups of data, (the varied particle height and varied contact time), analysis of variance was the best statistical technique to use to determine whether there is any significant difference in the performance of the bio reactor at varied operating conditions. The information obtained from the analysis of variance and other statistical graphs were used in developing a concentration - Time graph with a trend line showing the mathematical equation model. The equation was then used to determine the HRT at a given desired efficiency.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Sizing and Fabrication

The system operation was designed by considering the functional operation conditions as follows:

a) Analysis of different substrate sizes (with 38-50mm and 30-38mm substrate particle size).

b) Varied hydraulic retention time (at an interval of 12, 24, 48 and 60 hours).

c) Varied column height of the substrate (at 14, 18 and 22 cm substrate column height).

4.1.1 Set Up of the Trickling Filter Bioreactors

The trickling filter was set up such that the retention tank was 1.0m above the ground and the reactor tanks were 0.4m above the ground placed on a metallic frame stand. These height difference allowed the water to flow by gravity from the retention tank to the reactor tanks. PPR pipes with a diameter of ¾ inches were used and valves were used to regulate the flow of water. Trial and error method was used in adjusting the valves to manipulate to the desired flow rate and to achieve the set contact time of 12h, 24h, 48h and 60h as stated in the methodology. The reactor tanks were labeled and packed with the required substrate materials as shown in the Plate 4.1.

Plate 4.1: Bio reactor system

4.2 Evaluation of Optimal Substrate Conditions

4.2.1 Characteristics of Wastewater

The influent wastewater (raw water) was collected in sample bottles and tested in a set of three replications. The mean value± standard deviation was compared to set standards as presented in Table 4.2.

Table 4.1: Raw Water Contaminant Levels

Sn	Parameters	Mean ± SD	Standards (EMCA third schedule)
1	Nitrates	208±1.24 mg/l	10mg/l
2	Nitrites	24.5±0.4mg/l	10mg/l
3	Phosphates	20.66±0.31mg/l	5mg/l
4	BOD	152.6±2.4mg/l	30mg/l
5	COD	126±2.1mg/l	30mg/l

6	Temperature	22±0.4^{o}C	22^{o}C
7	PH	6.33± 0.16	5-8

From Table 4.2, it can be seen that for all water quality parameters under consideration, that is Nitrates, Phosphates, BOD, COD results from sampled raw aquaculture wastewater were above set standards. Temperature and PH were within the required levels. These results shows that there was need for the wastewater to be treated before disposal. Many authors have reported similar findings: According to Keramat (2008) effluent from fish ponds contain nutrients particularly nitrates and phosphates that leads to eutrophication of recieving water bodies. Siddiqui (2017) and Feng *et al*. (2016) reported that aquatic wastewater is characterised by high precence of nitrogen species contaminants from the fertilizer, feed biomass and excreter. According to Mburu *et al.* (2019) the aquatic wastewater from fish ponds in Lake Victoria region is high in nutrients responsible for eutrophication and overgrowth of water hyacinth.

4.3 Results of 30-38 mm and 38-50 mm particle size of subtrate

Table 4.3: Concentration of nitrites (mg/l) in the effluent from bioreactor at varied HRTs, column height and particle size

		38-50 mm particles size			30-38mm particles size		
		14 cm	**18 cm**	**22 cm**	**14 cm**	**18 cm**	**22 cm**
	0h	24.5	24.5	24.5	24.5	24.5	24.5
	12h	16.7	10.8	7	13.9	8.6	5
	24h	3.6	0.8	0.6	2.9	4.1	3.1
	48h	0.3	0.06	0	0	0	0
woodchips	**60h**	0.04	0.01	0	0	0	0
	0h	24.5	24.5	24.5	24.5	24.5	24.5
	12h	18.8	12.1	10	17.5	10	8
	24h	7.2	1.6	1.1	5.9	4.9	4.8
	48h	2.9	0.5	0.4	0.9	0.6	0.6
maize cobs	**60h**	0.7	0.09	0	0	0	0

	0h	24.5	24.5	24.5	24.5	24.5	24.5
	12h	15.5	12	10.3	14	9	7
	24h	1.4	1.2	0.8	4.1	3.7	2.1
	48h	1.2	0.81	0.2	0.8	1.3	0.4
sugarcane	**60h**	0.9	0.21	0	0.4	0.2	0

Table 4.4: Concentration of nitrates (mg/l) in the effluent from bioreactor at varied HRTs, column height and particle size

		38-50 mm particles size			**30-38 mm particles size**		
	Time (hours)	**14cm**	**18cm**	**22cm**	**14cm**	**18cm**	**22 cm**
	0h	208	208	208	208	208	208
	12h	205	131	88	189	76	65
	24h	132	92	65	121	59	36
	48h	89	81	43	71	44	15
woodchips	**60h**	60	56	19	54	31	7
	0h	208	208	208	208	208	208
	12h	209	145	98	196	98	78
	24h	199	136	69	187	78	42
	48h	160	112	48	149	54	24
maize cobs	**60h**	89	81	26	78	43	11
	0h	208	208	208	208	208	208
	12h	206	128	104	201	118	86
	24h	201	98	81	182	87	54
	48h	184	91	68	129	79	27
sugarcane	**60h**	130	86	34	92	65	13

Table 4.5: Concentration of phosphates (mg/l) in the effluent from bioreactor at varied HRTs, column height and particle size

		38-50 mm particles size			30-38mm particles size		
	Time (hours)	14cm	18cm	22cm	14cm	18cm	22 cm
	0	20.56	20.56	20.56	20.55	20.56	20.56
	12	22.06	22.93	34.54	29.95	28.13	72.70
	24	24.23	39.84	61.63	42.65	36.58	90.01
	48	28.47	45.87	83.78	67.00	51.15	105.98
Woodchips	**60**	31.16	54.33	116.41	86.30	70.90	158.96
	0	20.56	20.56	20.56	20.55	20.56	20.56
	12	21.70	22.83	25.86	25.09	27.04	38.56
	24	22.06	24.83	36.58	30.93	27.64	54.26
	48	22.63	31.27	43.38	43.60	32.28	79.59
maize cobs	**60**	24.46	36.58	67.43	54.30	40.03	93.49
	0	20.56	20.56	20.56	20.55	20.56	20.56
	12	21.19	21.88	25.59	31.10	28.30	54.26
	24	23.77	23.89	33.24	34.03	31.57	69.79
	48	24.33	29.74	37.87	42.36	37.94	85.55
sugarcane	**60**	29.18	34.27	45.81	55.21	54.64	90.46

4.4 Substrate Performance Evaluation

The performance of the different substrates (woodchips, maize cobs and sugarcane bagasse) was tested at the different operating conditions of contact time (12h, 24h, 48h and 60h), different heights (14cm, 18cm and 22cm) of substrate and different particle sizes. The mean values of contaminants was used in calculating the efficiency from Equation 3.5. The required efficiency for the removal of each contaminant was calculated using the contaminant concentration and desired minimum values as listed in Table 2.2 standards of effluent before discharge and the efficiencies were as shown in Table 4.6.

Table 4.6: Required efficiency for the removal of contaminants

Substrate	Equation	% efficiency
Nitrate	(210-10)/210	95
Nitrite	(25-10)/25	60
Phosphate	(21-10)/21	50

The performance efficiency as a percentage for each bioreactor in the removal of various pollutants by each substrate at a given operating condition is tabulated and the results presented in the form of figures. The results from were compared with those presented in Table 4.6. The objective of undertaking the comparison was to be able to identify the most suitable operating conditions that can achieve the required efficiency of 95% for nitrate removal. The APHA listed the desired percentage of microbial denitrification of wastewater as 70%. Further, it was also aimed at achieving the removal of nitrites and phosphate to the desired efficiency levels listed in EMCA water quality regulation schedule of about 60% and 50% respectively.

4.4.1 Nitrates removal

The graphs in Figure 4.1 to 4.6 shows the performance of the different bioreactors in the removal of nitrates at different operating conditions. The values on the graph are the mean experimental value obtained from three replications of the experiment.

4.4.1.1 Nitrate removal at Varied Column Height

Figures 4.1 and 4.2 Show the plotted results of nitrate removal by 38-50 mm particles and 30-38 mm particles of the substrates respectively at 14cm column heights.

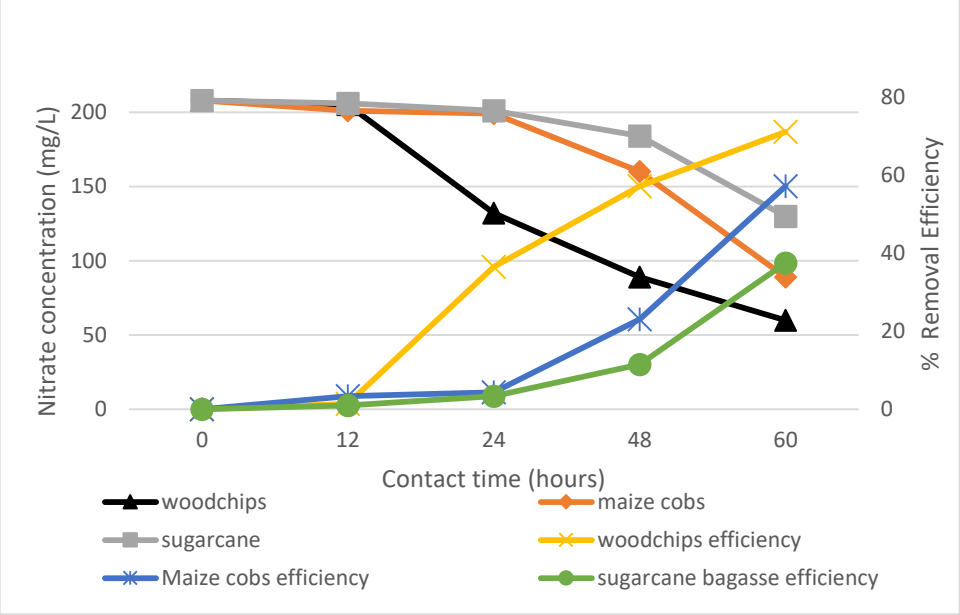

Figure 4.1: Nitrate concentration/efficiency at 14cm (38-50mm substrate particle size)

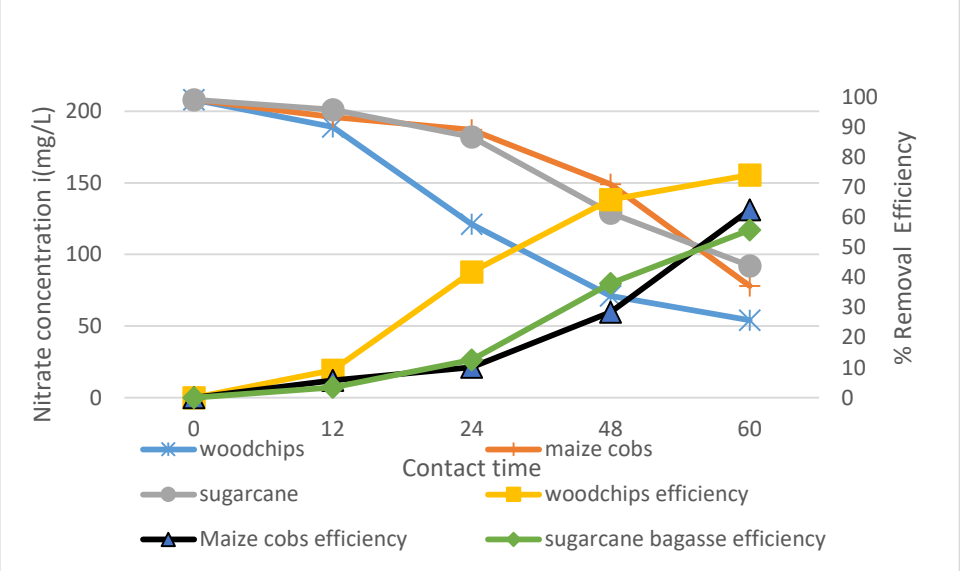

Figure 4.2: Nitrate concentration/efficiency at 14cm height (30-38 substrate particle size)

From Figure 4.1 and 4.2 the nitrate concentration reduces with increase in the contact time from 0 hours to 60 hours. However, at 14 cm column height the desired effluent

concentration of 10mg/L was not achieved. At 60 hours contact time the effluent concentration were 60±0.5mg/L, 89±0.5mg/L and 130±0.5mg/L for wood chips, maize cobs and sugarcane bagasse respectively for the large particles and 54±0.5mg/l, 78±0.5mg/L and 92±mg/L for wood chips, maize cobs and sugarcane bagasse respectively for 30-38mm substrate particle size. Wood chips had the highest efficiency of 71.15% in the 38-50mm substrate particle size bioreactor and 74.3% in the 30-38mm substrate particle size bioreactor. At these efficiencies the quantity of nitrate removed was 148mg/L and 154mg/L for the both particle sizes of woodchips respectively. These efficiencies are lower than the desired efficiency of 94%. The substrate column height was increased to 18cm to evaluate if there would be an increase in the efficiency. The results for 18cm column height are presented in Figure 4.3 and 4.4 for 38-50mm and 30-38mm substrate particle size respectively.

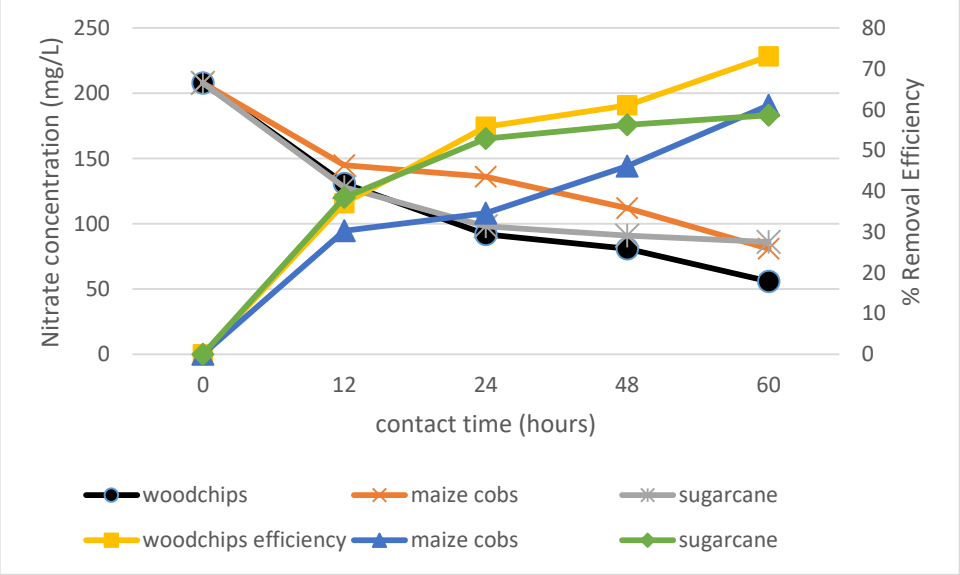

Figure 4.3: Nitrate concentration/efficiency at 18cm column height (38-50mm particle size)

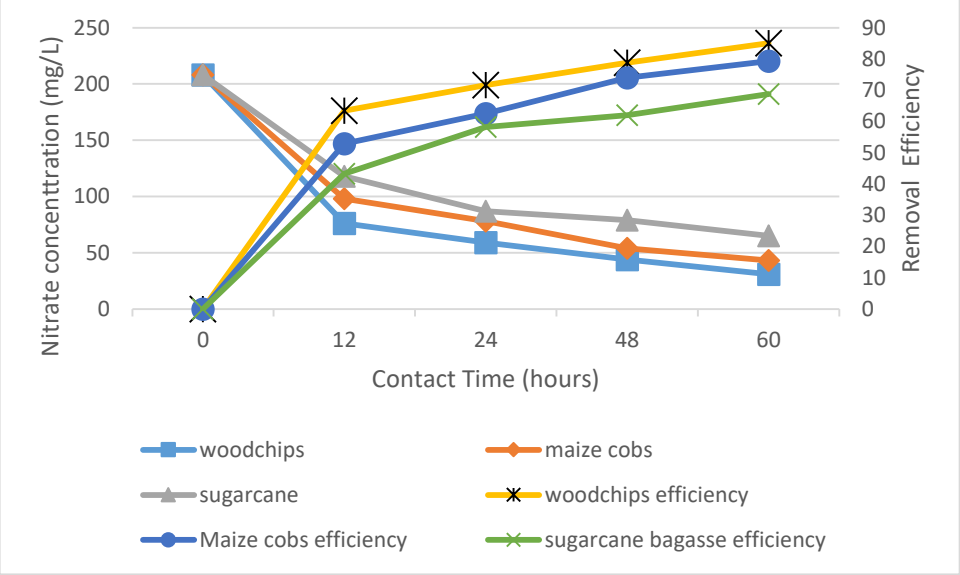

Figure 4.4: Nitrate concentration/efficiency at 18cm column height (30-38 mm particle size)

At 18cm substrate column height, the effluent concentration reduced at each contact time compared to the 14cm column height. The 30-38mm substrate particle size bioreactors were more efficient than the large particle bioreactors at constant contact time. The effluent concentration at 60 hours in the 30-38mm substrate column height bioreactors were 31±0.5mg/L, 43±0.5mg/L and 65±0.5mg/L for woodchips maize cobs and sugarcane bagasse. The large particle bioreactor at corresponding operating conditions had concentrations of 56±0.5mg/L, 81±0.5mg/L and 86±0.5mg/L for woodchips, maize cobs and sugarcane bagasse respectively. Despite the increase in the efficiency compared to the 14cm column height, the desired efficiency is still not achieved with the highest efficiency being 74% and 85% for the 38-50mm and 30-38mm, woodchips respectively. The column height was further increased to 22cm column height and the results are presented in Figure 4.5 and 4.6.

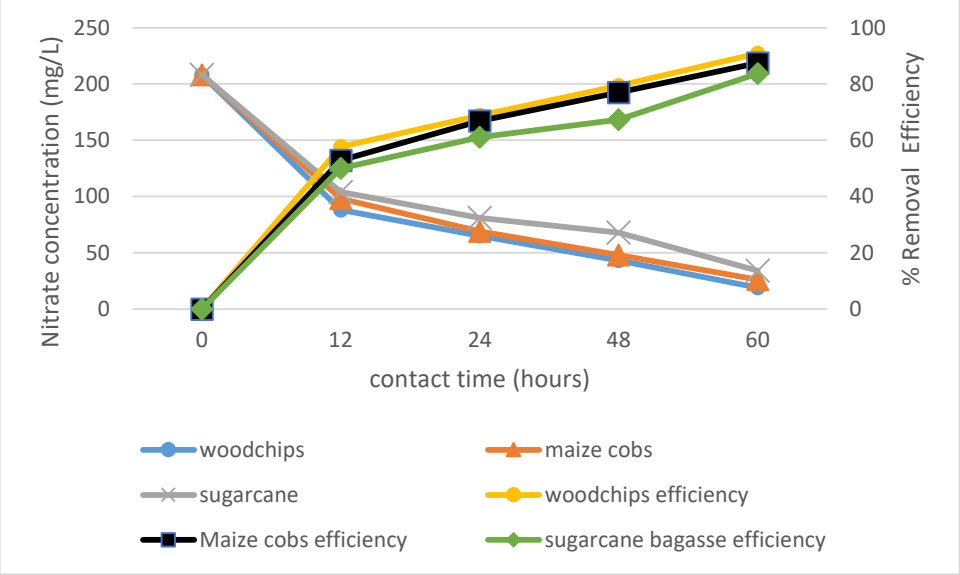

Figure 4.5: Nitrate concentration/efficiency at 22cm column height (38-50mm substrate particles size)

Figure 4.6: Nitrate concentration/ efficiency at 22cm column height (30-38 mm particle size)

From Figures 4.5 and 4.6, 22cm height of substrate for both groups of particle size yields the best results in the removal of nitrates from the wastewater. The 30-38 mm woodchip

bioreactor was most suitable at the operating conditions of 22cm substrate column height and a contact time of 48 hours at this point only 15± 0.5 mg/L of nitrate was present in the effluent.

The efficiency archived by bio reactor with 30-38 mm particle sizes at a column height of 22cm at 60 hours was 95.8%. At 48 hours and 22cm substrate column height, woodchip had an efficiency of 93.8%. To achieve the maximum efficiency with the 38-50 mm particle bioreactor there was need to increase the contact time for all the three substrates. The performance of the 30-38 mm particles was better than that of the 38-50 mm particle when they are subjected to constant time and column height. This is because the smaller particles provide a large surface area for the reaction hence increasing the efficiency of the bioreactors (Azizi and Sithebe, 2013). According to Ali *et al*., (2016), smaller particle substrate provide a wider adsorptive suface for the removal of conterminants.

From the graphs, it can be seen that the mean value of nitrates recorded at the different contact time shows that the performance of the bioreactors in the removal of nitrates increases with the increase in the time of contact and the depth of substrate for all the three bioreactors. The concentration of nitrate in the effluent at 12 hours contact time was higher than the level recorded at subsequent hours of 24, 48 and 60 hours for all the three substrates. The percentage conversion of nitrate to nitrogen in water increases with increase with the contact of the wastewater with the denitrifying microorganisms (Shih *et al.*, 2011). Hence, increase in the contact time increases the efficiency of the bioreactor in the removal of nitrates. Therefore, at 12 hours the microoganisims were still growing and therefore, the rate of nitrate removal was low. The sudden increase in the rate of removal between 12 hours and 48 hours is attributed to the balooning microbial population leading to a high rate of microbial action (Azizi, Valipour, and Sithebe, 2013).

Woodchips were more efficient compared to the other substrates. This is because woodchips are characterized with very high carbon concentration in the form of lignin and cellulose therefore provide more energy for the microbial population. Sugarcane and

maize cobs have cellulose and hemicellulose, a less complex carbon structures and are easily depleted (Greenan*et al*,, 2006: Kaetzl, *et al*., 2018). It is for this reason, that woodchip has a high efficiency of nitrate removal than sugarcane bagasse and maize cobs. The results were further subjected to statistical comparison as presented in Table 4.7.

Table 4.7: 2-way T-test values using 0.5 level of significance

Set of comparison	T- calculated	T-tabulated	Remark
Wood chips of 30-38 mm and 38-50 mm particles at 22cm and varied HRT	0.295425	0.741	There is no significant difference
Comparison of woodchips at 60 hours and varied height	0.825	0.816	There is a significant difference
Maize cobs and sugarcane	0.829557	0.741	There is a significant difference

Woodchips are the most efficient substrate in the removal of nitrates in both 38-50mm and 30-38mm substrate particle size bioreactors. At the Contact time of 48 hours the result obtained were as follows: 27±0.5mg/l and 24±0.6mg/l of sugarcane and maize cobs respectively and 43±0.5mg/l and 15±0.5mg/l in both particle size of woodchip bioreactors respectively. There is a difference in the removal of nitrates by varying the substrate column height as illustrated in the Table 4.7 and ANOVA tables in Appendix 1 and 2. According to (Ponnada *et at*., 2017), wood chip bioreactors can achieve 80-99% nitrate removal from wastewater. By keeping other operation conditions constant and manipulating only the substrate particle size the results show that the bioreactor with 30-38 mm particle had a better performance in the removal of nitrates than the bioreactor with 38-50 mm particles for all the three substrates. This means that the performance of 30-38 mm substrate is higher than that of 38-50 mm substrate particles in a bioreactor. Ponnada *et al.*, (2017) states that the larger the surface area in contact with the wastewater the more

efficient the system is in the removal of nitrogen species contaminants therefore the 30-38 mm particles had more efficiency.

4.4.2 Nitrites Removal

The effluent was tested for nitrites at each level of operating condition. The data was analyzed and the mean values were used to generate the following graphs listed as Figure 4.7 to 4.12.

4.4.2.1 Nitrite Removal at Varied Column Height

The analysis of nitrite removal at 14 cm substrate column height was done and result presented in Figures 4.7 for the 38-50 mm substrate particles and 4.8 for the 30-38 mm substrate particles respectively.

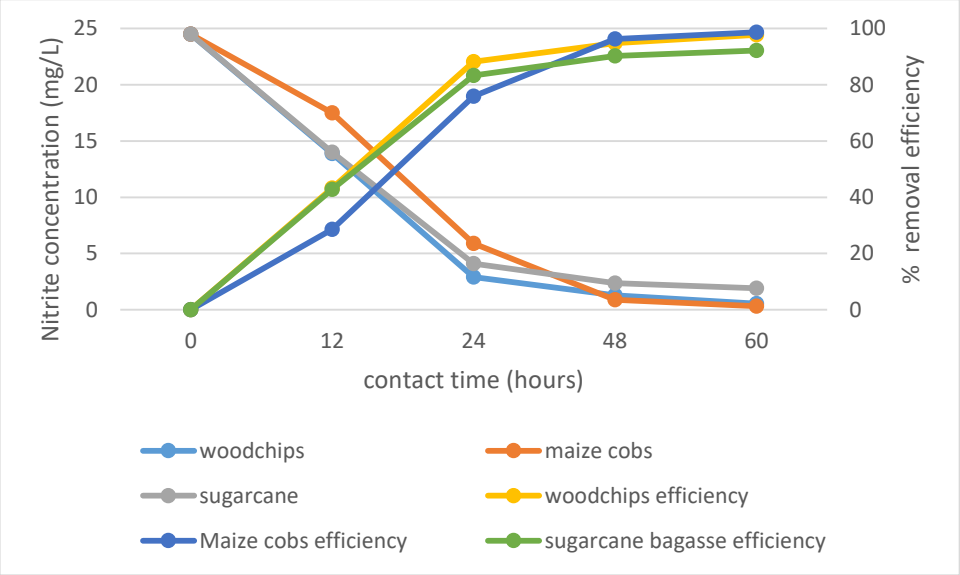

Figure 4.7: Nitrite concentration/efficiency at 14cm column height (38-50 mm**)**

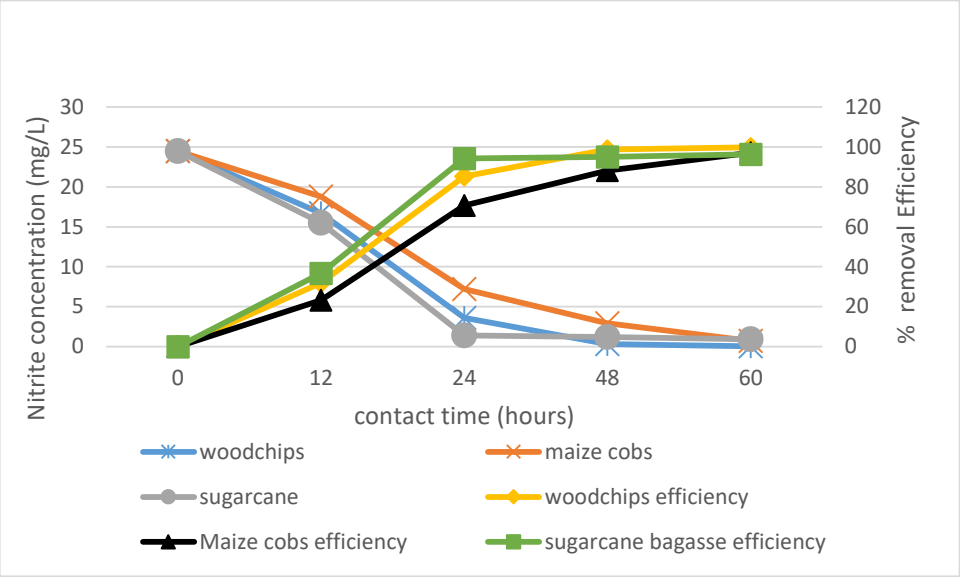

Figure 4.8: Nitrite concentration/efficiency at 14cm column height (30-38mm substrate particle size)

From Figure 4.7 and 4.8, the nitrite concentration reduces with increase in the contact time. At 24 hours the effluent concentration were 3.9± 0.5 mg/L, 4.1± 0.5 mg/L and 5.5± 0.5 mg/L for the small substrate particles of woodchips, maize cobs and sugarcane bagasse. The following concentrations were recorded as 4.6± 0.5 mg/L, 7.2± 0.5 mg/L and 15.5± 0.5 mg/L of nitrites for the 38-50 mm substrate particle of woodchips, maize cobs and sugarcane bagasse respectively. By 48 hours, the concentration of nitrites were less than the maximum allowable limit for the 38-50 mm substrate particles. For the 30-38 mm substrate particles, at similar operating conditions, the concentration was less than 5mg/L. Woodchips had the highest efficiency in both cases recording 95% and 98% for the 38-50 mm and 30-38 mm substrate particles respectively. Figure 4.9 and 4.10 for the 18 cm column height respectively.

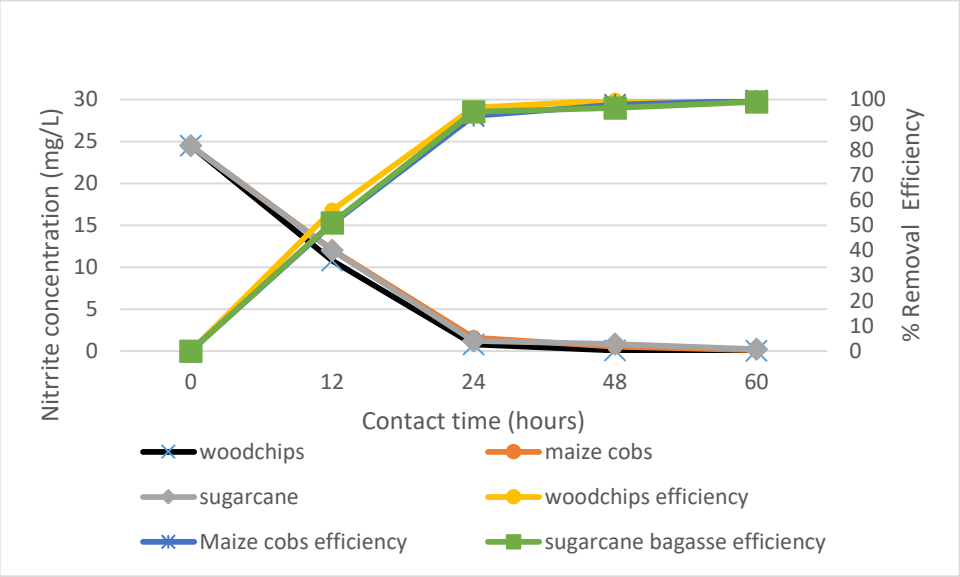

Figure 4.9: Nitrite concentration/efficiency at 18cm column height (38-50mm substrate particle size)

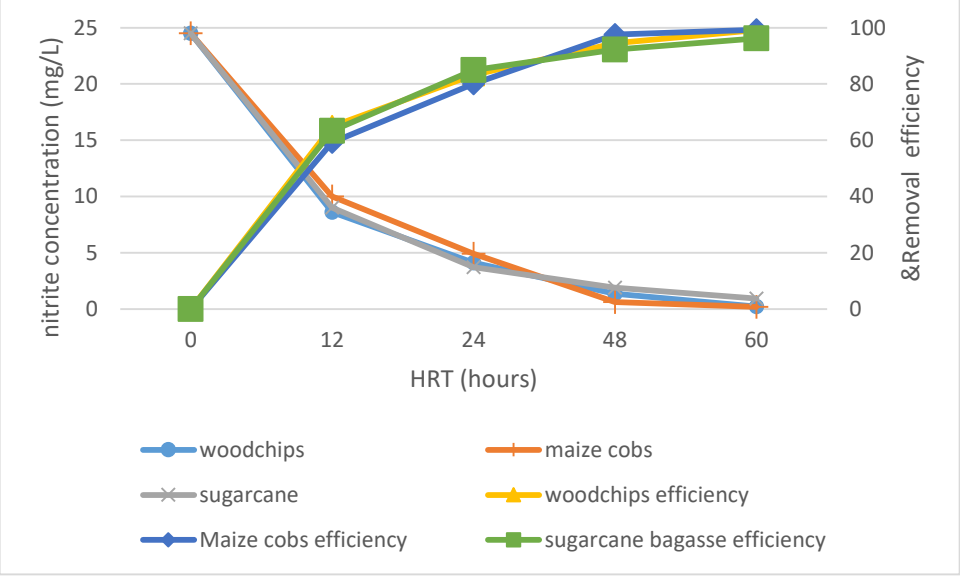

Figure 4.10: Nitrite concentration/efficiency at 18cm column height (30-38mm substrate particle)

From Figures 4.9 and 4.10 the nitrite concentration reduces with increase in the contact time. At 18cm substrate column height the efficiency increases compared to the corresponding operating condition at 14cm column height. Nitrite removal by all the substrates was achieved by a HRT of 24hours for all the column height and particle size. Figures 4.11 and 4.12 presents the removal of nitrites at 22cm substrate column height for the both substrate particles sizes.

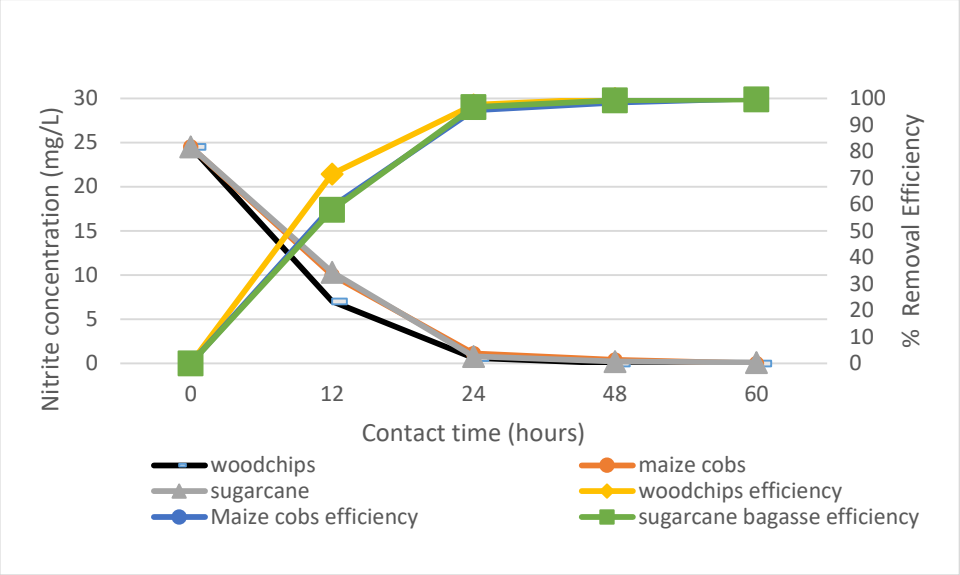

Figure 4.11: Nitrite concentration/efficiency at 22cm column height (38-50mm substrate particle size)

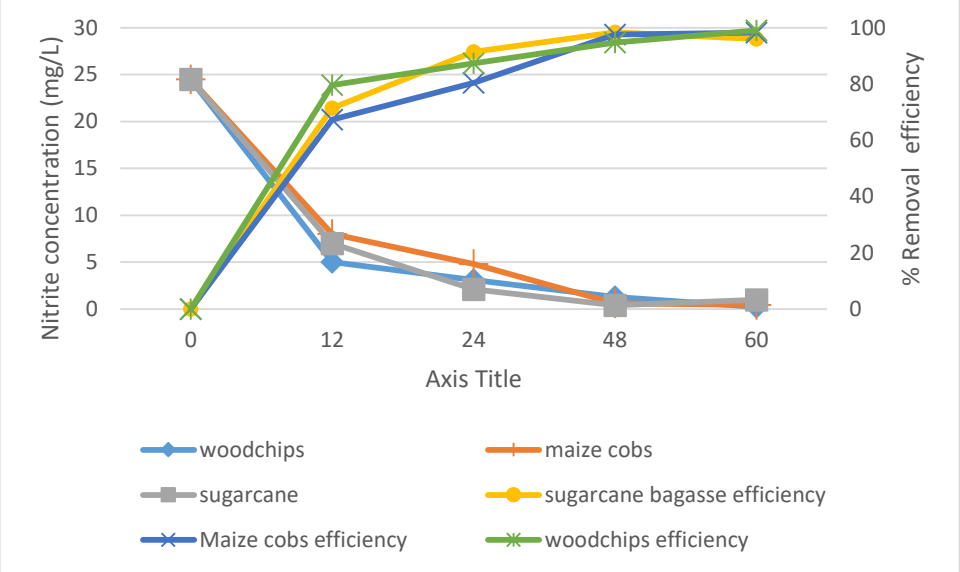

Figure 4.12: Nitrite concentration/ efficiency at 22cm column height (30-38mm substrate column height)

At 12hours, 22cm substrate column height of both 38-50 mm and woodchip particles is less than 5±0.5mg/l. The 18cm and 14cm column height of both particle size of woodchips particles size gets to less than 1±0.5mg/l at 18 hours and 24hours HRT respectively.

The removal of nitrite increases with increase in the contact time to from 0 hours to 24 hours, beyond which the removal rate appears to be constant in all cases. By 24 hours, most of the nitrite had been removed from the wastewater resulting in a drop of concentration from 25mg/l to less than 10mg/l. At 22cm column height of small substrate particle sizes was the most efficient. When the HRT was set at 12 hours the concentration of nitrite recorded was 4.6±0.5mg/l. The best operating condition in the removal of nitrite is at 24 hours 18cm of 38-50mm substrate particles which yields concentration of nitrite less than 1±0.5mg/l.

For all the three substrates, the corresponding mean values of nitrite concentration present in the effluent at 24 hours was less than the required maximum level. For both substrate particle size bioreactors, the efficiency of each substrate in the removal of nitrite is very

high with minimum values achieved at contact time of 24hours. The same outcome was observed by Lepine *et al.* (2016). Who concluded that between 12 and 24 hour the rate of conversion of nitrite to nitrate was maximum. The authors further concluded that the levels of nitrites present in the effluent were 1-2% that of the nitrates, indicating that the values of nitrites will always be lower than that of nitrates. This is because nitrites are highly unstable and they quickly oxidise to nitrates (Azizi, Valipour, and Sithebe, 2013). The performance of the of the larger particles (38-50mm) was compared to that of the 30-38 mm particles size at constant height and time and the results show that that there is minimal variation of efficiency in removal of nitrites of bioreactors with varied substrate particle size. This is because the nitrites are easily converted to nitrates by the denitrifying bacteria. For this reason, the nitrites is not a suitable parameter to determine the performance of the bioreactors.

4.4.3 Phosphate Removal

The effluent was tested for phosphate concentrations and the mean values of three replicates per each sampling was used in developing the relationships presented in the graphs listed under Figures 4.13 to 4.18.

4.4.3.1 Phosphate Removal at Varied Column Height

The concentration of phosphate was tested at 14cm substrate column height and results presented in Figure 4.13 and4.14 for the both particle sizes.

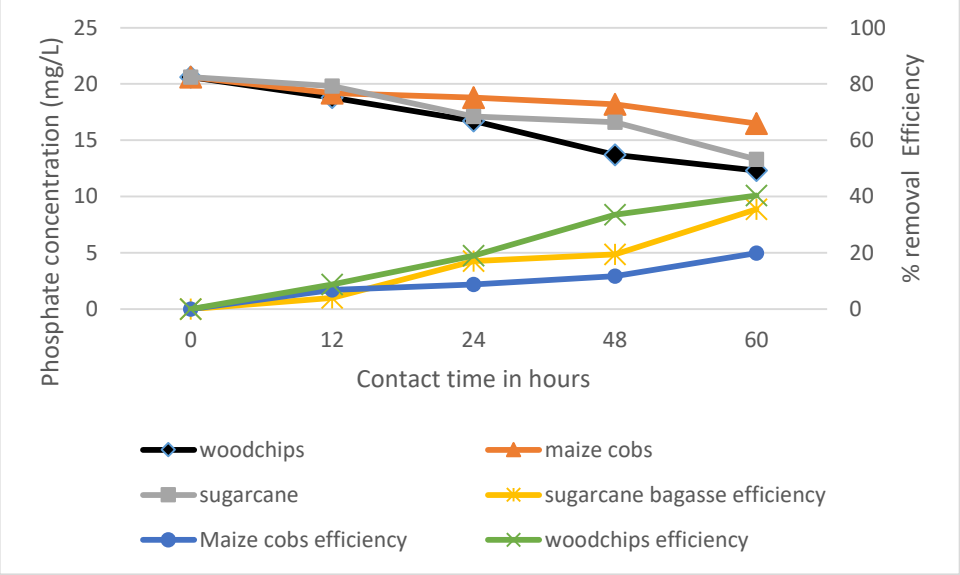

Figure 4.13: Phosphate concentration/efficiency at 14cm column height (38-50mm substrate particle size)

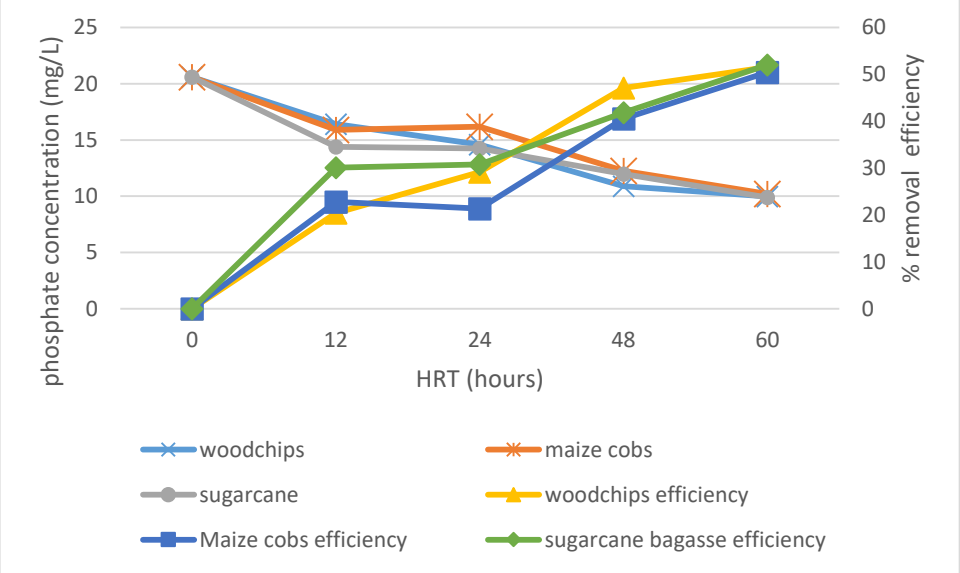

Figure 4.14: Phosphate concentration/efficiency at 14cm column height (30-38mm substrate particle size)

From the Figures 4.13 and 4.14, the concentration of phosphate reduces with increase in the contact time. However, it takes longer to achieve the desired concentration of less than 10mg/l for both groups of substrate particles at 14cm substrate column height. At 60 hours, the concentrations recorded were 12± 0.5 mg/l, 16± 0.5 mg/L and 13± 0.5 mg/L for 38-50mm particles size of woodchips, maize cobs and sugarcane bagasse respectively. At similar conditions, the 30-38mm substrate particle yielded concentrations of 9± 0.5 mg/L, 10± 0.5 mg/L and 11± 0.5 mg/L for woodchips, maize cobs and sugarcane bagasse respectively. For 18cm column height the results are as shown in Figures 4.15 and 4.16 for the both particles sizes.

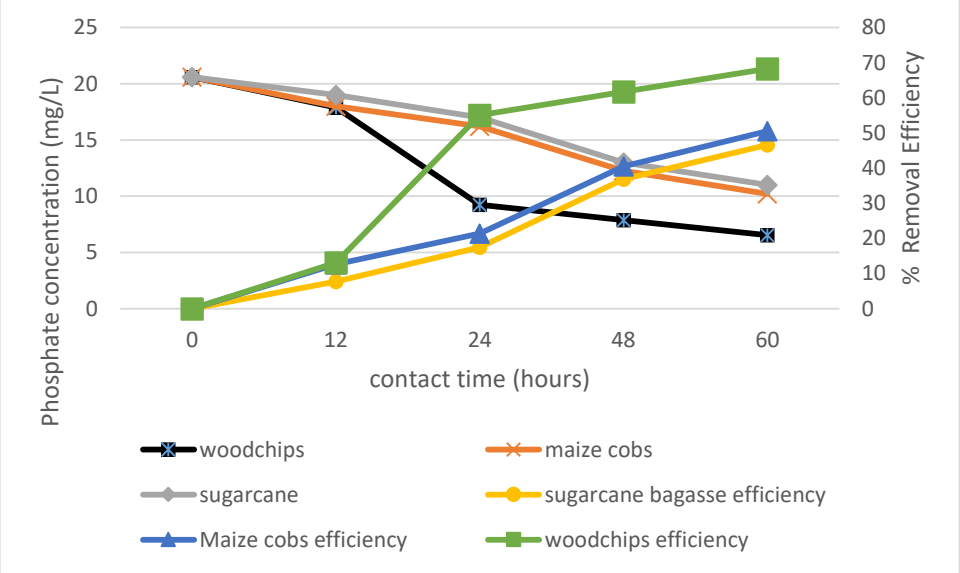

Figure 4.15: Phosphate concentration/efficiency at 18cm column height 38-50mm substrate particle size)

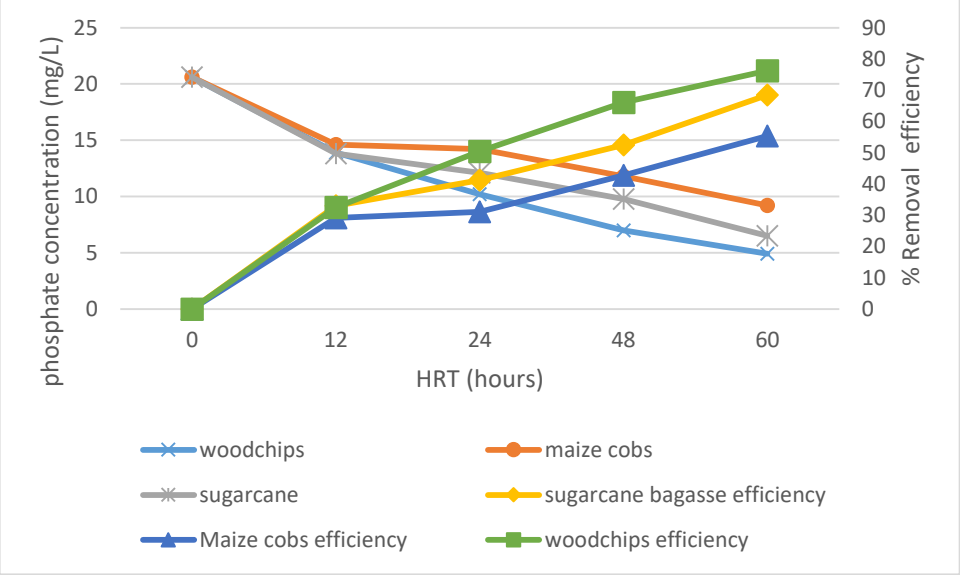

Figure 4.16: Phosphate concentration/efficiency at 18cm Column height (30-38mm substrate particle size)

Figures 4.15 and 4. 16 show the efficiency in the removal of phosphate at 18cm substrate column height. At 60 hours the efficiencies were 76%, 68% and 55% for the small particles of woodchips, maize cobs and sugarcane bagasse respectively. This is higher than the efficiency of the large substrate particle bioreactors which yielded 69%, 50% and 46% at 60hours for woodchips, maize cobs and sugarcane bagasse respectively. This therefore means that the performance of smaller substrates is better than the performance of larger substrates because the smaller the substrate the larger the surface area of action (Azizi, Valipour, & Sithebe, 2013). 22cm column height results were presented in figure 4.17 and 4.18 for both substrate particle respectively.

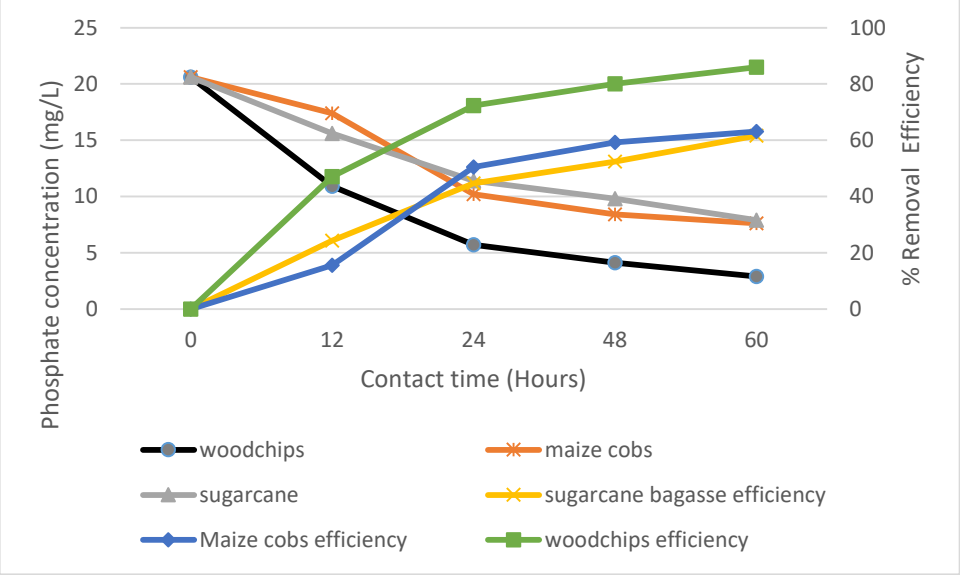

Figure 4.17: Phosphate concentration/efficiency at 22cm column height (38-50mm substrate particle size)

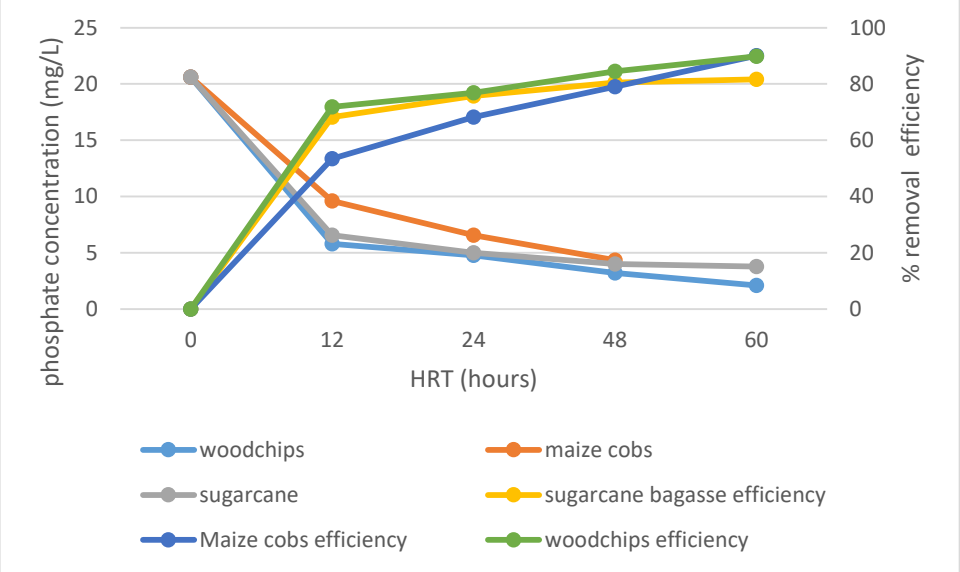

Figure 4.18: Phosphate concentration/efficiency at 22cm column height (30-38mm substrate particle size)

The results in Figure 4.17 and 4.18 show that at 22cm substrate column height, both particle sizes had high phosphate removal rates. For both particle size, a contact time of 24hours was enough to achieve 5.7±0.08mg/l and 4.77±0.05mg/l respectively for woodchips. The statistical range of the mean values of the efficiency at 12 hours was 5.1mg/l, and 1.03mg/l and 0.8mg/l for the 24 hours and 48 hours respectively. This concludes that the particle size of substrates does not influence the efficiency of the removal of phosphates. From the Figures 4.13 to 4.18, it is evident that the performance of the bioreactors increases with increase in the time of contact from 12 hours to 60 hours for all the three substrates. At maximum substrate column height of 22cm for all the three substrates the bioreactors had the highest performance, corresponding to higher removal rate of phosphate.

Woodchips was the most efficient in the removal of phosphates, maize cobs was slightly suitable than sugarcane bagasse. For woodchips, the desired outcome is achieved by the HRT of 24 hours where the result is 4.7±0.5mg/l. While for maize cobs and sugarcane bagasse, the desired outcome is achieved at 48 hours HRT were the phosphate concentration present were 4.3±0.5 mg/l and 4.1±0.5mg/l respectively. The most suitable process of phosphate removal in bioreactors is through adsorption of complex ions (Joshua *et al.,* 2018). In this case the complex phosphate ions is broken down using microorganisms and the simple phosphate ions adsorbed by the carbon structures of the substrates. This means that the substrate with the highest carbon content has the potential of removing more phosphate pollutants. According to Sufia (2015), to increase the efficiency of bagasse in the removal of phosphate, it requires chemical modification such as pretreatment of the sugarcane to make its chemical structure more adsorptive. The sorptive capacity of a substrate depends on polarity, surface area and pore size (Kolondyska, *et al*., 2012). This explains why there is a variation in the efficiency of the system at varied operating conditions. Since the carbon structure and pore size of wood chips, maize cobs and sugarcane bagasse varies greatly their efficiency as bioreactor packing material and substrate also varies. The smaller the particles, the larger the surface

area and the better the efficiency as reported by Jiang and Graham (1998), Ali *et al*. (2016) and Lepine *et al.*, (2016).

4.4.4 Statistical Analysis of the Performance of each substrate

4.4.4.1 Woodchips

The efficiency of woodchips in contaminant removal was calculated using the mean values of nitrates, nitrites and phosphates obtained from the results, the optimal operating conditions of the 30-38mm wood chip particle size in the removal of all the parameters was at 22cm substrate column height 48 hours contact time. 18cm substrate column height 60 hours contact time and 22cm column height 60 hours contact time for the 38-50mm woodchip bioreactor. At these operating conditions, the efficiency was then determined using mean nitrate values as shown in Table 4.4.

Table 4.4: Efficiency values for optimal conditions for nitrate removal in woodchip bioreactor

Particle size	Substrate column height	Contact time	Mean nitrate values	Efficiency
Influent concentration			210 mg/l	
Group B(30-38 wood chips)	22cm	48	12±0.5mg/l	94%
Group B(30-38 wood chips)	18cm	60	21±0.5mg/l	89%
Group A(38-50 mm wood chips)	22cm	60	17±0.5mg/l	93%

Two of these three operating conditions achieve the desired efficiency of 90% as listed in Table 4.4. According to Lepine *et al*, (2016) and Mark *et al*, (2015), the efficiency of

wood chip bioreactor increases with increase in the HRT. This is bacause the lignin and celusose in the woodchip structure does not decompose at a high rate hence it is not depleted easily. These properties makes woodchip a suitable substrate in a bioreactor. Similar results were witnessed by Gold, Schipper, and Addy, (2012) where the efficieny of a woodchip bioreactors vary with change in the contact time, surface area and reactor/filtration depth. Increased surface area to volume ration allows a wider interface of the wastewatwer to get into more contact with the biofilm hence increasing the efficiency of the system

4.4.4.2 Maize cobs

The performance/efficiency of maize cobs as a substrate for polishing effluent was calculated and is presented in Table 4.5. For both the particles sizes (30-38mm and 38-50mm), the optimal operating condition in the maize cob bioreactor was obtained as 22cm substrate column height at the HRT of 48 hours. The 30-38 mm maize cob particles (30-38mm) and 22cm substrate column height at the HRT of 60 hours for the 38-50 mm maize cob particles (38-50mm). The efficiency was calculated based on these information.

Table 4.5: Efficiency values for optimal conditions in maize bioreactor

Particle size	Column height	Contact time	Mean nitrate values	Efficiency
group B (30-38mm maize cobs)	22cm	48 hours	21±0.5mg/l	90%
group A (38-50 mm maize cobs)	22cm	60 hours	24±0.5mg/l	88%
group B (30-38 maize cobs)	22cm	60 hours	18±0.5mg/l	92%

From the Table 4.5 the efficiency of the maize cob bio reactor increases with increase in the column height and the contact time. Schipper *et al.* (2010) had similar results and concluded that the maize cobs in the bioreactors require a large surface area and longer HRT to increase its efficiency Therefore, the efficiency of a maize cob bioreactor increase with surface area to volume ratio of the substrates.

4.4.4.3 Sugarcane bagasse

The efficiency values for the sugarcane bioreactor were calculated as tabulated in Table 4.6. These was at the optimal conditions for operation which were 48 hours and 60 hours at 22cm height in small substrate particles bioreactors, and 22cm substrate column height for the group A particle size at 60 hours HRT.

Table 4.6: Efficiency Values for Optimal Conditions in Sugarcane Bagasse Bioreactor

Particle size	Column height	Contact time	Mean nitrate values	Efficiency
group B (30-38 mm particles)	22cm	48 hours	20±0.5mg/l	90%
group A (38-50 mm particles)	22cm	60 hours	27±0.5mg/l	87%
group B (30-38mm particles)	22cm	60 hours	13±0.5mg/l	92%

Similar to maize cobs, the efficiency of sugarcane bagasse in the removal of contaminates increases with increase in HRT and substrate depth. Kizito et al., (2016) had simmilar results. Also sugarcane bagasse exhibited more adsorptive properties in the removal of pigment, phosphates compared to maize cobs .

4.4.5 Analysis of the Optimal Operating Conditions for Nitrate Removal

The operating conditions were manipulated at three levels including the substrate particle size, substrate column height and HRT. The research worked with the hypothesis that these three conditions had a significant effect in the results obtained. Therefore, the hypothesis was tested statistically using ANOVA.

Analysis of Variance was used to determine the significance of varying the operating conditions. The results were presented as follows: varied substrate analysis in Table 4.7, Varied HRT analysis in Table 4.8 and varied substrate column height in Table 4.9.

Table 4.7: ANOVA Table for Varied Substrate

		Sum of Squares	Df	Mean Square	F	Sig.
substrate	Between (Combined) Groups	81278.492	2	40639.246	15.428	.000
	Within Groups	1699025.548	645	2634.148		
	Total	1780304.040	647			

The Table 4.7 shows that there is a significant difference in the removal of nitrates by varying the type of substrate in a bio reactor therefore justifying that the efficiency of woodchips, maize cobs and sugarcane bagasse significantly varies.

Table 4.8: ANOVA Analysis of Varied HRT

			Sum of Squares	Df	Mean Square	F	Sig.
Yield of time in hours	Between Groups	(Combined)	89914.727	3	29971.576	11.418	.000
	Within Groups		1690389.313	644	2624.828		
	Total		1780304.040	647			

Table 4.8 shows that there is a significant difference in the efficiency of contaminant removal when the time is varied. Therefore the change in HRT is an important variable in the study since there is a significant difference in the performance of the bioreactors at different HRTs.

Table 4.2: ANOVA Analysis of the Substrate Column Height

			Sum of Squares	Df	Mean Square	F	Sig.
Yield of the substrate column height	Between Groups	(Combined)	81278.492	2	40639.246	15.428	.000
		Within Groups	1699025.548	645	2634.148		
		Total	1780304.040	647			

Table 4.9 shows that varying the column height has a significant difference on the mean values of nitrates recorded. The substrate column height affects the efficiency of the bioreactor.

4.5 Determination of the optimal hydraulic retention times (HRTs) of the trickling filters

The hydraulic retention time of the system was manipulated by varying the valve opening to allow a minimal flow to match with the set time which was 12hrs, 24hrs, 48hrs and

60hrs. At HRT of 12 hours the treatment system had the lowest efficiency since it had the highest loading rate as described by Lepine *et al.* (2015).

Table 4.3: Inflow rates

Time in hours	Formula (Volume/time)	Inflow rate (liters/hour)
12	12/12	1
24	12/24	0.5
48	12/48	0.25
60	12/60	0.2

The optimal hydraulic retention time is the time it takes for the parameters measured to get to the maximum acceptable levels (Lepine *et al.*, 2015; Arif *et al.*, 2018). In this study the hydraulic retention time was determined for each substrates using linear graphs. The efficiency of the system is directly proportional to the substrate height, the contact time and the particle size. Working with the optimal conditions for each substrate (conditions with the highest efficiency tabulated in Table 4.4, 4.5 and 4,6 for woodchips, maize cobs and sugarcane bagasse respectively, the optimal HRT was determined graphically.

4.5.1 Optimal HRT for Woodchips

The optimal conditions for the operation of a woodchip bioreactor is at 18cm, 60hours or 22cm, 48 hours. The graph was generated and extrapolated to predict the optimal hydraulic retention time that would yields the desired efficiency listed in Table 4.4. According to the linear equations in the graph of Figures 4.20 and 4.21, $y = 0.7888x + 18.75$ *for* the 38-50mm particle size of woodchip particles and $y = 0.5682x + 20.346$ for the 30-38mm woodchip particles. Since the desired value of Y is 95% (nitrate efficiency) then the x (time) values which is the HRT is given by Equation 4.6.

$$X = \frac{(Y - C)}{M} \tag{4.6}$$

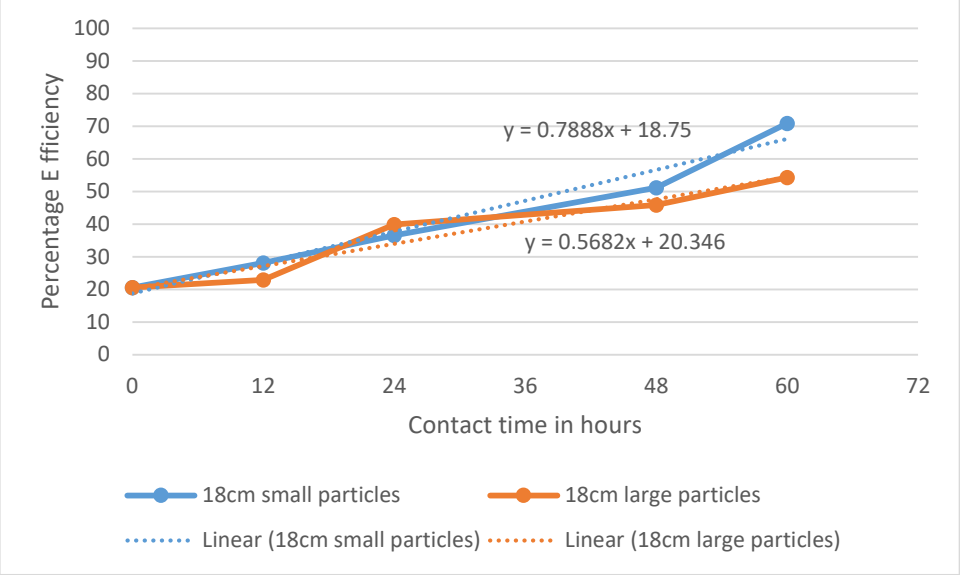

Figure 4.19: HRT of Woodchips at 18cm

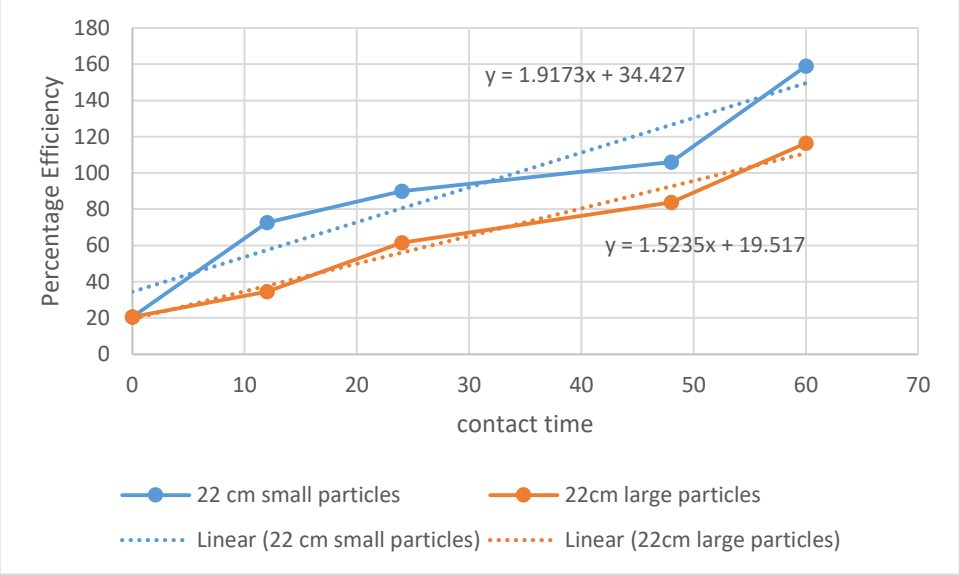

Figure 4.20: HRT of Woodchips at 22cm

The optimal HRT at 18cm column height of woodchip is 74.8 hours and 49 hours for 22cm column height of both particle sizes of woodchip. These results obtained for the HRT of woodchips can be compared to various research work such as (Lepine *et al.*, 2016) which states that the efficiency of woodchips in denitrification is about 50% between HRT of 12 to 24 hours. This theory has been proven by the graph in Figure 4.20 and 4.21. According to Cantrell *et al.* (2008), a desired efficiency can be achieved by increasing the height of the bed of the bio reactor and reducing contact time or increasing contact time and reducing the bed height. Also, the width of the bio reactor affects the hydraulic loading rate which will in turn affect the overall efficiency.

4.5.2 Optimal HRT for Maize cobs

Similar to woodchips, the efficiency of maize cobs was discussed. The optimal operation conditions that would yield desired efficiency for maize cobs was beyond 22 cm height and this is what was used to determine the optimal HRT in the extrapolated graph Figure 4.22.

$$y = 9.5714x + 56.905 \quad (4.7)$$

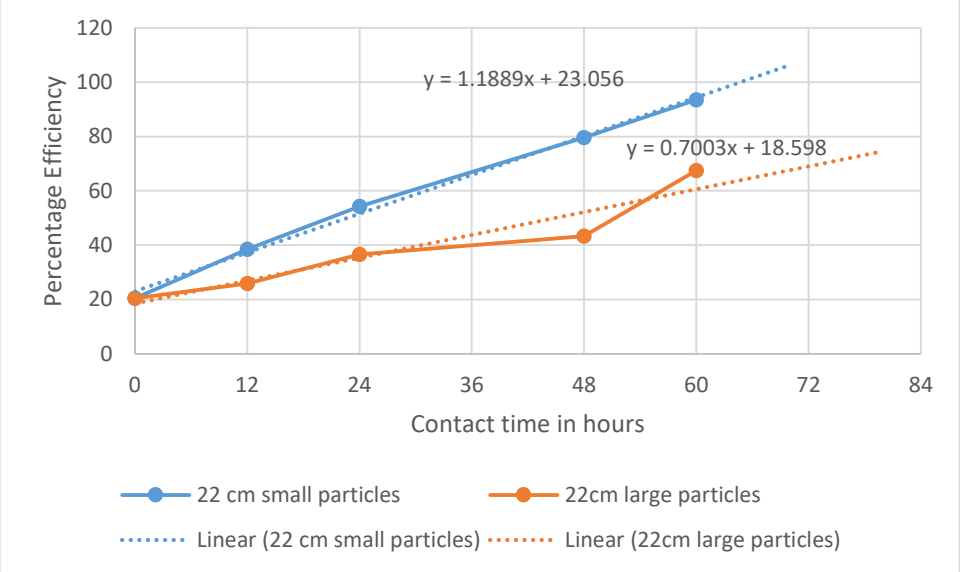

Figure 4.21: HRT of Maize Cobs

From the Equation 4.7 on the graph the value of X was determined and the extrapolated trend line obtained the optimal HRT for a maize cob bioreactor at 22cm height of substrate at 65 hours. The efficiency of the Maize cobs bioreactor at 22cm was increasing as the HRT is increased. Ali *et al*. (2016) also suggested that the performance of a maizecob bioreactor relies mostly on a suitable HRT since very high HRT means that the wastewater flows very slowly which leads to carbon buildup and overgrowth of microorganisims and a low HRT leads to wash out of the microorganisims.

4.5.3 Optimal HRT for Sugarcane bagasse

For the sugarcane bagasse bioreactor the optimal operating conditions mentioned in section 4.3.2.3 was closely achieved by 22cm height of substrate and this was used to develop the prediction graph 4.17 and Equation 4.8.

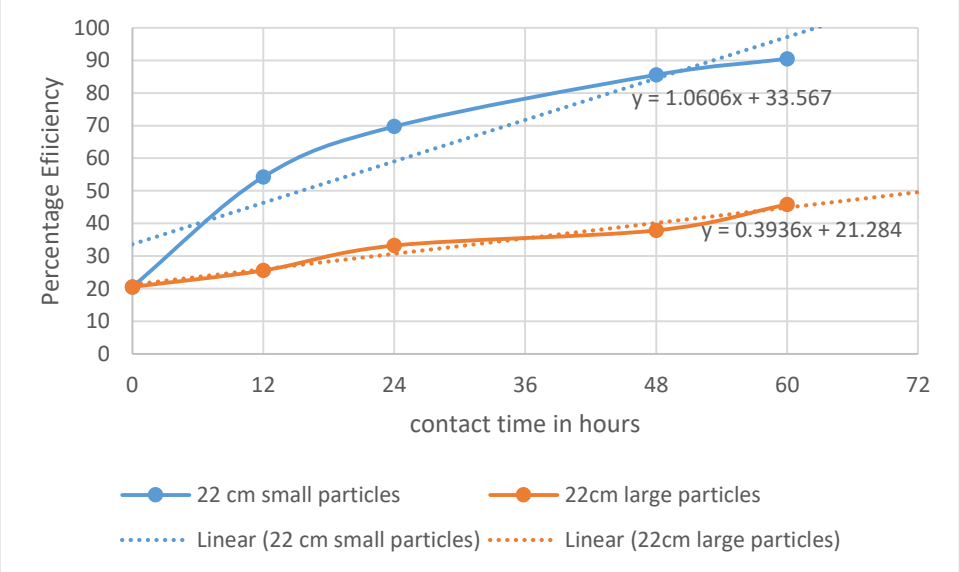

Figure 4.22: HRT for Sugarcane Bagasse Bioreactor

$$y = 10.619x + 39.286 \quad (4.8)$$

From the in Figure 4.23 it was evident that the optimal HRT that meets the 95% efficiency in removal of nitrates was obtained using Equation 4.8. These yielded the HRTs at 67 hours for the group B (30-38 mm particles) and 74 hours for the large substrate particle bioreactors. Daud *et al.* (2014) and Kanawade & Gaikwad, 2011 also suggest that the HRT is very important parameter in the design of sugarcane bioreactor. The efficiency for a sugarcane bioreactor varies with refference to specific design parameters.including the retention time and substrate packaging/porosity.

4.6 Modeling Phosphate Mean values

The mean values of Phosphate were used to calculate the constants of the Langmuir model equation as illustrated in Equation 3.6.The constants were obtained from the gradient and

the Y-intercept of the linear function of the graph. The constant values were tabulated in Table 4.11.

Table 4.11: Langmuir constant values

	$\frac{1}{x_m}$**(gradient)**	X_m	$\frac{1}{bx_m}$**(y-intercept)**	**B**	R^2
Woodchips at 14cm	1.896	0.527	2.12	0.894	0.876
Woodchips at 18cm	1.8688	0.535	2.49	0.752	0.8961
Woodchips at 22cm	1.0455	0.956	1.93	0.599	0.9865
Maize cobs at 14cm	1,65	0.606	2.12	0.778	0.812
Maize cobs at 18cm	1.61	0.606	2.07	0.797	0.867
Maize cobs at22cm	1.49	0.67	2.09	0.714	0.9141
Sugarcane bagasse at 14cm	1.0802	0.92	2.05	0.589	0.8946
Sugarcane bagasse at 18cm	1.067	0.93	2.05	0.528	0.895
Sugarcane bagasse at 22cm	1.079	0.93	2.17	0.51	0.876

From Table 4.11, the R^2 values are close to 1 and therefore the results can fit best the Langmuir model. The woodchips bioreactor at 22cm exhibited an almost perfect fit. Therefore, the efficiency of this design can be forecasted using the Langmuir model.

4.6.1 Testing the Langmuir Model on phosphate values

The Langmuir model was a perfect fit for the variables of this study. The model was tested with the variables and the results documented on the Table 4.12. From the table, the calculated values of Y (concentration of phosphates) is approximately similar to the actual value of Y. this confirms that the model can be used to predict the outcome of this bioreactor trickling filter in phosphate adsorption.

Table 4. 12: Testing the Langmuir model on the phosphate values

	Gradient	X values	(X value x gradient)	(y-intercept)	Calculated Y	Actual Y
Woodchips at 14cm	1.896	4.314346	8.18	2.12	10.35	10.3
Woodchips at 18cm	1.8688	1.289598	2.41	2.49	4.87	4.9
Woodchips at 22cm	1.0455	0.162602	0.17	1.93	2.13	2.1
Maize cobs at 14cm	1.65	4.89697	8.08	2.12	10.4	10.2
Maize cobs at 18cm	1.61	4.428571	7.13	2.07	9.2	9.2
Maize cobs at22cm	1.49	-0.03356	-0.05	2.09	2.04	2.04
Sugarcane bagasse at 14cm	1.0802	7.257915	7.84	2.05	10.01	9.89
Sugarcane bagasse at 18cm	1.067	4.170572	4.45	2.05	6.53	6.5
Sugarcane bagasse at 22cm	1.079	1.482854	1.6	2.18	3.78	3.78

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This research aimed to evaluate the treatment performance of a modified trickling filter packed with different substrates/media in treating aquaculture wastewater. The following conclusions were made:

The best operation condition for the aquaculture wastewater treatment trickling filter for the removal for nitrites, nitrates and phosphates was at 18cm and 22cm substrate column height for both particle sizes of woodchip particles at 60 hours and 48 hours contact time respectively. For maize cobs and sugarcane bagasse, the optimal conditions was at 22cm substrate column height at 60 hours contact time for 30-38 mm substrate particle size.

The most suitable substrate was woodchips. The efficiency of woodchips was highest at 94%, 89% and 93% efficiency in the removal of nitrates at 22cm substrate column height at contact time of 48 hours and 18 cm substrate column height at contact time of 60 hours for 30-38mm substrate particle size). At 22cm substrate column height, the suitable contact time was at 60 hours for 38-50 mm particle size.

The optimal contact time for treating 12 liters of wastewater with a 95% efficiency for each substrate were as follows: For the woodchip bioreactor at 22cm substrate column height a time of 49.8 hours is required and in the 18cm substrate column height a time of 74.8 hours: For the maize cobs bioreactor at 22cm height of substrate the required time is 65.5 hours and for the sugarcane bagasse at 22 cm substrate column height a time of 67hours for the 30-38mm particle size and 74 hours for 38-50mm particle size.

The phosphate mean values were a good fit in the Langmuir model with an average R^2 values as 0.897. Therefore this model can be used to determine optimal conditions with at varied operating requirement.

5.2 Recommendations

- Although the comparison of the different substrates was deliberate, this study recommends further studies to determine the efficiency of a system that integrates all the substrates in one bioreactor: and also to determine the efficiency of pyrolized ashes of wood, maize cob and sugarcane in wastewater bioreactors.

- This study recommends to the design criteria to policy maker to develop regulations pertaining to the standard designs for commercial bioreactors using woodchips, maize cobs and sugarcane Bagasse as substrate materials

REFERENCES

Abdel-Kader, A. M. (2007). A Review of Mebrane Bioreactors Technology and Their Application in the Wastewater Treatment System. *Water works journal*, 2(12), 269-278.

Alexandratos, N., & Bruinsma, J. (2012). World Agriculture Towards 2030/2050. *FAO report on food security*, 89-92.

Ali, I., Sultan, M., Khan, Z., Muhammud, H., Farid, H., Ali, M., & Nasir, A. (2016). Experimental Study on Maize Cob Trickling Filter-Based Wastewater Treatment System: Design, Development, and Performance Evaluation.. *25, (6) Polish Journal of environmental studies*, 1-9.

Al-Rekabi , W., Qiang, H., & Qiang, W. W. (2007). Improvements in Wastewater Treatment Technologies. *pakistan journal of nutrition*, 104-110.

APHA. (2005). Standard Methods for Examination of Water And Waste Water. USA, Washington DC: American Public Health Association.

Arif, A. U., Sorour, M. T., & Aly, S. A. (2018). Design and Comparision of Waste Water Treatment Plants (Activated Sludge And Membrane Bioreactor), Using GPS-X Simulation Program: Case Study of Tikrit WWTP. *journal of environmental protection 9*, 636-651.

Astolfi, A. (2006). Optimization of Vertical Flow Wetlands as a Wastewater Treatment System . *Journal of environmental engineering 25*, 112-123.

Azizi, S., Valipour, A., & Sithebe, T. (2013). Evaluation of Different Wastewater Treatment Processes and Development of a Modified Attached Growth Bioreactor as a Decentralized Approach. *The Scientific World Journal*, 156870-156878.

Babayemi, K., Onukwuli, D., & Menkiti, M. (2013). Coag-Flocculation Kinetics Of Mucuna Sloanei Seed For Phosphorus Removal From Wastewater. *American journal of analytical chemistry, 4 (12)*, 732-738.

Barongo, J., Munga, D., Opello, G., Kitheka, J., Massa, H., Mwangi, S., . . . Mwaguni , S. (2006). Vulnarability and Pollution Of Ground water in Mombasa Kenya. *journal of water*, 6 (9) 213-228.

Beatrice, K. K. (2018). Pollutant Removal from Water Using Plant Materials. *PHD dessetation*. Nairobi, Kenya: Jomo Kenyatta University of Agriculture and Technology.

Brazil, L. (2001, october 4). Evaluation of an Effluent Treatment Strategy To Controll Nitrogen From A Recirculating Aquaculture Facility. *MSC Thesis*. Kenya: Kenyatta University.

Bui, H. M. (2018). Applying Response Surface Methodology to Optimize the Treatment of Swine Slaughterhouse Wastewater by Electrocoagulation. *Polish Journal of environmental studies 27*, (1) 1975-1981.

Cantrell, K. B., Ducey, T., & Hunt P G. (2008). Livestoke Waste Water To Bioenergy Generation oppotunities. *journal of environmental science-Elsevier*, 546-549.

Chapman , S., Leslie, G., & Law, I. (2012). Membrane Bioreactors (Mbr) For Municipal Wastewater Treatment – An Australianperspective. Existing Mbr Installations – the Global Experience. Sydney, Australia: CH2M HILL Australia Pty Ltd pp 1-11.

Crispp, S. J., & Bergheim, A. (2000). Solid Managment And Removal For Intensive Land Land Based Aquaculture System. *journal of aquacult*, 5(18) 22-31.

Daud, S., Salleh, S., Salleh, M. N., Kasim , F. H., & Saad, S. A. (2014). Analysis Of Chemical Composition In Sugarcane Bagasse and Ricestraws For Their Suitability in Paper Production. University of Malaysia Perlis (uni MAP).

Dutta, S. (2007, February 14). Mathemticl Modelling of the Performance of the Rotating Biological Contactors For Process Optimisation In Wastewater Treatment. *journal of civil engineering*, 90-99.

Ebeling, J. (2014). Bio filtration and Nitrification Design Overview. *environmental engineering aquaculture system technology*, 675-687.

EMCA. (1999). Environmental Managment and Coordination Act. *Constitution of Kenya*. Kenya: Legislation.

EMCA. (2015). Environmental Managment and Coordination Act. *Constitution of Kenya*. Kenya: legislation schedule 9.

Emile Bisekwa, Paul M. Njogu, Taye Kufa-Obso 2021. Wet Coffee Processing Discharges

Affecting Quality of River Water at Kayanza Ecological Zone, Burundi" *Open Journal of Applied Sciences*, 11 (6), 20-21

E Bisekwa, PM Njogu, T Kufa-Obso. 2020. Effluent quality of wet process coffee processing

Factories in coffee growing ecological zones in Burundi. *International Journal of Water and Wastewater Treatment ISSN2381-5299* dx.doi.org/10.16966/2381-5299.176

Environmental Protection Agency. (1979). Methods for Chemical Analysis of Water and Wastes. Washington, D.C.: *Environmental Protection Agency Report*.

Feng, G., Chen, L., Zhao-Hui, Y., Guang-Ming, Z., & Li-Juan, F. (2016). Continous Microalgae Cultization In Aquatic Wastewater A Membrane Photobioreactor For Biomass Production And Nutrients Removal. *Elsevier Ecollogical engineering Journal*, 55-61.

Fraiture, V., Smekhtin, V., Bossio, D., McCornick , P., Hoanh, C., & Turral , H. (2007). Facing climate change by securing water for food , livelihood and ecosystems. *East Africa: international crop research institute for the semi arid tropics report*.

Gary, F., Laura, C., Thomas, M., Rodney , v., & Jeffery, C. (2017). Plastic Biofilm Carrier After Corn Cob Reduces Nitrate Loading In Bioreactors. *Journal of environmental quality*, 46-60.

Gold, A. J., Schipper, L. A., & Addy, K. (2012, july 24). Denitrrifying Bioreactors Oppotunitiesand Challenges For Managing Offsite Nitrogen Losses. *international symposium on managing soils for land security ans climate change adoptation*. Conference report, Rhode island.

Greenan, C., Moorman, T., Kaspar, T., Parkins, T., & Jayne , D. (2006). Comparing Carbon Substrates for Denitrification of Subsurface Drainage Water. *Journal of environmental quality*, 624-629.

Guo, B., Hong , L., & Jiang, H. (2003). Macroporous Poly(calciumacrylatedivinybenzene) Bead-Selective Orthophosphate Sorbent. *Industrial Enginnering Chemical Reseearch, 42(1),* 559-565.

Gupta, N., Jana, N., & Majumdar, C. (2008). Submerged Membrane Bioreactors System For Municipal Waste Water Treatment Process. *Indian journal of chemical Technology vol.15*, 604-612.

Hochheimer, J., & Wheaton, F. (2000). Biological Filters: trickling and RBC Designs. *second international confrence of recirculating aquaculture*, research gate. 16-19. Washington DC:

Jenkins, A., & Sanders, D. (2012). Introduction of Fixed Film Bio Reactors for Decentralized Wastewater Treatment. *Contech, engineered Solutions*, 789-899.

Jiang, J., & Graham, J. (1998). Pre-Polymerised Inorganic Coagulants and Phosphorus Removal By Coagulation-A Review. *Water SA*, 237-244.

John, B. (2014). Engineering mathematics. london: London publishers.

Juma, L. (2014). Waste Water Managment. A Case Of Reducing Waste Water Release Into The Environment. Mathare North, Nairobi, Kenya: Kenyatta university Msc Thesis.

Kaetzl, K., Manfred, Gehring, T., & Wichern, M. (2018). Efficient Low Cost Anaerobic Treatment Of Wastewater Using Biochar And Wood Chip Filters. *journal of water*, 818-835.

KALRO. (2013). Agricultural Potential In Kenya. *report on the country wide adricultural potential*.

Kanawade , S. M., & Gaikwad, P. W. (2011). Removal of Dye from Dye Effluent Using Sugarcane Bagasse Ash as an Absobent. *International journal of chemical engineering and application. 2 (3*), 202-206.

Kasima, E. (2014, April). Efficiency of Wastewater Treatment plants in Kenya:. *Waste water treatment in kenya*. Nairobi, Kenya: University of Nairobi Msc. thesis.

Keramat, A. A. (2008). Environmental Impact Of Nutrient Discharged Aquaculture Waste Water On Haraz River. *Journal of fisheries and aquatic science* 3(1), 275-279.

Lepine, C., Christianson, L., Sharter, K., & Summmerfelt, S. (2016). Optimising Hydraulic Retention Time In Denitrifying Woodchips Bioreactors Treating Recirculating Aquaculture System Wastewater. *journal of environmental quality* (special section*)*, 813-821.

Lowe, M. R. (1994). Changing Ecosystem Of Lake Victoria. *Fresh water forum*, 74-89.

Makisha , N., & Nesterenko, A. (2018). Wastewater Treatment In Membrane Bioreactor Features and Applications. *Materials Science and Engineering* 365-342. Moscow: IOP Publishing.

Mark, B. D., Lowell, E. G., Cooke, R., & Herbstritt, S. M. (2015). Temprature And Substrate Control Woodchip Bioreactor Performance In Reducing The Tile Nitrate Loads In Illinois. *J Qual*, 909-921.

Mburu , J. N., Hoinkins, J., Njogu, P. M., Kinyua, R., & Atiye, T. (2019). Pilot Trials on Testing and Optimization of Polyethersulfone Membranes for Treatment of Fish Processing Wastewater through Membrane Bioreactor Technology. *International Journal of Water and Wastewater Treatment* 5 (1), dx.doi.org/10.16966/2381-5299.158.

Mena, J., Rodriguez, L., Nuñez, J., Fernández, J., & Villaseñor, J. (2008). Design of Horizontal And Vertical Subsurface Flow Constructed Wetlands Treating Industrial Wastewater. *water pollution IX (WIT Transactions on Ecology and the Environment,* 1(11), 555-564.

Merino-Solís , L. M., Villegas , E., Anda , J., & López-López , A. (2016). The Effect of the Hydraulic Retention Time on the Performance of an Ecological Wastewater Treatment System: An Anaerobic Filter with a Constructed Wetland. *water journal*, 1149-1163.

Metcalf, & Eddy. (1991). *Wastewater Engineering Treatment Disposal and Reuse*. New york: McGraw- Hill.

Mohammed, B., Shuokur, A., Salem, A., & Choon , A. N. (2015). Wastewater Treatment Processes Optimization Using Response Surface Methodology (RSM) Compared with Conventional Methods: Review and Comparative analysis. *middle east journal scientific research*, 34-45.

Montegomery, D. (1997). Design and Anlysis Of Experiments (4th ed.). Hoboken,New Jersey: John Wiley and sons,inc.

Muraguri, P. M. (2013). Assessment of Groundwater Quality in Nairobi County, Kenya. *Thesis*. Kenya: Kenyatta University.

Musa, M. A., Idris, S., Che, M. H., Daud, N., & Norsyahariati, N. (2018). Wastewater Treatment And Biogas Recovery Using Anaerobic Membrane Bioreactors Strategies And Achievements. *Energies (MDPI)*, 1675-1699.

Mwangangi , D. (2015, December). Use Of Maize Cobs Derived Products For Removal of Selectedinorganic Ion Color and Turbidity from Contaminated Water. *use of maize cobs derived products for removal of selectedinorganic ion color and turbidity from contaminated water*. Nairobi, Kenya: Kenyatta university Msc, thesis.

Nazaroff, A., & Alvarez, C. (2016). Biological Wastewater Treatment. Hanover: http://www.hanovernh.org/Pages/HanoverNH_PublicWorks/WaterRecl/Index. Retrieved from (http://www.hanovernh.org/Pages/HanoverNH_PublicWorks/WaterRecl/Index)

O'Dell, J. (1993). Determination Of Nitrate-Nitrite Nitrogen By Automated. Ohio: Environmental Monitoring Systems Laboratory.

Opal, P. (2016). Water Hyacinth In The Lake : Causes Effects And Measures. Nyanza, kenya: Lake basin development authority.

PALIN, A. (1950). Chemical Aspects Of Chlorination. *journal of water engineering*, 50-62.

Park, H., Chang, I.-S., & Lee, K. (2015). Principles Of Membrane Bioreactors For Wastewater Treatment. In *bioreactors for wastewater treatment.* London: Taylor and Fransis group journal.

Ponnada, L., Lynn, T., Ergas, J., & Mihelcic, J. (2017). Application of Denitrifying Wood Chip Bioreactors For Management Of Residential Non-Point Sources Of Nitrogen. *Journal of biological engineering*, (DOI 10.1186/s13036-017-0057-4) 1-14.

Quresh, K., Bhatti, L., & Kazi, R. (2008). Physical And Chemical Analysis Of Activated Carbon For Decolonization. *International journal of chemical and bimolecular engineering*, 454-461.

Reza, A., & Abedin , Z. (2013). Bagasse as an Adsobent for the Wastewater Treatment of a Composite Knit Industry. *International Conference on Solid Waste Management in the Developing Countries* 126-134. Bangladesh: WasteSafe 2013 – 3rd edition.

Rodger, B; Bridgewater, L; APHA; AWWA;. (2017). Standard Methods for the Examination of Water and Wastewater. Washington D C: American Public Health Association, American Water Works Association, Water Environment Federation.

Saddoud, A., Ellouze, M., Dhouib, A., & Sayadi, S. (2006). A Comparative Study On The Anaerobic Membrane Bioreactor Performance During The Treatment Of Domestic Wastewaters Of Various origins. *journal of Environmental Technology*, 991-999.

Schipper, L., Robertson, W., Gold , A., Jaynes, D., & Cameroon, S. (2010). Denitrifying Bioreactors An Approach Of Reducing Nitrate Loads To Recieving Waters. *journal of Ecological Engineering-Elsevier*, (doi: 10.1016/j.ecoleng.2010.04.008) 1647-1659.

Shih, R., Robertson, L., Schiff, S., & Rudolph, D. (2011). Nitrate Controls Methyl Mercury Production In A Streambed Bioreactor. *J. Environ. Qual. 40*, 1586– 1592.

Siddiqui, S. A. (2017). Wastewater Treatment Technology In Aquaculture. *journal of qualt*, 123-134.

Sorensen, L. (2018). Hydrodynamic Optimisation Of Membrane Bioreactors. Hydrodynamical Optimisation Of Membrane Bioreactors. Aalborg, Denmark: DOI (link to publication from Publisher): 10.5278/vbn.phd.eng.00053.

Sufia, A. (2015). Evaluation of Bagasse In The Treatment Of Agricultural Wastewater. *journal of environ mental engineering-Elsevier*, 256-268.

Sutton, M., & Schuman, E. (2006). Denitrifying Microbial Synthesis For Waste Water. *journal of environmental engineering-Elsevier*, 49-58.

Sweetapple, C., & Fu , G. (2016). Multi Objective Optimisation Of Waste Water Treatment Controll To Reduse GHG Emmision. *water research*, 908-912.

Trinh, T., & Kang, L. (2011). Response Surface Methodological Approach To Optimize The Coagulation-Flocculation Process In Drinking Water Treatment. *Journal of Chemical Engineering Research And Design.* 6(1) 55-59.

US-EPA. (2000). Trickling filter guideline. Washington DC: Environmental Protection Agency.

Wamberee, D. (2014). Trickling Filters Design Considerations For Local Waste Water Treatment. *thesis*. kenya: kenyatta university .

Wright, S. J., & Nocedal, J. (2007). Numerical Optimization. In P. Glynn, & S. Robinnson, *Springer Series in Operations Research.* Newyork: Springer.

Yamashita, T., & Yamamoto, I. (2014). Nitrogen And Phosphorus Removal From Wastewater Treatment Plant Effluent Via Bacterial Sulphate Reduction In An Anoxic Bioreactor Packed With Wood And Iron. *international journal of environmental research and public health*, 9835-9853.

Yildirim, M., & Topkaya, B. (2012). Assecing environmental impact of waste water traetment alternatives for small scale communities. *CLEAN soil air water*, 171-178.

APPENDICES

6.1 Appendix 1: ANOVA FOR 30-30mm SUBSTRATE PARTICLE SIZE PARTICLE BIOREACTORS:

ANOVA FOR SMALL PARTICLE BIOREACTORS								
Type of bioreactor			Sum of Squares	standard deviation	df	Mean Square	F	Sig.
Woodchip	14 CM	Between Groups	99630.495	1	2	49815.248	44.979	0
		Within Groups	36547.946		33	1107.514		
		Total	136178.441		35			
	18cm	Between Groups	55124.171	1.15	2	27562.085	100.296	0
		Within Groups	9068.6		33	274.807		
		Total	64192.814		35			
	22cm	Between Groups	20101.815	1.78	2	10050.907	40.302	0
		Within Groups	8229.901		33	249.391		
		Total	28331.715		35			
Maize cobs	14cm	Between Groups	183625.454	1.1547	2	91812.727	111.834	0
		Within Groups	27091.999		33	820.97		
		Total	210717.454		35			
	18cm	Between Groups	96537.37	0.57735	2	48268.685	204.407	0
		Within Groups	7792.612		33	236.14		

		Total	104329.982		35			
	22cm	Between Groups	22817.788	2.08167	2	11408.894	41.908	0
		Within Groups	8983.716		33	272.234		
		Total	31801.504		35			
Sugarcane	14cm	Between Groups	229473.803	1.1547	2	114736.901	332.398	0
		Within Groups	11390.901		33	345.179		
		Total	240864.704		35			
	18cm	Between Groups	67416.72	2.08167	2	33708.36	300.125	0
		Within Groups	3706.379		33	112.315		
		Total	71123.1		35			
	22cm	Between Groups	33696.6	1.1547	2	16848.3	69.17	0
		Within Groups	8038.104		33	243.579		
		Total	41734.704		35			

6.2 Appendix II: TABLE OF PERCENTAGE EFFICIENCY

	Nitrates				Nitrites				Phosphate		
	12h	24h	48h	60h	12h	24h	48h	60h	12h	24h	60h
14cm 38-50 mm particles	2.38	37.14	57.61	71.42	32.38	85.42	98.78	99.83	9.17	19.32	40.5
18cm 38-50 mm particles	37.61	56.19	61.42	73.33	56.27	96.76	99.75	99.95	13.52	55.31	68.4
22cm 38-50 mm particles	58.09	69.04	79.52	90.95	71.65	97.57	100	100	47.343	72.46	85.9
14cm 30-38 mm particles	10	42.38	66.19	74.28	43.72	88.25	100	100	20.77	29.46	50.2
18cm 30-38 mm particles	63.80	71.90	79.04	85.23	65.18	83.40	100	100	32.85	50.72	76.3
22 cm 30-38 mm particles	69.04	82.85	92.85	95.23	79.75	87.44	100	100	81.64	76.95	89.8
14cm 38-50 mm particles	0.47	5.23	23.80	57.61	23.88	70.85	88.25	97.16	7.24	9.17	20.2
18cm 38-50 mm particles	30.95	35.23	46.66	61.42	51.01	93.52	97.97	99.6	13.04	21.73	50.7
22cm 38-50 mm particles	53.33	67.14	77.14	87.61	59.51	95.54	98.38	100	25.60	50.72	61.8
14cm 30-38 mm particles	6.66	10.95	29.04	62.85	29.14	76.11	96.35	100	13.52	21.73	50.7

18cm 30-38 mm particles	53.33	62.85	74.28	79.52	59.51	80.16	97.57	100	29.46	31.40	55.5
22 cm 30-38 mm particles	62.8	80	88.5	91.90	67.61	80.5	97.57	100	53.69	68.35	90.1
14cm 38-50 mm particles	31.90	48.28	12.38	38.09	37.24	94.33	95.14	96.35	4.347	17.39	35.7487
18cm 38-50 mm particles	39.04	53.33	56.66	59.04	51.417	95.14	96.72	99.14	30.43	31.15	52.2222
22cm 38-50 mm particles	50.47619	61.42857	67.61905	83.80952	58.2996	96.76113	99.19028	100	24.63768	44.92754	63.2850
14cm 30-38 mm particles	4.285714	13.33333	38.57143	56.19048	43.31984	83.40081	96.76113	98.38057	30.43478	31.15942	52.2222
18cm 30-38 mm particles	43.80952	58.57143	62.38095	69.04762	63.56275	85.02024	94.73684	99.19028	33.33333	41.49758	68.5990
22 cm 30-38 mm particles	59.04762	74.28571	87.14286	93.80952	71.65992	91.49798	98.38057	100	68.35749	75.89372	81.7391