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MESOCOSM STUDY ON THE POTENTIAL USE OF VERTICAL SUB-SURFACE FLOW CONSTRUCTED WETLANDS FOR REMOVAL OF ORGANIC MATTER IN SLAUGHTERHOUSE WASTEWATER

Master of Science Thesis

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DECLARATION AND RECOMMENDATION

This thesis is my original work and has not been submitted or presented for examination in any institution.

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RECOMMENDATION

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations

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DEDICATION

I would like to dedicate my work to The Almighty God for the opportunity to advance my ken and to my family members for their immense support both material and emotional during the study period.

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ABSTRACT

The fast pace of economic growth in Kenya has created a large demand for meat products. This stands at an annual average of about 600,000 metric tonnes of red meat which is expected to continue rising according to global animal product consumption trends. Consequent challenges in management of increasing volumes of high strength wastewater have necessitated ardent research into sustainable technologies, for which vertical flow wetlands offer a promising solution. Three month experimentation conducted at Egerton University, explored the potential for use of vertical flow constructed wetlands in removing organic matter from slaughterhouse wastewater. The wastewater used was sourced from a mid-scale size slaughterhouse in Njoro Township. Experimental design consisted of three tanks of 2 mm sand, 8 mm quarry dust and 16 mm gravel at shallow 0.65 m and deeper 0.8 m depths, each with four replicates. Retention times of 1, 3 and 5 days were also investigated. The tanks were operated batch-wise and effluent water samples collected five times for each retention time studied. The water samples were analysed soon after using standard protocols for BOD_5, COD, NH_4-N and TSS. The untreated slaughter house wastewater characteristics ranged between 28,336-3,2502 mg/L for COD, 2,070-3,653 mg/L BOD_5,1,371- 2,160 mg/L TSS and 52.98-52.42 g/L NH_4-N. The results from the experimental mesocosm treatment set-up demonstrated that organic matter removal was highest at 5 day retention time, with removals of about 50%, 55% and 82% for BOD_5, COD and TSS respectively. Deeper 0.8m mesocosms were noted to have significant differences in treatment for TSS and NH_4-N compared to shallow 0.65 m mesocosms. Differences in substrate type were observed to have no significant effect on organic matter removal. In the case of ammonia, increase in substrate size was observed to decrease removal efficiency, although significant nitrification did not occur. NH_4-N was observed to fluctuate with removal efficiency averaging at 26.5%. This study demonstrates that vertical flow wetlands operated at longer retention times and by tidal flow pattern facilitate removal of organic matter in slaughter house wastewater. However, a pre-treatment stage is necessary in order to reduce the organic matter load, and ensure lifecycle of the wetland is not threatened. Targeting ammonia reduction at the pre-treatment stage can highly increase the overall treatment efficiency.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ACA	Activated carbon adsorption
AD	Anaerobic digestion
AH	Alkaline hydrolysis
AP	Aerated ponds
APHA	American Public Health Association
AS	Activated sludge
BOD_5	five day Biological oxygen Demand
BTF	Bio-trickling filter
COD	Chemical Oxygen Demand
CWs	Constructed Wetlands
DO	Dissolved Oxygen
EC	Electrical conductivity
HF	Horizontal flow
HL	Hydraulic load
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
NEMA	National Environmental Management Authority
NH_4-N	Ammonium nitrogen
OL	Organic load
OLR	Organic loading rate
OM	Organic matter
RBR	Rotating bio-reactor
SP	Stabilization pond
SS	Suspended solids

TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSS	Total Suspended Solids
UASB	Up flow anaerobic sludge blanket
VFWs	Vertical Flow Wetlands
VSSFCWs	Vertical Sub-surface Flow Constructed Wetlands
WW	Wastewater
ET	Evapotranspiration

CHAPTER ONE

INTRODUCTION

1.1 Background Information

In developing countries, it has been reported that release of untreated wastewater into rivers and streams poses a great risk to human and animal health in addition to degrading quality of surface and groundwater (Koech, Ogendi and Kipkemboi, 2012). High operational and maintenance costs associated with common chemically engineered treatment alternatives for wastewater are tremendous and more often than not, overwhelm the local authorities mandated to operate them. These challenges have necessitated a search for low cost yet efficient methods of waste water treatment for which Constructed Wetland (CW) technologies have shown great potential in east Africa (Oketch, A., 2002; Abira, A., 2008; Hunt, Riungu and Mathiu, 2011; Kimwaga, Mwegoha, Mhange, Nyomora and Ligali, 2013)

Verhoeven, Arheimer, Yin and Hefting, (2006) indicated that the use of constructed wetland technology can be of particular significance in the conservation of catchments, rivers and lakes especially because of their similarity in function to natural wetlands. As such, they have the added benefit of increasing natural habitats. Morel and Diener, (2006) also pointed out that CW technologies show great promise in *inter-alia*, reducing the agricultural use of much needed drinking water, reducing cost of water, increasing food security and improving public health.

Previous studies have established that constructed wetlands can be successfully used in the treatment of large scale industrial wastewater (Bojcevska, H., and Tonderski, K., 2007; Al Jawaheri, 2011; Lavrova and Koumanova, 2013; Chunkao *et al.*, 2014) and domestic waste water (Vymazal, 2010; Gikas and Tsihrintzis, 2012; Lavrova and Koumanova, 2013). These evidences notwithstanding, little information exists on the treatment efficiency of CW systems in tropical regions. In addition, there are no documented CW systems treating slaughterhouse wastewater in Kenya. Noting further, very few studies exist regarding the application of vertical sub-surface flow constructed wetlands (VSSFCWs) to meat industry wastewater (Johns, 1995). This is despite the fact that vertical flow wetland technologies have been proven to efficiently remove high organic loads which are a major challenge for slaughterhouse wastewater (Stefanakis and Tsihrintzis, 2012; Lavrova and Koumanova, 2013, Chunkao and Dumpin, 2015). Considering that VSSFWs are also smaller than Horizontal Flow systems, they are a cost effective alternative. There is great value therefore, in conducting further studies on VSSFWs to fill existing gaps in their application on abattoir wastewater.

1.2 Statement of the problem

The fast pace of economic growth in developing nations like Kenya has created a large demand for meat products. A livestock revolution attributable to rising incomes and protein based diets has seen meat consumption triple in the global south (Delgado, 2003). The consequent intensification of meat production and animal agriculture to meet this demand is said to be putting significant pressure on freshwater ecosystems (Mekonnen and Hoekstra, 2012). Studies by the (World Bank Group, 2007) indicate that slaughterhouses typically consume between 2.5 m^3 to 40 m^3 of water per metric tonne of meat produced. Wastewater produced from slaughterhouse processes is usually a mixture of cleaning water of the facility and processing water from slaughtering and cleaning of guts. About 1200L are used for mid-sclae facility cleaning while 250 L of fresh water is used per carcass. A large volume of wastewater with high organic load is the result.

Predications by (Bouwman *et al.*, 2013) indicate that this trend will continue to increase steadily until 2050. Slaughterhouses have therefore been presented with a unique challenge of managing increasing volumes of high strength wastewater. In most cases, raw or partially treated effluent is discharged directly into aquatic ecosystems. Occasionally, disposal mechanisms such as exhauster services are employed by some facilities. Poor management of slaughter house wastewater in general poses a very big threat to aquatic life due to the competition for dissolved oxygen created. Vertical flow wetlands present an efficient and cost effective solution to organic rich wastewater such as those generated from slaughterhouses, but knowledge gaps exist on their design and use in slaughterhouse wastewater management.

1.3 Objectives

1.3.1 General objective

To assess the potential use of vertical subsurface flow wetlands in treatment of slaughterhouse wastewater using mesocosm setup.

1.3.2 Specific objectives

1. To assess temporal variation in the physico-chemical characteristics of slaughterhouse wastewater over the study period.
2. To determine the effect of substrate type and depth on organic matter removal efficiency of slaughterhouse wastewater using a mesocosm experimental setup.
3. To assess the effect of different HRTs on removal efficiency of BOD_5, COD, TSS and NH_4-N.

1.4 Hypotheses

H_0: There is no significant variation in physico-chemical characteristics of slaughterhouse wastewater over time.

H_0: Differences in substrate type and depth have no significant effect on organic matter removal efficiency of slaughterhouse wastewater.

H_0: Variation of HRTs does not have a significant influence on removal efficiency of BOD_5, COD, TSS and NH_4-N

1.5 Justification

Following incidences of poor surface water quality and foul odour in peri-urban areas of Dagoretti, Kenya as the results of untreated slaughterhouse wastewater, the National environmental Management Authority (NEMA) ordered closure of all slaughterhouses discharging raw effluent into aquatic receptacles (Kiplagat, 2008). Legal efforts by NEMA, (2006 a and b) compelling large water consuming enterprises to recycle their wastewater to set standards before release into the environment, have necessitated research into cost effective technologies involved in the pre-treatment of wastewater. Large scale operations without proper pre-treatment facilities for their wastewater were forced to shut down or invest in the same (Shiundu and Mwai, 2008).

Evidences strongly indicating that VFCWs have the ability to efficiently treat high loads of concentrated industrial pollutants such as slaughterhouse wastewater (WW) may provide a much needed solution. Conversely, their application in East Africa for treatment of slaughterhouse wastewater remains low. In the case of Kenya, it is perhaps because of the waste's bio-chemical complexity combined with a scanty knowledge base on system design and operational mechanisms. The unpredictable treatment behaviour of CWs in general further points to existing knowledge gaps that hinder optimization of this technology. Also, existing literature elaborates extensively on the more popular conventional alternatives for slaughterhouse WW management. Not to mention that, the largest proportion of studies conducted on slaughterhouse WW is of temperate regions, hence cautioning on replicability of findings to temporal regions.

The small size requirements and characteristic design and operation aspects which enhance an aerobic environment make VFCWs a potentially sustainable technology for high organic matter breakdown. This in addition to the limitations mentioned above make it of great importance to advance existing studies on design and operational factors that optimize VFCWs' ability to effectively reduce organic load, which happens to be a significant component of slaughterhouse WW.

1.6 Structure of thesis

Chapter one introduces the study, giving a general perspective of the problem in developing nations then narrowing down to specific cases in Kenya. It also highlights the scope of the problem and supports significance of the study. The section also highlights specific research inquiry and provides hypotheses aimed at answering these questions.

Chapter two details the general characteristics of slaughterhouse wastewater observed in different studies. It also looks at the conventional treatment options used for management of abattoir waste and finally narrows down to the specific use of vertical flow wetlands. It described various design and operation aspects that are important in achieving high treatment efficiency and also outlines removal processes and some of their affecting factors.

Chapter three describes the area of study and location of experiment site. The chapter further outlines the experimental setup design used, methods of sampling, water collection, laboratory analysis and finally the statistical analyses applied for output generation and presentation.

Chapter four details results obtained for the study, presented as tables and graphs according to the objectives under investigation.

Chapter five discusses the results and expounds on them in relation to past and present studies. It highlights similarities and differences of the findings with those of other researches.

Chapter six concludes on the findings of the study and provides recommendations for further action.

CHAPTER TWO

LITERATURE REVIEW

2. 1 Slaughterhouse wastewater characteristics and production trends

Common slaughterhouse wastewater characteristics have been documented in various studies as having high organic load. The wastewater comprises mostly of proteins, blood, fats, lard, paunche, undigested food and colloidal particles with high fat, grease and protein content.BOD_5 levels have been observed to reaching up to 2000 mg/l (Irshad, A., Talukder, S., and Selvakumar, K., 2015). Slaughterhouse wastewater is usually evaluated as bulk parameters due to the specific volumes and pollutant loads which may vary greatly for different facilities. Common to many slaughterhouses, are considerable amounts of Total phosphorous (TP), Total nitrogen (TN), Total organic carbon (TOC), suspended solids, COD and BOD_5 (Bustillo-Lecompte and Mehrvar, 2015). A summary of the general slaughterhouse WW characteristics is given in Table 1 below.

Table 1: General characteristics of slaughterhouse wastewater

Parameter	**Range**
COD (mg/L)	18,904-27,800
BOD_5 (mg/L)	11.340-16,680
TN (mg/L)	500-15,900
TSS (mg/L)	614-2,562
TP (mg/L)	270-6,400
Ortho-PO_4 (mg/L)	20-100
NH_3-N (mg/L)	296-308
Oil and grease (mg/L)	232-246
pH	4.90-8.10
Colour (mg/L Pt scale)	175-400
Turbidity (FAU[a])	200-300

[a] FAU, Formazine Attenuation Units.

Table adapted from slaughterhouse wastewater characteristics by (Bustillo-Lecompte *et al.*, 2015; Irshad et. al., 2015).

Characteristics of effluent wastewater can be assessed in terms of physical, biological and chemical components. This preliminary process is essential in informing possible treatment options, design of facility, extent of treatment application and even the general waste management approaches that can be adopted for maximum efficiency of resource use within the abattoir (Irshad *et al.*, 2015). In addition, knowledge on physico-chemical parameters helps elucidate patterns observed in effluent data, due to the strong inter-relationship between physical, chemical and biological characteristics of water. Noting further, the characteristics of slaughterhouse wastewater such as their temporal variability observed by (Zhao *et al.*, 2004; Abdelhakeem, S., Aboulroos, A. and Kamel, M., 2015) where influent concentrations fluctuated irregularly during the experimental period point to the importance of determining the extent and impact of influent wastewater quality variation on treatment capacity in order to better understand and manage design and operation processes for high treatment results.

A livestock revolution attributable to rising incomes and protein based diets has seen meat consumption triple in the global south. FAO, (2013) stated that from 2002-2007, annual global beef production increased from 14.7 metric tonnes to 10,000 metric tonnes. It is estimated that by 2020 the current share of meat product consumption in developing countries will rise to 63% from the current 52%. A projection of 107 million metric tonnes more is anticipated, which dwarfs the developed countries' increase by 19 million metric tonnes by 2020.

The consequent intensification of meat production and animal agriculture is said to be putting significant pressure on freshwater ecosystems (Mekonnen and Hoekstra, 2012). In Kenya, the per capita consumption of meat has been observed to steadily rise from about 14 kg to 16 kg over the last two decades for rural and peri-urban areas, and a slightly higher consumption in urban areas at 25 kg. This stands at a national average of about 600,000 metric tonnes of red meat which is expected to continue rising according to global animal product consumption trends.

Studies by the (World Bank Group, 2007) indicate that slaughterhouses typically consume between 2.5 m^3 to 40 m^3 of water per metric tonne of meat produced. These massive volumes have warranted classification of meat industries as significant effluent wastewater producers under the global food and agriculture sector. Bouwman *et al.* (2013) predicted that this trend will steadily double until 2050. It is therefore reasonable to presume that volume and strength of wastewater produced will follow a similar trend thus requiring an intensified reliance on effective wastewater treatment technologies if the world's freshwater receptacles are to be safeguarded.

2.2 Current wastewater management technologies in the meat processing industry

As is common to meat processing industries across the globe, wastewater production and disposal are issues of great concern, especially if effluent disposal should be practiced sustainably (FAO, 2013). In Europe, many slaughterhouses and rendering plants discharge their wastewater to municipal treatment systems after primary treatment. On the other hand, (Brix and Arias, 2005; Koech *et al.*, 2012; Chunkao *et al.*, 2015) observed that it has been common practice in many parts of the world where industries located near waterways dispose of their effluent directly into water bodies prior to treatment. The increased awareness on sustainable development coupled with a need for more effective WW treatment technologies has seen a great advancement in slaughterhouse WW management. Huge investments have been channelled into automation of slaughtering processes and minimal solid and liquid waste production (Brix, 1994). Nevertheless, operational challenges unique to each alternative used make it difficult to achieve the latter objective.

Some of the more popular alternatives currently in use belong to either the aerobic or anaerobic categories. Various treatment methods include Activated Sludge (AS) systems, Bio-trickling filters (BTF), Up flow Anaerobic Sludge Blanket (UASB), Anaerobic digestion (AD), Stabilization ponds (SP), Alkaline Hydrolysis (AH), Rotating Bio-Reactor (RBR), Aerated ponds (AP) and Activated Carbon Adsorption (ACA) (Johns, 1995; Al Jawaheri, 2011; Franke-Whittle and Insam, 2013; Bustillo-Lecompte and Mehrvar, 2015). Other simpler alternatives include rendering, incineration and composting (Franke-Whittle and Insam, 2013).

Anaerobic treatment is mostly used in Europe because of high removal rates of organic concentrations present in the WW and generation of small quantities of highly stabilized dewatered sludge (Johns, 1995). For instance, both ACA and ASRB are able to achieve between 72%-93% removal efficiency for BOD_5 and COD. However, complete degradation of the OM using anaerobic technology solely is not achievable. This is because some of the residue effluents usually contain solubilized organic matter that is preferentially aerobically treated (Irshad *et al.*, 2015). However, the production of foul odour limits the application of aerobic treatments in tropical regions or during the summer season in temperate climates. Nevertheless, both technology types work best in a complementary manner in order to achieve final effluent characteristics that comply with discharge limits and standards (Bustillo-Lecompte, Mehrvar and Quiñones-Bolaños, 2013; Irshad *et al.*, 2015).

It should be noted that little data exists on directly traceable sources of waste and minimization strategies used, that allow one to determine the best and most cost effective alternatives for wastewater management in the meat industry (Johns, 1995). The information on conventional technologies presented in this review therefore is meant to give a better viewpoint on the challenges faced in dealing with wastewater in meat processing and as such support investigation into the use of constructed wetlands as a sustainable alternative technology for slaughterhouse waste management.

Many studies indicate that the more chemically engineered technologies attract large operation and maintenance costs not to mention a great need for skilled operators. These factors reduce both attractiveness and longevity of the aforementioned wastewater treatment alternatives and therefore more sustainable options are constantly sought after. Furthermore, Seif and Moursy, (2001) established that these conventional treatment processes often do not achieve environmentally compliant effluents. In his study, Koech *et al.* (2012) proposed that existing slaughterhouse facilities should be up-scaled to match the quantities of effluent produced daily. In addition, adoption of cleaner meat processing technologies was seen to be a significant step in curbing environmental and health risks associated with slaughterhouse waste.

Organic wastewater treatment using 'sustainable' biological alternatives like lagoons and constructed wetlands is gaining preference over physico-chemical treatment technologies due to the conventional systems' apparent inability to reduce BOD_5/COD loads to environmentally acceptable concentrations (Chunkao *et al.*, 2014).The passive nature of wetland systems mimicked by CWs with regard to low maintenance cost provides for a much better prospect in this regard (Van Oostrum, 1990).Their treatment capacity can be optimized by carefully considering an intermittent loading inflow to increase oxygen transfer, proper substrate selection and recirculation the effluent water. However, there is need for further research in order to help define and optimize design criteria with a view of long-term performance capabilities and limitations (Brix *et al.*, 1994).

Biological systems have also been seen to perform faster in the tropical regions. However, fat emulsification may pose a challenge due to relatively high temperatures typical of such regions (Johns, 1995).In addition, biological processes require long retention times and large reactor volumes with sludge control problems (Irshad *et al.*, 2015). Notwithstanding, researches by (Kayser and Kunst, 2005; Soroko 2007; Cui *et al.*, 2010; Vymazal 2010; Lavrova and Koumanova, 2013) have illustrated the efficiency of CWs as a biological treatment option. Treatment efficiencies for constructed wetland studies have shown removal efficiencies varying from 85% - 95% for COD, BOD_5, TSS, NH_4-N, colour, coliform, and faecal bacteria. 80% - 90% removal efficiency has been observed for TKN, EC, and organic compounds (Molle, Prost-Boucle and Lienard, 2008; Lavrova and Koumanova, 2013).

It is also indicated that BOD_5 can be efficiently removed by VFWs treating effluent from oxidation ponds which produce effluent with a BOD_5 concentration of about 200mg/L. (Chunkao *et al.*, 2015). Given that the effluent from the ponds is still too high for aquatic ecosystems, vertical sub-surface flow wetlands are particularly useful as a secondary treatment measure in such a case (Soroko 2007; Molle *et al.*, 2008; Chunkao *et al.*, 2014). Chunkao et al, 2015 observed that VSSFWs have been satisfactorily applied across Thailand for the same purpose.

2.3 Constructed wetlands for wastewater treatment

Constructed wetlands are artificial systems that mimic natural wetland systems in treatment function of wastewaters. Treatment occurs through biotic pathways such as plant nutrient uptake, microbial adsorption, bio-degradation or assimilation. Abiotic pathways include inter alia sedimentation of organic matter by substrate media, volatilization, and UV treatment.

Constructed wetlands can be classified as either Free water surface (FWS) or as subsurface flow (SSF) systems. As the name suggests, FWS wetlands have above ground water flow while SSF are characterised by gravitational water flows within porous substrate media. Under SSF, there are two other types of wetland systems namely; horizontal (HSSF) and vertical (VSSF) based on direction of water flow. The major difference between both systems as noted generally is oxygen transport within the wetlands. VSSF have better oxygen transfer ability by multiple mechanisms and as such is used in the scope of this study. (Description adapted from Kyambogo, Mbwette, T., Katima, Ladegaard and Jшrgensen, n.d).

These treatment systems have gained much recognition over the decades. This is because they are cost effective in terms of design, construction, operation and management. Furthermore, they require substantially less treatment area than conventional systems. Social acceptance of CWs is good owing to their ability to create recreation habitats and/or enhance natural ecosystems thereby improving quality of life.

The use of CW technology in the treatment of various wastewaters in Africa has been under investigation for a number of years. To date, this technology remains largely unused because little is still known about design and pollutant removal processes. Tanzania has in the last two decades stepped up investment in CW technology. This is owing to the great need for sustainable wastewater management alternatives for over 80% of the country (Kyambogo et al., n.d). Success achieved by Tanzania in implementation of CW in treatment of domestic and industrial wastewater sets precedent for its use in Kenya. Studies by (Kimwaga *et al.,* 2013 and Senteu, 2014) treating domestic effluent water, (Abira, 2008) treating paper mill wastewater and (Bodin, 2013) treating sugar factory wastewater have further confirmed this.

2.3.1 Vertical Flow Wetlands

Vertical flow wetlands were initially designed to provide higher levels of oxygen transfer for enhanced effluent treatment (Al Jawaheri, 2011). Nevertheless, they have remained less popular than conventional technologies due to information gaps on design and operation variables. There are a number of basic dimensions (feed mode, time, space, and biological complexity) to consider in the use of VFWs.

VFWs can either be planted or unplanted. Coleman, Hench, Garbut, Sextone, Bissonnette and Skouusen, (2001); Zhu, Sun, Zhang, Wu, Jia and Zang, (2012) observed that the presence of vegetation had minor variations on treatment efficiency of wastewater compared to action of gravel media. Abdelhakeem, *et al.* (2015) observed the contrary where, results indicated a significant difference in mass removal rates for most pollutants except for ammonia and phosphorous. Removal efficiencies of COD, BOD_5, TSS and NH^+_4 were observed to be 75%, 84%, 75% and 32% for the planted beds compared to 29%, 37%, 42% and 26% respectively, for the unplanted beds.

There is an indication that plants contribute more to nutrient uptake rather than organic matter reduction but even this is arguable. Langegraber, (2005) suggested that plant role is minimal compared to wastewater loading. He observed that nutrient uptake was 1.9 % in treatment of municipal waste compared to 46 % for lower loaded systems. Further to this, plants have been noted to be a source rather than sink for organic matter in poorly managed systems. These findings give an indication that plant function is minimal to negligible for treatment of high strength wastewaters and more so, organic matter.

The choice of either continuous or batch feed, time of wastewater retention in the system and level of microbial activity all contribute to the quality of effluent water obtained. The most common mode of operation is an intermittent loading of wastewater to the wetland surface until flooded, after which the water is allowed to percolate down through a substrate medium. The wetland is fed pulse-wise after the previous batch has drained thus allowing oxygen diffusion into the bed. As such, VFWs are far more aerobic than their HF counterparts, which make them very effective in organic matter and suspended solids removal. In a study by (Stefanakis and Tsihrintzis, 2012) on various design and operational characteristics, it was observed that, among all the constituent parameters monitored, OM removal achieved the highest efficiency. BOD_5 and COD exceeded 75% and 79% respectively. This indicates that OM is easily bio-degradable and is easily removed from the system.

VSSFWs are particularly poor in nitrogen removal especially in the form of NH_4-N (Van Oostrum and Cooper 1990) in cases where the wastewater has high COD. However, if anoxic microhabitats exist as a result of high organic matter available (acting as bacterial energy source), some denitrification may occur. Moreover, if the system design and mode of operation is targeted at NH_4-N removal, substantial treatment results can be obtained. In a study by Connolly *et al.*, 2004, NH_4-N removal occurred mainly by adsorption to the reed bed media (64%) while the rest was transformed to NO_2-N (4%) and NO_3-N (24%).

Design and operational mechanisms in vertical flow constructed wetlands

Vertical sub-surface flow wetlands can either be shallow excavations in the ground or built above ground depending on slope required for influent water flow and recirculation requirements. Treatment performance in VFCWs is said to depend on a number of operational factors that are tied to system design, wastewater characteristics and application (Stefanakis and Tsihrintzis, 2012). System related factors include substrate type, substrate pore size, bed depth, climate and maturity of the system (Bojcevska and Tonderski, 2007; Prochaska, Zouboulis and Eskridge, 2007). The wastewater characteristics are related to nutrient load, while application related factors include the hydraulic loading rate (HLR), influent concentration (Q) and level of wastewater pre-treatment. These application factors result in a hydraulic retention time (HRT) that is unique to a system if treated as a 'black box' where HRT is a response variable; which has a significant influence on extent of wastewater treatment.

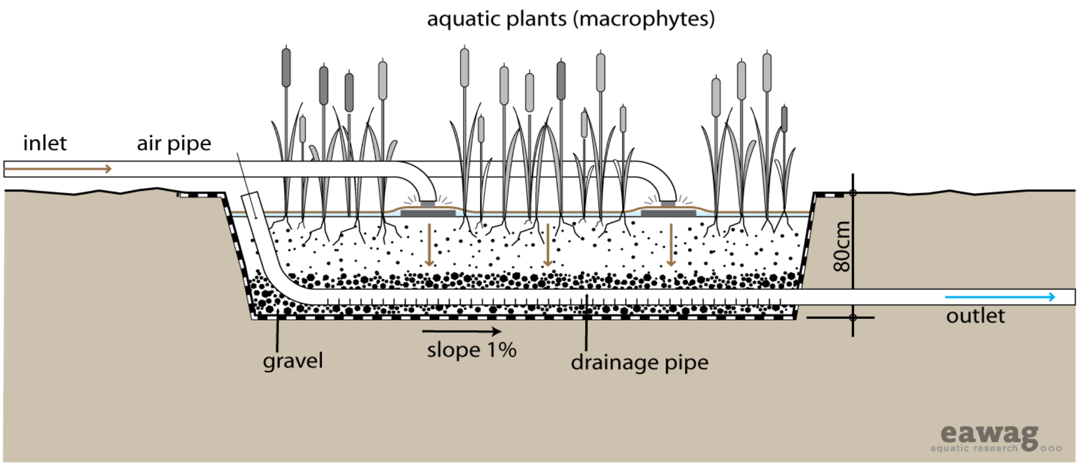

Figure 1: Schematic diagram of a conventional vertical sub-surface flow wetland design. Sourced from: Tilly, Ulrich, Luethi, Reymond and Zurbruegg, (2014).

Substrate characteristics

The choice of substrate media is crucial in wetland design. Grain size, media depth and pore size all contribute significantly to HRT, establishment of effective microbial communities in addition to removal efficiencies of different pollutants. The media must be fine enough to retain organic matter yet rough enough to ensure no clogging occurs while maintaining good oxygen penetration (Torrens, Molle, Boutin and Salgot, 2009). Each substrate has uniquely behaving structure and texture which evolve over time, making it difficult to generally characterize into given filter types. Global knowledgebase on behaviour of different substrates is little known also because water content and flow mechanisms vary greatly in complexity (Molle, Liénard, Grasmick and Iwema, 2006). The need for locally available substrate in any region where CW technology is applied creates precedent for further intensified studies on use of different media for optimal pollutant removal.

Wastewate*r *loading method and mode of operation

Mode of wastewater application plays a key role in determining the aerobic condition of the wetland and rate of substrate clogging. Given that VFWs require aerobic conditions for OM breakdown and subsequent BOD_5 reduction, a feeding mode that enhances maximal oxygen transfer is important. There are several pathways for oxygen penetration into the substrate media. These include, gas diffusion that occurs between doses and rest periods and which is considered as the primary aeration process (Kayser and Kunst, 2005). Others include convection as a result of batch feeding and dilute oxygen present in the wastewater.

A study by Zhao *et al.* (2003) demonstrated that the highest pollutant removal rates were achieved after a short saturation time followed by a long unsaturated time. This allowed good oxygen transfer in the media bed. Feeding mode is also known to a role in determining the extent and type of treatment processes within the wetland. COD removal and nitrification appeared to be dependent on feeding frequency in a study by (Bancolé, Brissaud and Gnagne, 2003). He showed that a higher feeding frequency of small volumes greatly enhanced both OM breakdown and nitrification. However, the removal trend of nitrogen observably reduced while that of COD remained constant, agreeing with findings by (Molle *et al.,* 2006). This was attributable to the preferential nitrification that occurred during rest periods between batches. Caution should be applied in the fractionating of batches because higher fractions may increase HRT, but at the expense of oxygenation within the system.

Bancolé *et al.* (2003) observed that lower daily fractions promoted even development of biofilm over the substrate depth which accumulates on the upper substrate layers for high loading frequencies. The latter diminishes hydraulic conductivity thus negatively affecting infiltration rate and oxygen transfer potential. This in turn threatens the wetland's lifecycle (Torrens *et al.*, 2009). In contrast, Bojcevska and Tonderski, (2007) proposed that the diminishing hydraulic conductivity was caused by anoxic microhabitats rather than increasing hydraulic loads. Both schools of thought illustrate the behaviour of newly created systems, which are known to have an initially high nutrient removal capacity which reduces steadily until they stabilize.

Resting periods between feeding batches are also important especially in the case where a change of treatment includes an increase in load application. General studies indicate that resting period deters excessive biomass accumulation and retards substrate clogging (Bojcevska and Tonderski, 2007). Prochaska *et al.* (2007) noted that organic matter which was not decomposed in previous feeding applications was transferred to lower depths of the treatment units. That contributed to an increase in effluent COD concentrations during subsequent treatments. In such instances, a significant carryover effect may be experienced and this may impact results on treatment efficiency.

Another factor of feeding mode is recirculation. Numerous studies on wastewater treatment show that effluent recirculation at a ratio of 1:1 greatly enhances purification capacity of a CW, more so in the case of high strength wastewater (Connolly *et al.*, 2004; Zhao *et al.*, 2004; Sun *et al.*, 2005; Lavrova and Koumanova, 2010; Lavrova and Koumanova, 2011; Prost-Boucle and Molle, 2012; Lavrova and Koumanova, 2013).

Hydraulic Retention Time (HRT), flow rate and Loading rates

Hydraulic retention time is generally known to have a positive linear relationship with nutrient removal efficiency (Wu, Zhang, Li, Fan and Zou, 2013). This is regulated by flow rate of influent wastewater. A high flow rate would promote faster percolation of water through the media, reducing contact time for microbial action (Lavrova and Koumanova, 2013). Consequently, measures like recirculation would be required to improve treatment efficiency.

The HLR substantially impacts treatment efficiency of any give wetland system, particularly in tropical regions due to suitability of temperature for rapid OM breakdown and thus substrate evolution. For a given HL, a high volume of wastewater applied in batch mode may favour oxygen penetration and increase infiltration rate but at the same time may also reduce exchange between mobile and less mobile water (Molle *et al.*, 2006). On the other hand, decreasing the batch volume increases retention time of the water which allows greater exchange between the mobile and less mobile pore water. There is therefore extended interaction between biofilm and percolating water, which increases removal efficiency of pollutants.

System clogging is a very big operational concern for vertical flow systems. This is largely influenced by operational factors aforementioned i.e. feeding mode, loading rates and substrate (Prochaska *et al.*, 2007). It is therefore important to have a good balance between all factors in order to minimize chances of system clogging (Stefanakis and Tsihrintzis, 2012). Jing, Lin, Wang and Lee, (2002) and Lin, Jing, Lee and Wang, (2002) established that application of different HLRs successively from low to high in experimental design will most likely introduce an undesirable effect of system ageing. As such, it would be particularly important to design experiments that have simultaneous loads (Bojcevska and Tonderski, 2007) rather than step wise increments in HLR, in order to determine optimal operation capacity of substrate while avoiding rapid system collapse.

2.4 Pollutant removal processes in Constructed Wetlands

Many studies have shown that the main pollutants of concern in wetlands treating slaughterhouse wastewater are usually organic matter and nitrogen fractions as they constitute the largest pollutant fraction. There exist significant variations in pollutant removal processes between and within different treatment systems. This is attributable to complex physical, biological and chemical interactions facilitating the treatment (Moshi, 2015).

The first recommended step in pollutant removal is usually pre-treatment. Solid particles are removed in order to retard their further breakdown and consequent increase of COD (Al Jawaheri, 2011). The second step is to check and correct for pH. The optimum pH operation range for biological systems is usually between 6.8- 8.5. Any values above or below this could retard functional efficiency of microbial communities present (Goronszy, Eckenfelder and Froelich, 1992). Nitrification process is known to reduce alkalinity of wastewater and as such significant nitrification may result in lowered pH and hinder denitrification (Kadlec and Knight, 1996). According to literature denitrification can be hampered at pH < 6.0 and pH > 8.0, with an optimal rate observed at pH range 7.0-7.5 (U.S. EPA, 1975) (cited by Saeed and Sun, [2012]). It is therefore recommended to lime acidic wastewaters and add sulphuric acid or CO_2 gas to alkaline waters (Britz, Van Schalkwyk and Hung, 2006).

Nitrogen removal processes are generally known to be significantly influenced by temperature and dissolved oxygen (Bodin, 2013). Tuncsiper, (2007) reported 7% higher NH_4-N removal, during summer in comparison to winter in constructed wetlands treating tertiary effluents. Langergraber, Tietz and Haberl, (2007) indicated that NH_4-N concentration in the effluent of VF wetlands increased when temperature dropped below 12 ^{0}C.

According to (Saeed and Sun, 2012), nitrogen transformation and consequent removal occurs in three main pathways. These are biological (ammonification, nitrification, denitrification, plant uptake and biomass assimilation), physico-chemical *inter-alia* (ammonia volatilization and adsorption) and those dependent on microbial metabolism. The latter are newly discovered and include partial nitrification, denitrification anammox and canon process.

The order of transformation depends on high amount of organic nitrogen in the wastewater, in which case, ammonification initiates transformation. This is followed by nitrification. Conversely, high amounts of NH_4-N in the wastewater initiate the nitrification step first. Obligate chemolithotrophic bacteria consume oxygen to form NO_2-N, which is then transformed to NO_3-N by facultative chemolithotrophs. Heterotrophic nitrifying bacteria are also known to nitrify NH_4-N. The denitrification process which follows, occurs by bacterial action to produce nitrogen gas (N_2), nitrous oxide (NO_2) and nitric oxide (NO) (Matheson and Sukias, 2010) all of which from bicarbonate salts resulting to raised water pH (Kadlec and Wallace, 2009).

Biomass assimilation proceeds through incorporation of NH_4-N in the heterotrophic biomass to fulfil nutrient requirements. Nitrogen assimilation via biomass had been reported in VF wetlands, fed with diluted pig slurry supernatant (Sun *et al.,* 2005). The authors noted that nitrification accounted for only < 10% of the NH_4-N removal, while overall NH_4-N removal ranged between 27 and 48%. Since the organic loading and removal rates in the experimental systems were higher, assimilation of NH_4-N into heterotrophic biomass could have played a vital role, in terms of nitrogen removal (Sun et al., 2005).

The physico-chemical process of ammonia volatilization occurs through mass transfer of the gas into the atmosphere (off-gas). It is highly dependent on wastewater pH. Wastewater with high alkalinity (pH > 9.3) results in NH_4-N conversion to NN_3 gas which is then volatilized (Cooper *et al.,* 1996; Bialowiec *et al.,* 2011). Ammonia volatilization is generally insignificant in subsurface flow wetlands, when the pH value is below 7.5-8.0 (Reddy and Patrick, 1984) (cited by Saeed and Sun, [2012]).

Adsorption in wetland systems is governed by media-cation exchange (Bayley, Davison and Headley, 2003) in the water. Media with cation exchange properties has been employed in wetland systems to optimize nitrogen removal (Yalcuk and Ugurlu, 2009; Cui et al., 2010; Saeed and Sun, 2011). In VF systems, the adsorbed NH_4-N can be nitrified by the attached biofilms (Connolly et al., 2004), due to predominant aerobic conditions inside the media. In addition, the reduction of NH_4-N concentration in the bulk water can stimulate the release of adsorbed NH_4-N, for maintaining chemical equilibrium (Vymazal, 2007). In such cases, adsorption can only facilitate the conversion of nitrogen, without changing the net quantity in wastewater. Matrix oriented adsorption processes are not frequently observed in wetland systems since common wetland media gravel has very low adsorption capacity (Keffala and Ghrabi, 2005).

Suspended solids, BOD and COD removal are not as sensitive to temperature (Kadlec and Wallace, 2009) therefore indicating that physical processes like retention time and sedimentation rate are the major determinants for TSS while bio-chemical interactions control the latter. Presence of macrophytes is known to increase the sedimentation process, particularly through retarding re-suspension of the sediment particles by trapping them in the root/litter layer (Kadlec and Wallace, 2009).

Organic compounds can be degraded aerobically and anaerobically in subsurface flow wetlands. Oxygen for aerobic degradation can be supplied via atmospheric oxygen diffusion, convection (wind effect), and/or macrophyte root transfer into the plant rhizosphere (Cooper et al., 1996). Aerobic degradation is facilitated by chemoheterotrophs which have a faster metabolic rate than chemoautotrophs (Saeed and Sun, 2012). Oxidised organic matter utilised the available oxygen to release carbon dioxide ammonia and other stable compounds (Garcia et al., 2010). Due to the higher availability of oxygen provided by vertical flow systems, aerobic degradation of organic matter occurs preferentially (Saeed and Sun, 2012).

Anaerobic degradation takes place in media zones devoid of oxygen. It is a two-step process performed by heterotrophic bacteria through fermentation. Acid forming bacteria convert organic matter into organic acids and alcohols (Saeed and Sun, 2012). Breakdown can also occur due to action of methane forming bacteria through methanogenesis. This group converts organic matter to new cells, methane and carbon dioxide as well. Both fermentation and methanogenesis occur in anaerobic media zones (Kadlec and Knight, 1996) and have very diverse pathways of compound transformation.

2.5 Effects of environmental variability in Constructed Wetlands

Climatic conditions have a cascading effect on the treatment efficiency of constructed wetlands due to their influence on abiotic factors such as solar radiation, temperature, precipitation and evapotranspiration (ET) (Kadlec and Wallace, 2009). These factors in turn affect biotic processes such as microbial and vegetation activity within the wetland. Studies on the influence of climate strongly indicate a significant difference in performance of CWs in Temperate and Tropical regions. These differences reveal that design, operational and maintenance strategies used for these regions are not directly replicable (Bodin, 2013).

Tropical climates experiencing warm and dry climates are particularly vulnerable to environmental vagaries such as rainfall and evapotranspiration. Both of these are important in that they influence the water balance in a CW system. Small scale wetland systems frequently show enhanced ET due to advection from the relatively warm and dry terrestrial surrounding (Kadlec and Wallace, 2009; Borin *et al.*, 2011). Evapotranspiration is a significant consideration in constructed wetlands because it has the potential to substantially affect functioning and treatment efficiency of the wetland (Kadelc and Knight, 1996; Bialoweic *et al.*, 2006). Water volume passing through a CW system may decrease under high ET, thus increasing the concentration of outflow dissolves compounds and even lack of effluent water may be experienced as has been proven by (Bialoweic *et al.*, 2006). High ET in this case was observed to be in excess of 2.5 mm d^{-1}.

Macrophytes are another pathway for ET loss because of their low water use efficiency (Bialoweic and Wojnowska-Baryla, 2007; Headley *et al.*, 2012). Despite these observations, many studies conducted on CWs base their treatment results on differences between inflow and outflow pollutant mass removal rates without consideration for ET and water balance dynamics (Kyambadde *et al.*, 2004). In many instances, water loss within a CW is typically not negligible and therefore assessment of results using the latter method can lead to significant errors and observably differ from those factoring in ET, water balance dynamics (Bialoweic *et al.*, 2014)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Njoro slaughterhouse is located in the agricultural town of Njoro Town. This lies approximately 18 km south west of Nakuru, Kenya (-0.31358, 35.95829), with a growing population of about 64,881 people, spread across 124.6 km^2 (Kenya Bureau of Standards- KEBS, 2013). The region receives 1000mm of rainfall per year. There is one river, Njoro River which drains into the saline Lake Nakuru. Both surface and groundwater are an important source of portable water. The main economic activities are agri-based industries, saw-milling, crop and livestock farming, with the latter being practiced by about 80 % of the households mainly in mixed farming systems. In the past, the land was predominantly forests but due to the expansion of agriculture and the general population growth, these have receded (Rosa, 2009). Egerton University is located about 7 km away from Njoro slaughterhouse making it a suitable location for experimental setup of the study (Figure 2). Proximity of the slaughterhouse to the University also informed the choice of location for the experimental setup.

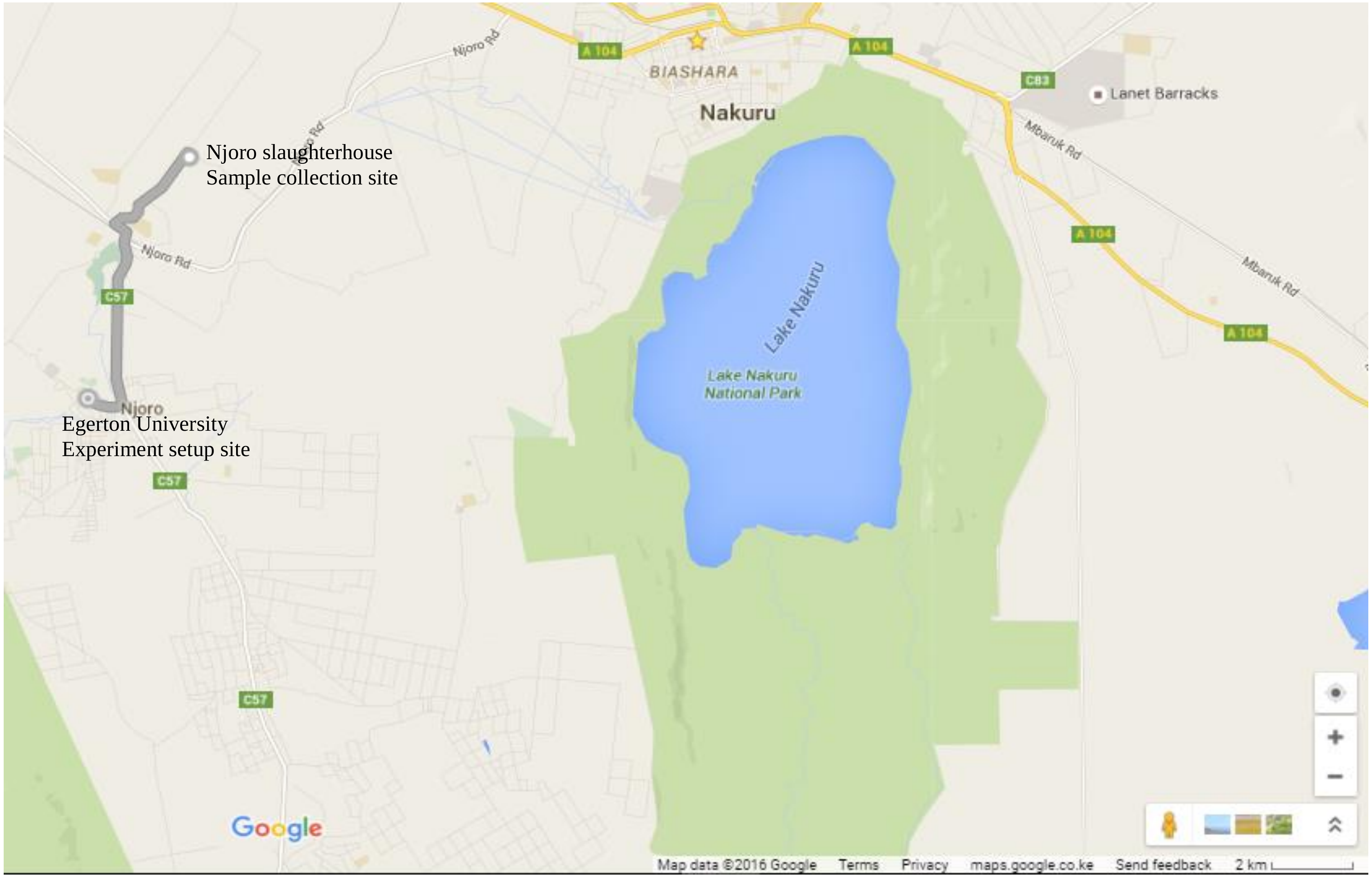

Figure 2: The location of the slaughterhouse wastewater sample collection and experimental site

3.2 Sourcing of wastewater

Njoro slaughterhouse is a mid-scale level facility and as such represents the larger portion of operating abattoirs around the country. The facility has an average daily production output of 22 bovine heads. There are two waste separation channels, one for blood and the second for carcass processing water, each leading into the respective collection tank. However, these channels are not an efficient separation technique as blood mixes with processing water during washing of the facility. On average about 1200L are used for facility cleaning under low meat demand while 250 L of fresh water is used per cow (Personal communication Ndirangu- Manager at Njoro slaughterhouse, 2016). The facility has three settling tanks for wastewater that are connected in series. For this study, wastewater was exhausted from the last of these tanks for use in experimental setup as it had sufficient amount of wastewater for running the setup. The exhausted water sample was transported to the experimental site and stored in plastic tanks with a total holding volume of 800 L.

3.3 Study design

The study design consisted of a preparation stage where substrate sieve analysis was done to obtain the appropriate diameter sizes for the test substrates used. Sieve analysis was conducted in the civil engineering laboratory at Egerton University to verify diameter size of each substrate type before filling the respective mesocosms. The substrate sizes were according to specifications outlined in Table 2 below. The experimental setup stage included: a) preliminary wastewater characterization in order to obtain values on influent concentrations of the study parameters, b) System configuration in which the treatments were set up (Figure 3), c) Operation mode used and d) sampling method of the wastewater.

Table 2: Experimental setup and substrate characteristics

No of mesocosms	**3**	
Replicates	4	
Dimensions	0.3 m diameter x	
	0.9 m height (W2 and 4)	
	0.75 m height (W1 and 3)	
Available area	$0.0567m^2$ @ 0.9m and	
	0.0471 @ 0.75m	
Substrate thickness	**Substrate depth**	**% porosity**
	W_1 W_2 W_3 W_4	
a) Coarse sand (2-5 mm)	65, 80, 65, 80	32
b) Gravelly sand (8-9 mm)	65, 80, 65, 80	35
c) Fine gravel (16-19 mm)	65, 80, 65, 80	38
Support layer (20-25 mm)	10, 10, 10, 10	40

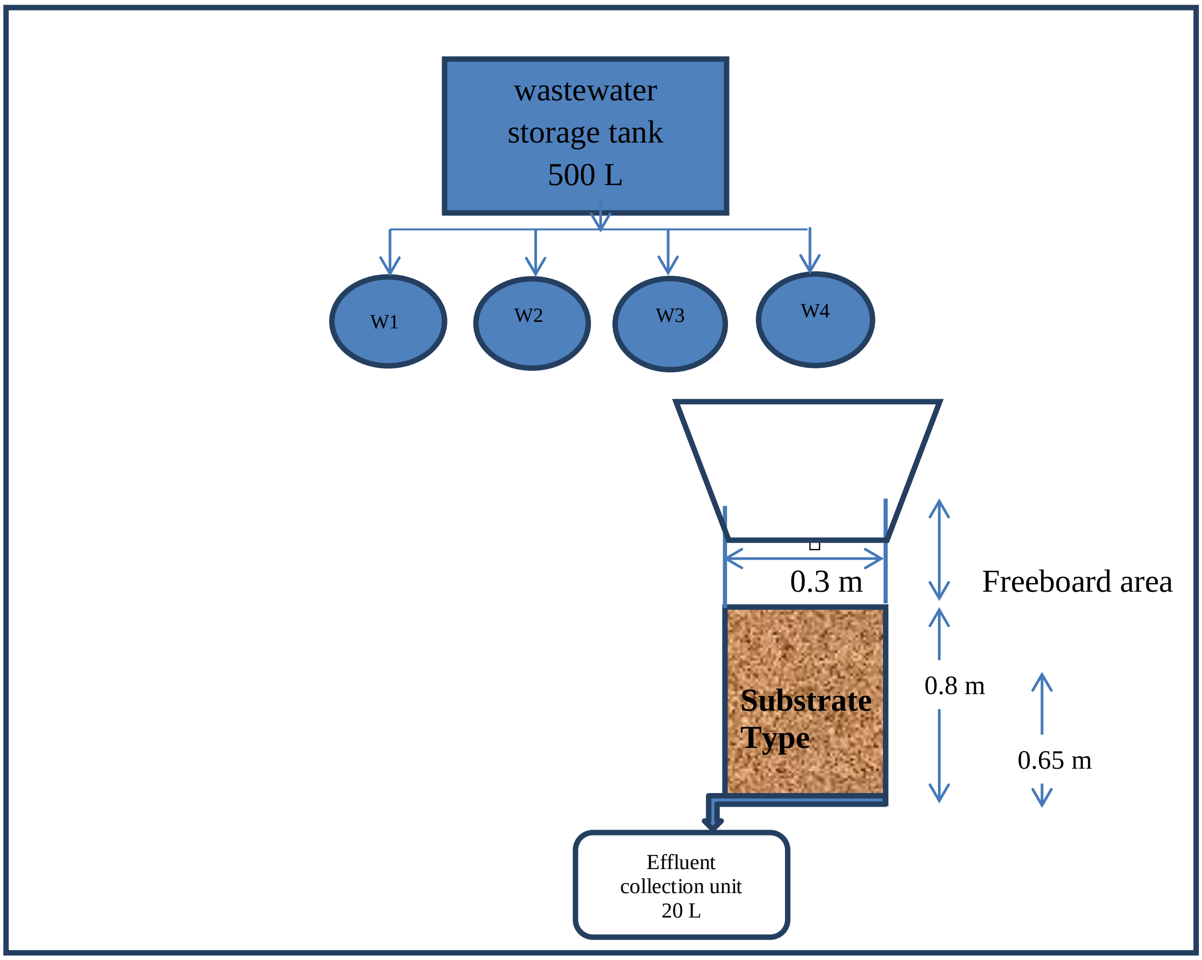

Figure 3: Layout of experimental setup

3.3.1 Substrate sourcing and sieving analysis

Ballast was sourced form quarries within Nakuru County and transported to the Civil Engineering lab in Egerton University, Njoro for the sieve analysis. Grading was done using 5 mm and 2 mm sieves to obtain sand of 2-4 mm diameter. Fine gravel of 8-9 mm was obtained by using sieves of 6mm and 9.2 mm. Mid-sized gravel of 16-18 mm was obtained by using 16 mm and 19 mm sieves. Finally, coarse gravel of 20 -24mm was obtained using 20mm and 25 mm sieves. After separation, all the substrates were washed to reduce silt and other organic impurities and dried. Each substrate type was then filled into the respective mesocosms at required media depths for the study, (APPENDIX 1).

3.3.2 Experimental setup

System configuration

The experiment was set up outdoors and consisted of four cylindrical metal tank mesocosms of 0.3m diameter, each with four similar replicates totalling to sixteen tanks. Eight of these tanks had a media depth of 0.9m (0.8 + 0.1) and the other eight had 0.75m (0.65 + 0.1) depth. The 0.9 m columns represented deep mesocosms while 0.75 m columns represented shallow mesocosms. All units has a supporting layer of 20 mm gravel to a height of 0.1 m on top of which, two replicates of each depth were filled with substrate media of either coarse sand 2-4 mm, gravelly sand 8-9.2 mm, fine gravel 16-19 mm or mid-size gravel 20 ± 5 mm. Each mesocosm was fitted with a half inch tap 0.1 m from the bottom to acts as the outlet. An effluent bucket with a holding capacity of 20 L was placed 0.2 m below each mesocosm to act as a collection unit during sampling. The media beds were not planted due to a lack of determinable importance in organic matter reduction.

The mesocosms were labelled A1, A2, B1, B2, C1, C2, D1 and D2. A1 and A2 represented 2 mm sand at 0.65 m and 0.8 m depth. B1 and B2 represented 16 mm gravel at 0.65 m and 0.8 m depth. C1 and C2 represented 8 mm gravel at 0.65 m and 0.8 m depth. D1 and D2 represented 20 mm gravel at 0.65 m and 0.8 m depth, (APPENDIX 2). The setup was shaded from extreme heat and rain in order to reduce the anticipated effects of environmental variability. The mesocosms were also arranged in random order in two rows of eight in order to account for the same.

Wastewater characterisation

Samples were collected from the slaughterhouse' holding chambers and analysed for 5 day Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), ammonia nitrogen (NH_4-N), total suspended solids (TSS), pH, electrical conductivity (EC), dissolved oxygen (DO) and temperature using standard protocols outlined in APHA, (2004). The wastewater was allowed to settle overnight and afterwards it was filled in all the mesocosms, submerging the media beds. The wastewater rested in the mesocosms for ten days prior to commencement of the operation and sampling phase. This was in order to allow microbial communities develop. Integrated effluent samples were collected after the period and analysed again for BOD_5, COD, NH_4-N, TSS, pH, EC, DO and temperature. The data obtained was used to establish influent concentrations at the beginning of the study. The study ran for three months from January to March 2016.

Calculation of loadings, removal rates and efficiencies

Calculations are adapted from formulas presented by (Abdelhakeem *et al.*, 2015) in a related study.

1) Both organic (OLR) and hydraulic (HLR) loading rates were determined using the equations below.

$$OLR\ (g/m^2/batch) = (\mathrm{Q} * C_i)/\mathrm{As} \quad (1)$$

$$HLR\ (m^3/batch) = \mathrm{Q}/\mathrm{As} \quad (2)$$

Where Q = discharge rate (m^3 per day)

C_i = concentration of influent (mg/L)

As= surface area of the mesocosm (m^2)

2) Removal efficiencies of pollutants in the mesocosms were calculated using the equation below and represented as percentages

$$Removal\ efficiency\ (\%) = ((C_i - C_{out})/\ (C_i) * 100) \quad (3)$$

Where C_i and C_{out} = influent and effluent concentrations respectively

3) Mass removal rates of each pollutant were calculated using the equation below as amount of pollutant removed in $g/m^2/day$. The results were presented in Appendix 6.

$$Mass\ removal\ rate\ (g/m^2/day)\ = Q * (C_i - C_{out}) \quad (4)$$

Where Q is discharge rate (m^3 per day), C_i and C_{out} are influent and effluent concentrations respectively

3.4 System operation

The setup was operated in a batch flow method. Buckets with a 4mm perforation at the bottom and a set flow rate of 41 ml min^{-1}, were chosen as the wastewater distribution mechanism. These buckets were initially corked at the bottom using an improvised plug and placed on the rim of each mesocosm. They were then filled with wastewater to coincide with respective volume for the sample depths and the bottom unplugged to release water into the mesocosms. The deep mesocosms were fed with 30 L of water while the shallow mesocosms received 25 L of wastewater. Both flow rate and influent concentrations of BOD_5 and COD were used to calculate the hydraulic and organic loads of organic matter going into the mesocosms. Spatial replication was considered in the design by having a replicate of each mesocosm, while temporal replication was considered by repeated sampling for each retention time under study.

3.4.1 Water sample collection

Experimental sampling involved the collection of an integrated influent water sample and individual effluent water samples of every mesocosm unit. During every sampling session, physical-chemical parameters: pH, EC, DO and temperature were measured *in situ* using a calibrated HQ 40d (HACH) multi-meter. Five sample replicates were collected for each of the retention times studied. These were in the order of HRT at 1, 3 and 5 days. During sampling, the columns were drained in such a way that an integrated sample was collected from the bottom and upper half of each column. This method facilitated an analysis of the vertical treatment profile of the pollutants in each mesocosm. A known volume of both bucket and mesocosm capacities were used to estimate half of the total sample volume contained in each mesocosm. The drained water samples were then re-circulated into their respective mesocosms at a ratio of 1:1 and set flow rate of 41 ml min^{-1}.

Between each HRT, the mesocosms were rested for 3 days to allow re-oxygenation of the substrate. Wastewater previously collected from the slaughterhouse and stored was diluted with the partially treated effluent water obtained at the end of each experiment stage. The resulting mixture was characterised and fed into the mesocosms for the next experiment cycle.

Effluent samples collected every sampling period were immediately transported to Egerton University aquatic sciences Laboratory in 500ml plastic bottles for analysis using APHA, (2004) methods. Parameters determined included COD, BOD_5, TSS and NH_4-N. Standard calibration curves for each parameter were prepared using the same methods and absorbance readings were taken using a GENESYS 10uv scanning spectrophotometer.

3.4.2 Water sample analysis

Description of analytical procedures and apparatus used in this study is detailed below. Samples were analysed in duplicate for each mesocosm at bottom and upper half sampling depths. APHA, (2004) standard methods were used for sample analysis. Volume of sample used for analysis of each test parameter was adjusted according to appropriate dilution ratios identified in the preliminary test phase. Blank samples were also analysed for each test parameter in order to provide a background concentration and correction standard for anomalous values identified in the analysis.

BOD_5 determination

A sample volume was added to BOD_5 bottles of known volume and topped up with aerated distilled water having an oxygen concentration of (7.2-7.6 mg/L). The initial DO was determined and sample bottles carefully filled to exclude air bubbles then capped tightly and stored in the dark at 20ºC for five days. After the 5 days, final DO was determined and BOD_5 calculated using the equation below. A volume of 0.5 ml was used in the initial dilutions and later increased to 1 ml sample as the BOD_5 decreased.

$$BOD_5\ (mg/L) = ((B - S) * vb))/c \qquad (5)$$

Where:

B = DO in blank after 5 days

S = initial oxygen in bottle

vb = volume of BOD sample bottle

c = volume of sample used

COD determination

Oxidation of organic matter was done by adding 1.5 ml $K_2Cr_2O_7$ digestion solution to 2.5 ml sample contained in a digestion tube and then adding 3.5 ml H_2SO_4/Ag_2SO_4, forming an acid layer at the bottom. 40 fold dilution of the sample was used. The tubes were swirled to homogenise the contents and then placed in a heating block at 150°C for 2hrs. The samples were left overnight to cool and absorbance read at 600 nm using a GENESYS 10uv scanning spectrophotometer. The values obtained were checked against respective standard curve absorbencies to obtain actual pollutant concentrations. Bio-degradability index was calculated as the fraction BOD_5/COD in order to determine whether the organic matter present could be biologically degraded easily.

NH_4-N determination

Sodium salicylate method was applied 0.06 ml sample was used for analysis. Reagents of sodium salicylate and hypochlorite solution were added consecutively and the samples stored in the dark at 25°C for 90 minutes. Thereafter, absorbance was read at 665 nm using a GENESYS 10uv scanning spectrophotometer for concentration relation with standard curves.

TSS determination

Total suspended solids were determined gravimetrically on Whatman GFC filters which had been pre-dried at 95°C for 24 hours to achieve a constant weight and eliminate filter moisture. A definite volume of sample was filtered and then dried for 3 hrs to a constant weight. Difference in weights of the filters before (Wf) and after combustion (Wc) were calculated in grams, taking into account the volume filtered.

$$TSS\ (mg/L) = ((W_c - W_f) * 10^6)/v^{-1} \tag{6}$$

Organic matter content was also determined by subtracting weight of the ashed filters at 500 °C (AFDW) from the TSS value.

$$Organic\ matter\ (g) = TSS - AFDW \tag{7}$$

3.5 Data management and analysis

MS Excel was used for raw data entry and management before transfer to R software version 0.98.1103.0 for both descriptive and inferential analysis. It is important to note that aggregation of the data set resulted in data that had a higher variability than the individual mesocosm performance.

3.5.1 Descriptive statistics

Measures of central tendency were described using pastecs package (Grosjean and Ibanez, 2014) for arithmetic means, standard error and coefficients of variation then presented as tables and boxplots for visual interpretation. Boxplots were used to identify outliers using sciplots (Morales, M., R Development Core Team and Murdoch, D., 2012), Histograms and QQ plots to determine distribution of the data set, which was observed to be non-normally spread. Excel was also used to assist in graphical representation of this information.

3.5.2 Inferential statistics

ANOVA

Multifactorial ANOVA was conducted on transformed data for HRT 1 and 5, testing effect of substrate and depth on pollutant removal. Location of significance was also tested using TukeyHSD post-hoc test. ANOVA was preceded by Shapiro-Wilk normality test and Bartlett post-Hoc test followed by Log transformation. The data for HRT 3 still had a high variance therefore Kruskal Wallis rank sum test was used and location of significant differences verified using post-hoc Nemenyi test at $p < 0.05$ (Pohlert, 2014).

CHAPTER FOUR

RESULTS

4.1 Slaughterhouse wastewater characteristics

A preliminary analysis of the wastewater was done in order to establish characteristics of the slaughterhouse effluent (Table 3). The slaughterhouse wastewater was then fed into each mesocosm, submerging the media and left for 10 days in order to allow development of microbial communities, adapted from (Wu *et al.*, 2013). No seeding was required as the wastewater had sufficient microbes. Afterwards, the mesocosms were emptied and effluent samples analysed. Table 3 below gives a summary of arithmetic means with standard deviations for the wastewater parameters selected. The fresh sample was analysed in triplicate (n = 3) while the detained wastewater was analysed in duplicate for each mesocosm (n = 32). The wastewater characteristics were observed to be higher than reported ranges similar wastewater.

Table 3: Characteristics of fresh slaughterhouse wastewater

Parameter	Fresh slaughterhouse effluent	After 10 day detention time
BOD_5 (mg/l)	2,098.49 ± 40.53	2,000.66 ±56.91
COD (mg/l)	25,558.33 ± 5,007.71	5,214.02 ±208.40
NH_4-N(mg/l)	52.70 ± 0.28	8.96 ±0.34
TSS (mg/l)	1,677.14 ± 244.26	407.95 ± 19.79
DO (mg/l)	0.07 ± 0.02	0.34 ± 0.09
Temperature (^oC)	25.7 ± 0.38	17.77 ± 0.26
EC (mS)	10.17 ± 0.01	6.79 ± 0.10
pH	10.23 – 10.25	8.84 – 8.89

The difference in concentration of the pollutants analysed initially and after 10 days detention indicates that microbial communities established themselves well within the substrate media. This step was seen to act as pre-treatment stage and was very beneficial in pollutant reduction especially for COD, NH_4-N and TSS.

4.2 Temporal variations of slaughterhouse wastewater characteristics

During the course of the three month study, concentration of *in-situ* parameters of the slaughterhouse effluent wastewater was monitored and results summarised in (Table 4) below. Wastewater was collected from the slaughterhouse septic tank in December and stored in plastic tanks at the experiment site for the duration of the experiment. It was replenished in February after required experimental volumes decreased. Characterisation was done immediately before commencement of each experiment cycle. This was aimed at establishing whether there were any significant variations in concentration of these parameters over time. Knowledge on physico-chemical parameters helped elucidate patterns observed in effluent data, due to the strong inter-relationship between physical, chemical and biological characteristics of water.

Table 4: Physico-chemical wastewater characteristics over the study period.

Parameter	Dec	Jan	Feb	Mar
BOD	2,098.49 ± 40.53	1941.19 ± 10.61	1100 ±70.77	1157.25 ± 77.95
COD	25,558.33 ± 5,007.71	9389.58 ± 331.45	2967.71 ± 39.06	1583.4 ± 14.73
NH_4-N	52.70 ± 0.28	0.062 ± 0.0012	0.055 ± 0.004	0.28 ± 0.00036
TSS	1,677.14 ± 244.26	899.93 ± 16.1	1042 ± 72.16	5575 ± 157.09
DO	0.07 ± 0.02	0.865±0.48	0.06±0.01	0.06±0.01
Temp	25.7 ± 0.38	15.5±0.42	18.45±0.44	21.1
EC	10.17 ± 0.01	7.58±1.98	6.78±0.0081	7.71±0.007
pH	10.23 – 10.25	8.50- 8.55	9.74- 9.88	8.81- 8.87

Physico-chemical wastewater characteristics were taken monthly and presented as averages plus/minus standard error (n = 605). The months are representative of experimental sampling cycles in which Dec is wastewater characterisation; Jan is 1 day retention time study. Feb is 3 day retention time study and Mar is 5 day retention time study. *pH was presented as range.

4.3 Hydraulic and organic loadings of BOD_5 and COD

This study conducted a stepwise decrease in loadings by virtue of the depth treatments and wastewater concentration. Results indicated in (Table 5) below, that reduced loads under longer retention gave better results.

Table 5: Influent wastewater loads

HLR (m^3/batch)	OLR (g COD/m^2/batch)			Treatment depth (m)
	HRT1	HRT3	HRT5	
0.0527	418.90	132.40	25.87	0.80
0.0447	495.46	156.59	30.59	0.65

Influent wastewater loads were calculated using Eq. (4) and (5) at each retention time for the deep mesocosms having 0.8 m and shallow mesocosms having 0.65 m. Hydraulic lading rate was higher for deeper mesocosms. Organic loading rate decreased between the retention times because the wastewater characteristics changed with increase in detention time of the stored slaughterhouse effluent.

4.4 Pollutant removal efficiency

Pollutant removal efficiency was calculated as a percentage of the difference between influent and effluent concentrations in (Table 6) below using Eq. (3). Retention time of 5 days gave best results for TSS and NH_4-N while there was no determinable difference in removal efficiency between 3 day and 5 day retention time for both BOD_5 and COD. TSS removal efficiency varied at $p < 0.001$ between 5-1day retention and between 5-3 day retention for which 5 day retention gave best results in both cases. NH_4-N was calculated in grams while BOD, COD and TSS were calculated in milligrams. The output was graphically presented in (Figure 4) below. Under one and three day retention times, NH_4-N was observed to generally increase rather than decrease indicating that there were processes within the mesocosms that generated ammonia rather than reducing it.

Table 6: Influent and effluent concentrations of pollutant for different substrate treatments

Substrate	HRT 1	HRT 3	HRT5
		BOD	
	1941.19 ± 10.61	**1100 ±70.77**	**1157.25 ± 77.95**
A1	1695.29±784.78	671.43±307.66	534.04±173.18
A2	1831.26±150.76	497.03±187.29	496.8±186.15
B1	1907.32±812.44	551.18±301.96	584±182.56
B2	1712.69±825.08	628.06±169.61	590.73±154.60
C1	1695.51±845.06	550.08±278.88	557.83±169.06
C2	2042.11±468.38	672.04±262.58	651.98±370.94
		COD	
	9389.58 ± 331.45	**2967.71 ± 39.06**	**1583.4 ± 14.73**
A1	2682.61±1617.76	2169.01±1351.22	649.73±279.84
A2	4567.01±1710.19	1977.28±1313.68	710.73±346.70
B1	4020.50±2994.49	2243.48±1440.37	832.08±318.67
B2	4193.021±1668.01	1803.77±1093.50	791.46±300.32
C1	3540.88±4457.50	2241.51±1385.78	808.65±303.25
C2	3208.33±2058.13	1913.49±1110.15	748.36±260.87
		NH4	
	0.062 ± 0.0012	**0.055 ± 0.004**	**0.28 ± 0.00036**
A1	0.065±0.053	0.153±0.087	0.227±0.049
A2	0.031±0.013	0.130±0.071	0.240±0.053
B1	0.107±0.084	0.141±0.075	0.183±0.079
B2	0.053±0.032	0.122±0.058	0.196±0.053
C1	0.110±0.080	0.136±0.079	0.221±0.055
C2	0.079±0.053	0.169±0.073	0.169±0.051
		TSS	
	899.93 ± 16.10	**1042 ± 72.16**	**5575 ± 157.09**
A1	513.56±321.87	463.03±142.67	788.21±297.61
A2	427.56±123.88	475.63±173.99	733.55±452.76
B1	602.09±377.12	592.46±248.76	1196.79±1126.46
B2	569.78±238.86	462.20±98.94	1028.50±652.34
C1	673.32±390.22	484.91±156.50	850.21±296.62
C2	551.64±211.74	488.96±288.30	823.37±218.01

The difference in concentration between influent (in bold) and effluent concentrations was used in E.q. (3) to calculate % removal efficiency and presented in (Figure 4) below. The treatments A1, A2, B1, B2, C1 and C2, represent sand at 0.65 m and 0.80 m, 16mm gravel at 0.65 m and 0.80 m and 8mm gravel at 0.65 m and 0.80 m respectively.

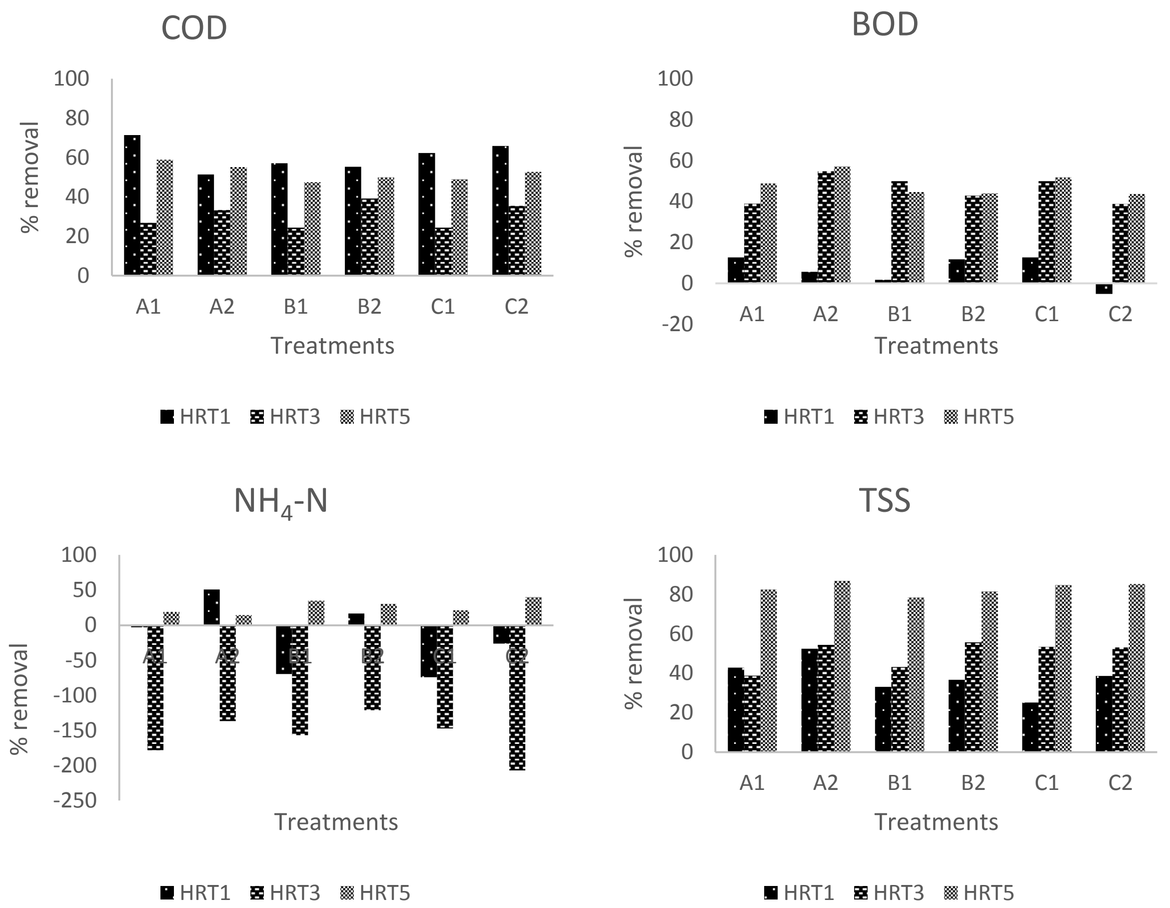

Figure 4: Removal efficiency of pollutant by different substrate treatments.

4.5 Effect of substrate type and depth on pollutant removal efficiency

4.4.1 Combined effect of substrate and depth on pollutant removal for HRT 1

Two way ANOVA was conducted to establish the effect of substrate- depth interactions on pollutant removal for 1 day retention time. Results in (Figure 5) indicated that substrate- depth interactions did not have a significant effect on removal of BOD *(ANOVA, $F = 1.839$, d.f. = 2, $p = 0.16$)*, COD (*ANOVA, $F = 1.853$, d.f. = 2, $p = 0.16$)*, NH_4-N *(ANOVA, $F = 0.564$, d.f. = 2, $p = 0.56$)* and TSS *(ANOVA, $F = 0.897$, d.f. = 2, $p = 0.41$)*. Further analysis was therefore carried out to investigate the individual effects of substrate and depth on pollutant removal.

4.4.2 The effect of substrate on pollutant removal for HRT 1

Effect of substrate type on pollutant removal was assessed using one way ANOVA (Figure 6). Differences were considered to be strongly significant at $\alpha < 0.05$ and weakly significant at $\alpha < 0.1$. Significance values were tabulated in (APPENDIX 3). BOD removal was observed to be unaffected by substrate treatments *(ANOVA, $F = 0.613$, d.f. = 2, $p > 0.05$)*. COD showed weak differences in performance between quarry dust and gravel *(ANOVA, $F = 2.542$, d.f. = 2, $p < 0.1$)*. NH_4-N showed treatment differences between sand and gravel *(ANOVA, $F = 4.731$, d.f. = 2, $p < 0.1$)* and between sand and quarry dust *(ANOVA, $F = 4.731$, d.f. = 2, $p < 0.01$)*, in which sand was observed to perform best overall. TSS showed no significant differences in removal between the substrate treatments *(ANOVA, $F = 1.302$, d.f. = 2, $p > 0.1$)*.

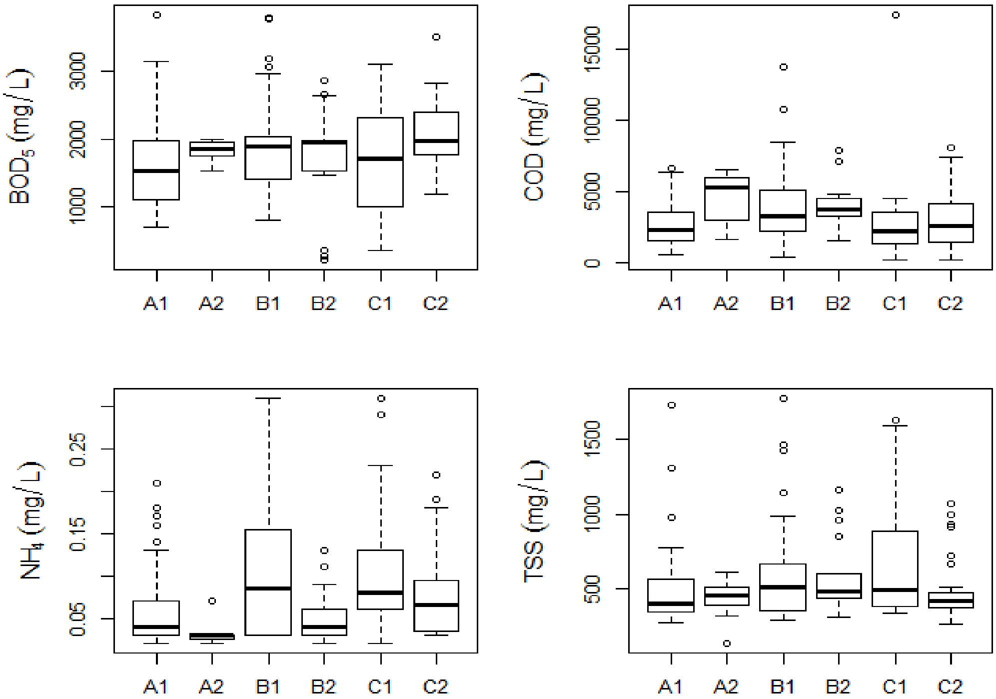

Figure 5: Overall pollutant removal of substrate treatments at HRT 1.
The treatments A1, A2, B1, B2, C1 and C2, represent sand at 0.65 m and 0.80 m, 16mm gravel at 0.65 m and 0.80 m and 8mm gravel at 0.65 m and 0.80 m respectively

4.4.3 The effect of depth on pollutant removal for HRT 1

The importance of depth was examined for both 0.65 m and 0.8 m mesocosms. One Way ANOVA was used to determine which mesocosms differed significantly in performance from each other. Results were presented as boxplot figures below and significance levels tabulated in (APPENDIX 4) for 0.65m depth analysis and (APPENDIX 5) for 0.8 m depth analysis. Generally, it was observed that BOD, COD and TSS showed no differences in treatment due to changes in depth.

Mesocosm performance at 0.65 m depth

Analysis of mesocosm performance at 0.65 m depth (Figure 6) indicated that all three substrates performed the same in terms of BOD, COD and TSS removal. Conversely, NH_4-N removal differed weakly between sand, quarry dust and gravel in which sand performed better than both quarry dust and gravel *(ANOVA, F = 3.661, d.f. = 2, p > 0.1).*

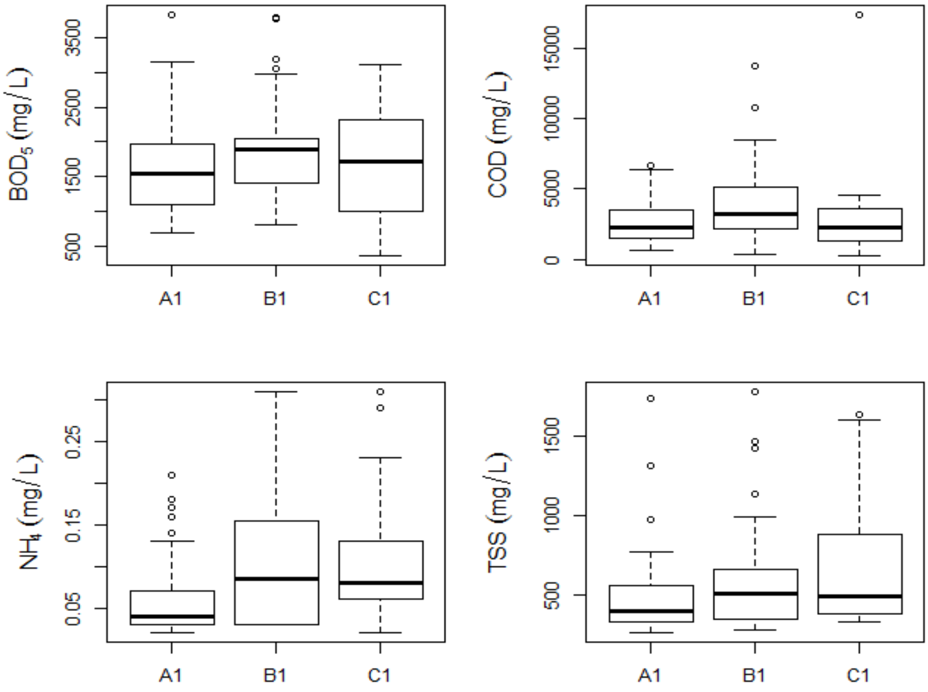

Figure 6: Pollutant removal of substrate treatments at 0.65 m for HRT 1.

Mesocosm performance at 0.8 m depth

Investigation of the deeper mesocosm performance (Figure 7) indicated that BOD and TSS showed no significant differences in treatment for all the substrates *(ANOVA, F = 2.144, d.f. = 2, p > 0.1, F = 1.966, d.f. 2, p > 0.1).* COD and NH_4-N performance on the other hand responded to depth treatment. There were weak relationships observed between sand, quarry dust and gravel. Both sand and gravel performed better than quarry dust *(ANOVA, F = 3.861, d.f. = 2, p < 0.1).*

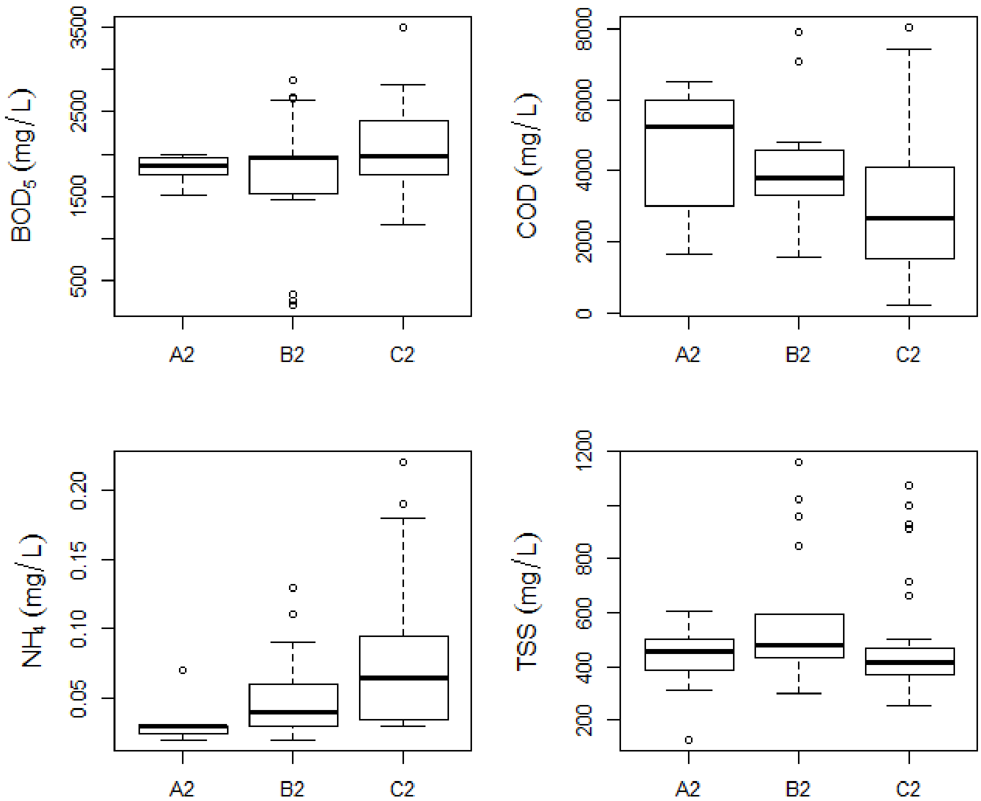

Figure 7: Pollutant removal of substrate treatments at 0.8 m for HRT 1.

4.4.4 Combined effect of substrate and depth on pollutant removal for HRT 3

Determination of the combined effect of substrate and depth on removal efficiency of pollutants was done using Kruskal Wallis rank sum test. Results as indicated by (Figure 8) below showed that only BOD_5 and TSS were affected by substrate- depth interactions in pollutant reduction. In the treatment of BOD, 0.65 m gravel mesocosm performed better than 0.8 m quarry dust mesocosm *(X^2 = 17.095, d.f. = 5, p = 0.092)*. The 0.65 m quarry mesocosm outperformed the 0.8 m gravel mesocosm *(X^2 = 17.095, d.f. = 5, p = 0.077)*, indicating that shorter mesocosms gave better results but neither of substrates outperformed the other. Sand gave better results than either gravel or quarry dust in the removal of TSS. The 0.8 m sand mesocosm performed better than 0.65 m gravel mesocosm (X^2 *= 15.604, d.f. = 5, p = 0.015).* Gravel at 0.65 m performed better than quarry dust at 0.8 m (X^2 *= 15.604, d.f. = 5, p = 0.073).*

4.4.5 The effect of substrate on pollutant removal for HRT 3

The significance of substrate type and size on the treatment efficiency of pollutants tested was determined and results of exact p values tabulated in (APPENDIX 3). Results showed that only TSS removal was affected by changes in substrate. Sand was observed to perform better than gravel *($X^2 = 7.90$, d.f. $= 2$, $p < 0.05$)*.While there were no differences in treatment identified between sand and quarry dust *($X^2 = 7.90$, d.f. $= 2$, $p > 0.1$)*, quarry dust gave better results than gravel *($X^2 = 7.90$, d.f. $= 2$, $p < 0.05$)*.

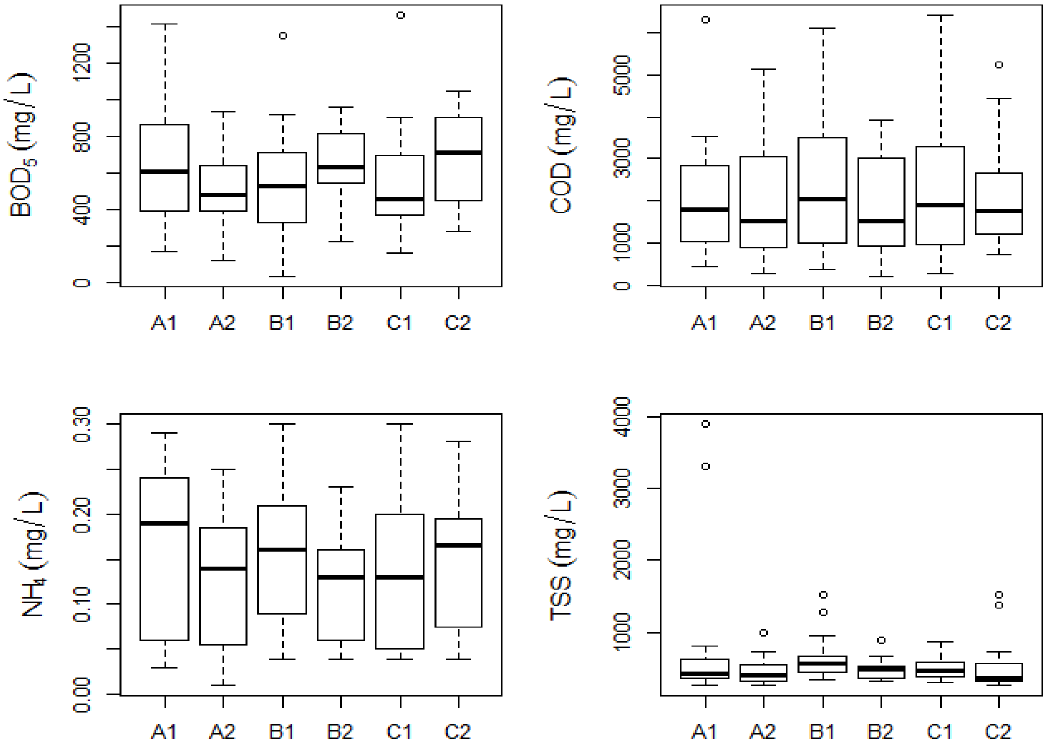

Figure 8: Overall pollutant removal of substrate treatments at HRT 3.
The treatments A1, A2, B1, B2, C1 and C2, represent sand at 0.65 m and 0.80 m, 16mm gravel at 0.65 m and 0.80 m and 8mm gravel at 0.65 m and 0.80 m respectively

4.4.6 The effect of depth on pollutant removal for HRT 3

Mesocosm performance at 0.65 m depth

The depth under study seemed to affect only TSS removal efficiency (Figure 9). Both sand and quarry dust showed no differences in TSS removal *($X^2 = 6.34$, d.f. $= 2$, $p > 0.1$)* and performed better than gravel *($X^2 = 7.90$, d.f. $= 2$, $p < 0.1$).*

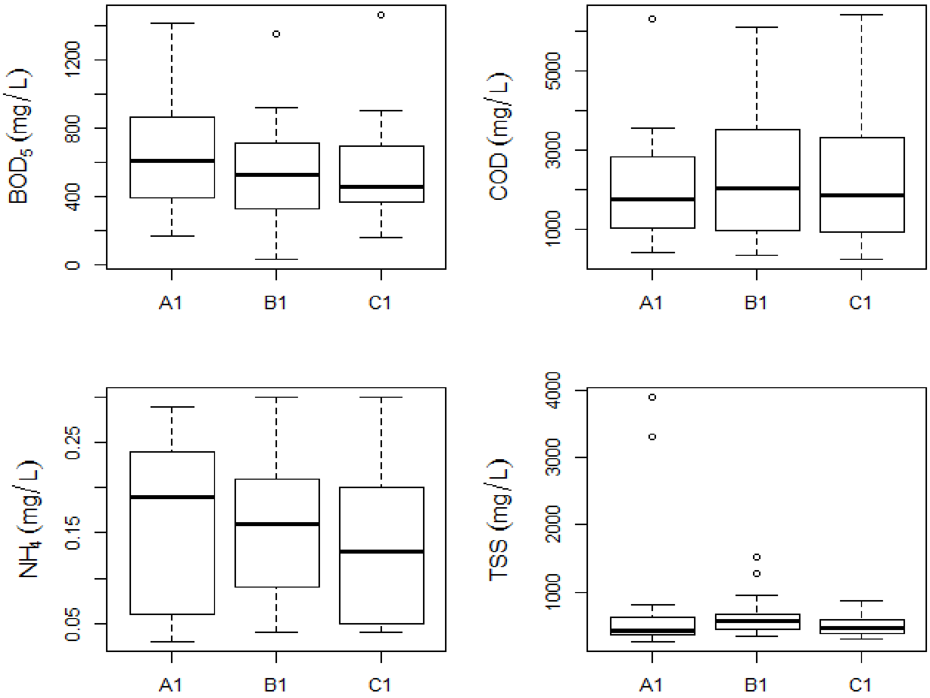

Figure 9: Pollutant removal of substrate treatments at 0.65 m for HRT 3.
The treatments A1, B1, and C1 represent sand at 0.65 m, 16mm gravel at 0.65 m and 8mm gravel at 0.65 m respectively.

Mesocosm performance at 0.8 m depth

The depth under study seemed to affect only BOD removal efficiency (Figure 10). Sand performed better than both gravel and quarry dust (*X^2 = 9.91, d.f. = 2, p < 0.05*). There were no marked differences in treatment between gravel and quarry dust (*X^2 = 9.91, d.f. = 2, p < 0.1*).

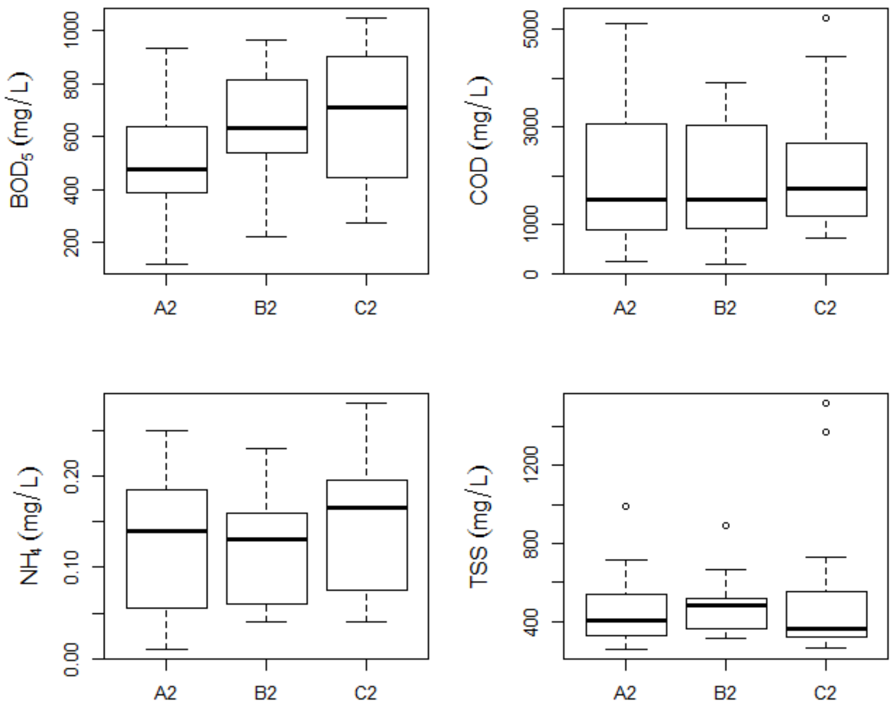

Figure 10: Pollutant removal of substrate treatments at 0.8 m for HRT 3.
The treatments A2, B2 and C2, represent sand at 0.80 m, 16mm gravel at 0.80 m and 8mm gravel at 0.8 m respectively

4.4.7 Combined effect of substrate and depth on pollutant removal for HRT 5

Two way ANOVA was conducted to establish the effect of substrate- depth interactions on pollutant removal for 5 day retention time (Figure 11). Results indicated that substrate- depth interactions did not have a significant effect on removal of BOD *(ANOVA, F = 0.377, d.f. = 2, p = 0.68)*, COD (*ANOVA, F = 1.17, d.f. = 2, p = 0.31)*. There were however, some differences observed in the removal of NH_4-N and TSS.

For NH_4-N removal, sand performed better than both quarry dust and gravel *(ANOVA, F = 10.604, d.f. = 2, p = 0.000, p = 0.000)*, while quarry performed better than gravel *(ANOVA, F = 10.604, d.f. = 2, p < 0.058)*. It should be noted that *p = 0.000* indicates interaction of other factors that affects the substrate performance, therefore substrate effect cannot be considered alone. Quarry dust mesocosm at 0.8 m performed better than sand mesocosm at 0.65 m

(ANOVA, F = 10.604, d.f. = 2, p = 0.011). Sand at both depths performed better than gravel at 0.65 m *(ANOVA, F = 10.604, d.f. = 2, p 0.008, p = 0.000).* Inversely, the 0.8 m gravel mesocosm performed better than sand at both 0.65m and 0.8 m *(ANOVA, F = 10.604, d.f. = 2, p = 0.000, p = 0.011).* Gravel at both 0.65m and 0.8 m depths also performed better than quarry dust *(ANOVA, F = 10.604, d.f. = 2, p = 0.001, p = 0.021).*

In the removal of TSS sand differed in performance from both quarry dust and gravel *(ANOVA, F = 2.920, d.f. = 2, p = 0.012, p = 0.000),* while quarry dust differed from gravel *(ANOVA, F = 2.920, d.f. = 2, p = 0.090).* Sand at both 0.65m and 0.8 m depths outperformed gravel at 0.65 m *(ANOVA, F = 2.920, d.f. = 2, p = 0.059, p = 0.000).* Sand at 0.8 m depth outperformed quarry dust at both 0.65m and 0.8 m *(ANOVA, F = 2.920, d.f. = 2, p = 0.012, p = 0.005)* and gravel at 0.8 m *(ANOVA, F = 2.920, d.f. = 2, p = 0.002).*

4.4.8 The effect of substrate on pollutant removal for HRT 5

One way ANOVA was conducted to determine whether substrate alone affected pollutant removal efficiency of the mesocosms. Exact p values of ANOVA output were tabulated in (APPENDIX 3). The results showed that, difference in substrate affected removal efficiency of all pollutants.

In the removal of BOD, sand performed better than both quarry dust and gravel *(ANOVA, F = 6.514, d.f. = 2, p < 0.01, p < 0.01)*, while there was no significant difference in treatment between the latter two substrates *(ANOVA, F = 6.514, d.f. = 2, p > 0.1).* Likewise, for COD removal, sand performed better than both quarry dust and gravel *(ANOVA, F = 3.706, d.f. = 2, p < 0.01, p < 0.01)*, while there was no significant difference in treatment between the latter two substrates *(ANOVA, F = 3.706, d.f. = 2, p > 0.1).* Gravel performed better than sand and quarry dust in the removal of NH_4-N *(ANOVA, F = 19.02, d.f. = 2, p < 0.000, p < 0.1).* Quarry dust also performed better than sand *(ANOVA, F = 19.02, d.f. = 2, p < 0.1).* It should be noted though, that *p = 0.000* indicates interaction of other factors that affects the substrate performance, therefore substrate impact cannot be considered alone. In the removal of TSS, sand was observed to give better performance than both quarry dust and gravel *(ANOVA, F = 11.58, d.f. = 2, p < 0.01. p < 0.000).*

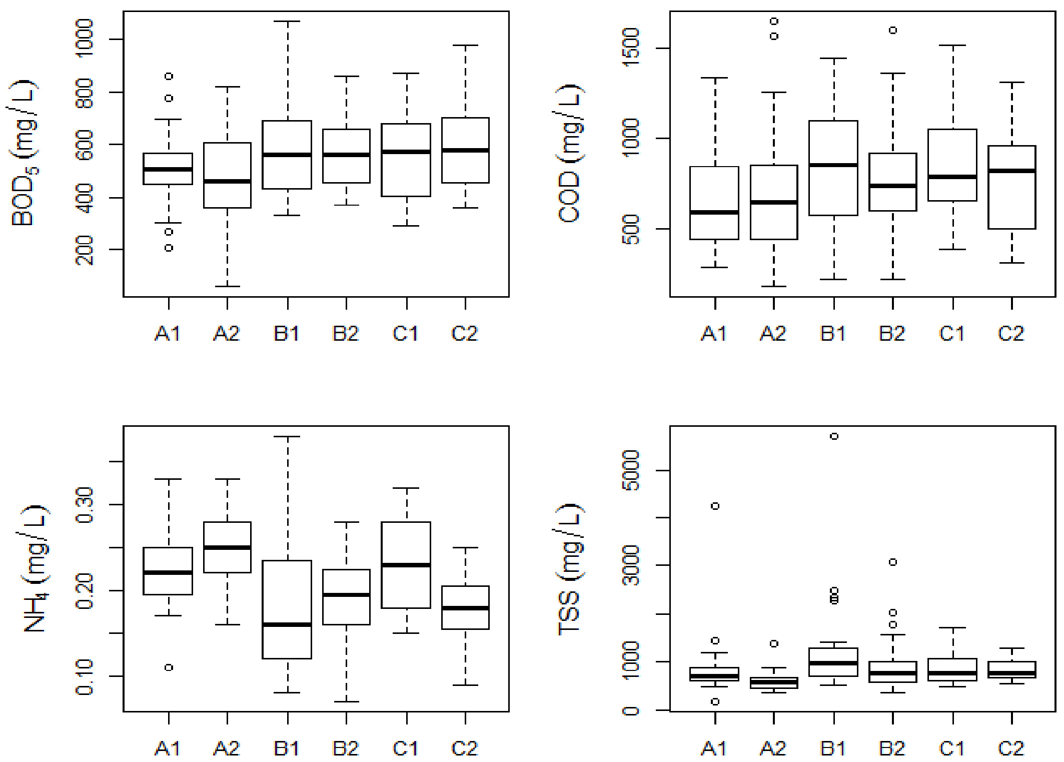

Figure 11: Overall pollutant removal of substrate treatments at HRT 5.
The treatments A1, A2, B1, B2, C1 and C2, represent sand at 0.65 m and 0.80 m, 16mm gravel at 0.65m and 0.80 m and 8mm gravel at 0.65 m and 0.80 m respectively.

4.4.9 The effect of depth on pollutant removal for HRT 5

The importance of depth was examined for both 0.65 m and 0.8 m mesocosms. One Way ANOVA was used to determine which mesocosms differed significantly in performance from each other. Results were presented as boxplot figures below and as (APPENDIX 3) for 0.65m depth analysis and (APPENDIX 4) for 0.8 m depth analysis. Generally, it was observed that removal efficiency of all pollutants studied responded to changes in depth.

Mesocosm performance at 0.65 m depth

One way ANOVA analysis of mesocosm performance at 0.65 m depth for 5 day retention was done and graphically presented in (Figure 12) below. In the removal of BOD, sand only differed significantly in performance from gravel *(ANOVA, $F = 2.382$, d.f. $= 2$, $p < 0.1$).* No marked differences were observed between sand and quarry dust *(ANOVA, $F = 2.382$, d.f. $= 2$, $p > 0.1$)* or between quarry dust and gravel *(ANOVA, $F = 2.382$, d.f. $= 2$, $p > 0.1$).* For COD removal, sand performed better than gravel *(ANOVA, $F = 4.135$, d.f. $= 2$, $p < 0.05$)* and quarry dust *(ANOVA, $F = 4.135$, d.f. $= 2$, $p < 0.05$).* Performance of quarry dust was statistically insignificant from that of gravel *(ANOVA, $F = 4.135$, d.f. $= 2$, $p > 0.1$).* NH_4-N removal by gravel was better than bot sand and quarry dust *(ANOVA, $F = 7.082$, d.f. $= 2$, $p < 0.05$, $p <$*

0.01). On the other hand, sand and quarry dust shoed no significant differences in removal of NH_4-N *(ANOVA, $F = 7.082$, d.f. = 2, $p > 0.1$).* Sand and quarry dust showed no marked differences in the treatment of TSS *(ANOVA, $F = 4.103$, d.f. = 2, $p > 0.1$)*, while they both performed better than gravel *(ANOVA, $F = 4.103$, d.f. = 2, $p < 0.05$).*

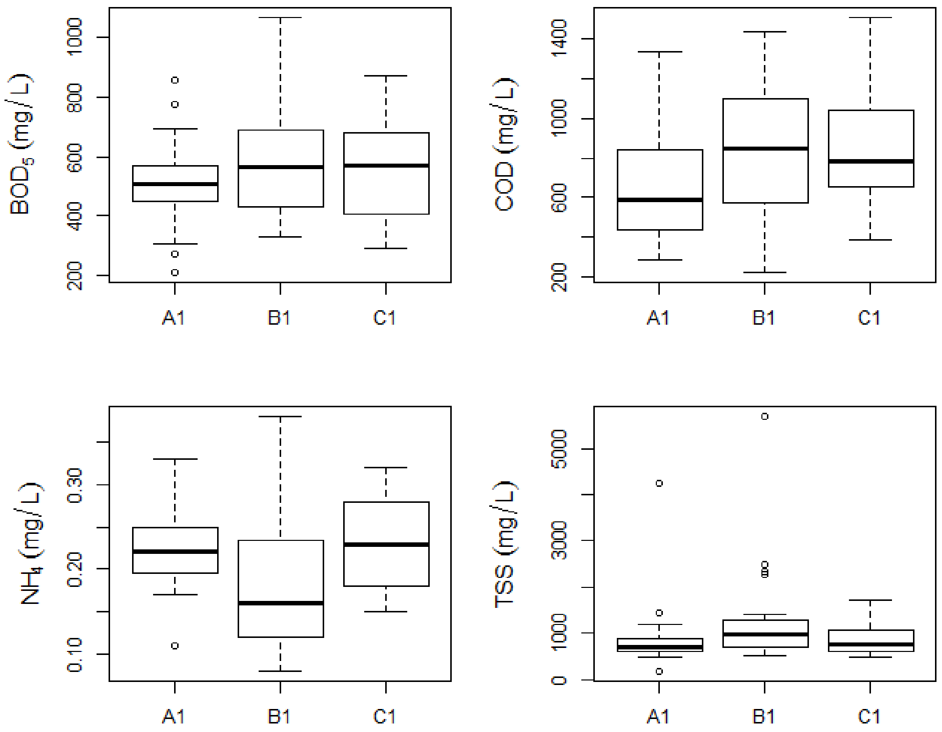

Figure 12: Pollutant removal of substrate treatments at 0.65 m for HRT 5.
The treatments A1, B1 and C1 represent sand, 16mm gravel and 8mm gravel respectively

Mesocosm performance at 0.8 m depth

Performance of 0.8 m mesocosms (Figure 13) indicated that depth affected the removal of BOD, NH_4-N and TSS while COD showed no difference in treatment between the substrates *(ANOVA, $F = 0.579$, d.f. = 2, $p > 0.1$).* For BOD and TSS removal, sand outperformed both quarry dust and gravel, with no determinable differences between the latter two. BOD and TSS recorded *(ANOVA, $F = 4.555$, d.f. = 2, $p < 0.05$) and (ANOVA, $F = 12.38$, d.f. = 2, $p < 0.001$)* respectively. Gravel and quarry dust shoed no differences in performance *(ANOVA, $F = 32.34$, d.f. = 2, $p > 0.1$)* and they both outperformed sand in the removal of NH_4-N *(ANOVA, $F = 32.34$, d.f. = 2, $p < 0.001$).*

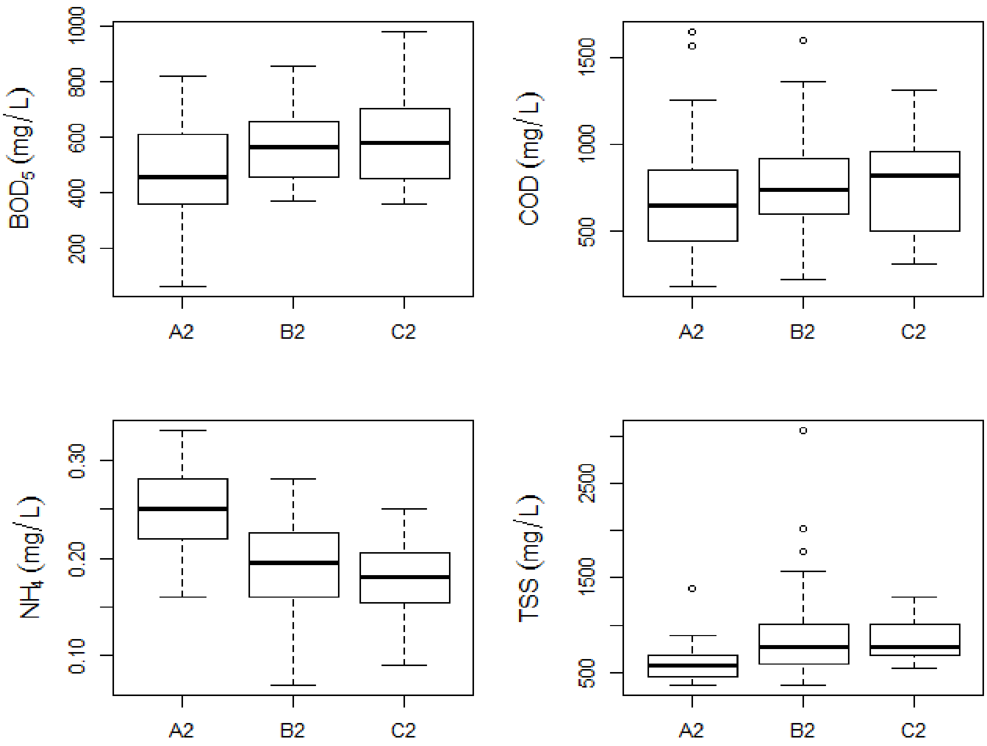

Figure 13: Pollutant removal of substrate treatments at 0.8 m for HRT 5.
The treatments A2, B2 and C2, represent sand, 16mm gravel and 8mm gravel respectively

4.6 Effect of HRT on pollutant removal efficiency

The effect of retention time on pollutant removal efficiency by all substrates was studied. Performance was analysed in terms of mass concentration of pollutants retained by the mesocosms per day. One way ANOVA was used to identify significant differences in performance of the mesocosms and the results tabulated in (APPENDIX 7). Visual description of the results was presented in (Figure 14) below.

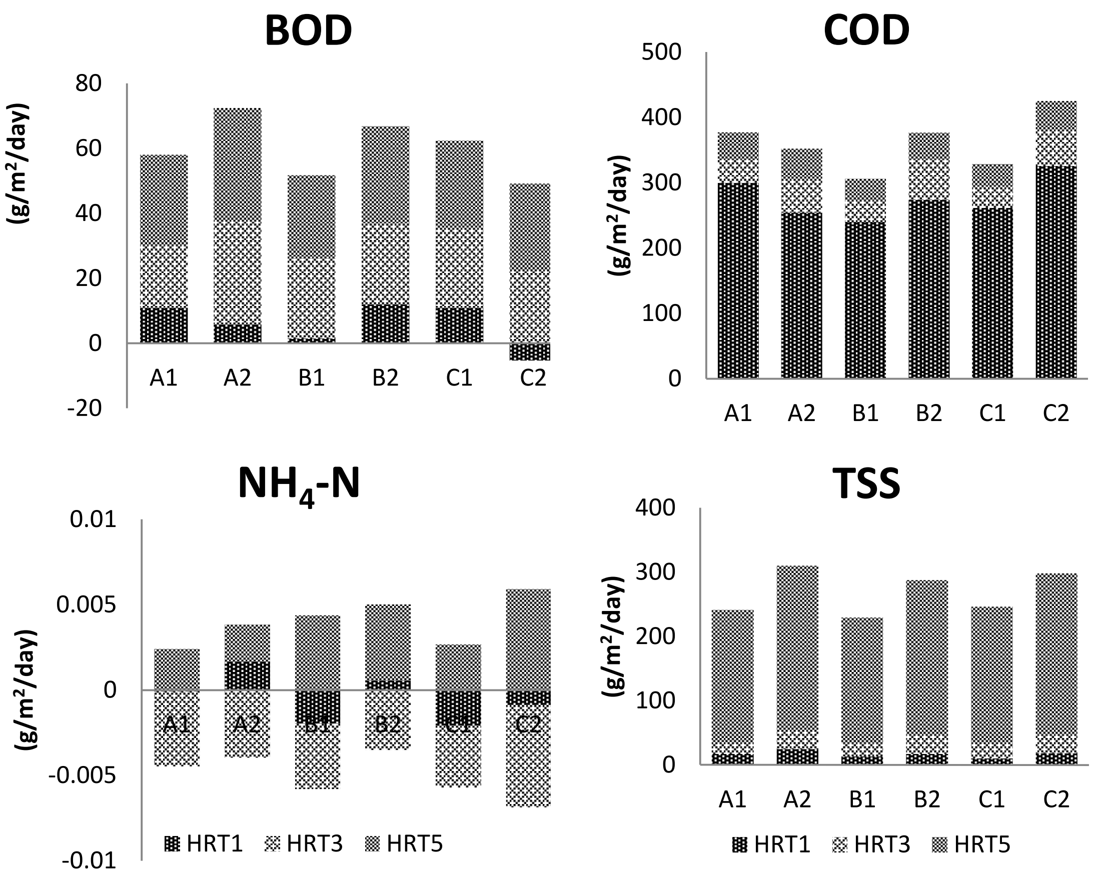

Figure 14: Mass removal rate of pollutants at HRT 1, 3 and 5

Retention times of 3 and 5 days were observed to greatly reduce BOD_5 compared to 1 day retention time. There was no significant difference between 3 and 5 day retention times however. Removal efficiencies for BOD_5 were in the order of 48.9%, 57.07%, 44.58%, 44%, 51.79% and 43.66% for treatments A1, B1, B2, A2, C1 and C2 respectively in HRT 5. 5 day retention time observably achieved the best results for all pollutants, with 2mm sand at 0.8 m depth giving the best BOD and TSS removal. NH_4-N removal was better in both gravel mesocosms and 0.8 m quarry dust mesocosm. Further investigation on the effect of retention time on individual substrate performance at both 0.65 m and 0.8 m depths was conducted and presented below.

4.5.1 Performance of 2 mm sand at 0.65 m depth in pollutant removal

The effect of retention time on removal efficiency of 2mm sand at 0.65 m depth was investigated. Results (Figure 15) indicated that retention time of 1 day produced significantly higher BOD_5 effluent *(ANOVA, $F = 55.36$, d.f. $= 2$, $p < 0.001$)* than both 3 and 5 day retention experiments. The latter two retention times showed no difference in BOD treatment *(ANOVA,* *F = 55.36, d.f. = 2, p > 0.1)*. One day retention outperformed both 3 and 5 day retention in COD removal *(ANOVA, F = 24.27, d.f. = 2, p < 0.001)*. Also, three day retention was observed to perform better than 1 day retention *(ANOVA, F = 24.27, d.f. = 2, p < 0.1)*. NH_4-N reduction fluctuated with change in retention time. For HRT 3, NH_4-N was observed to increase rather than decrease by up to twice the influent concentration. HRT 5 gave the best treatment results compared to HRT 3 *(ANOVA, F = 45.71, d.f. = 2, p < 0.001)* and HRT 1 *(ANOVA, F = 45.71, d.f. = 2, p < 0.001)*. TSS removal improved markedly at 5 day retention time when compared to 1 and 3 day retention *(ANOVA, F = 9.325, d.f. = 2, p < 0.001, p < 0.01)*.

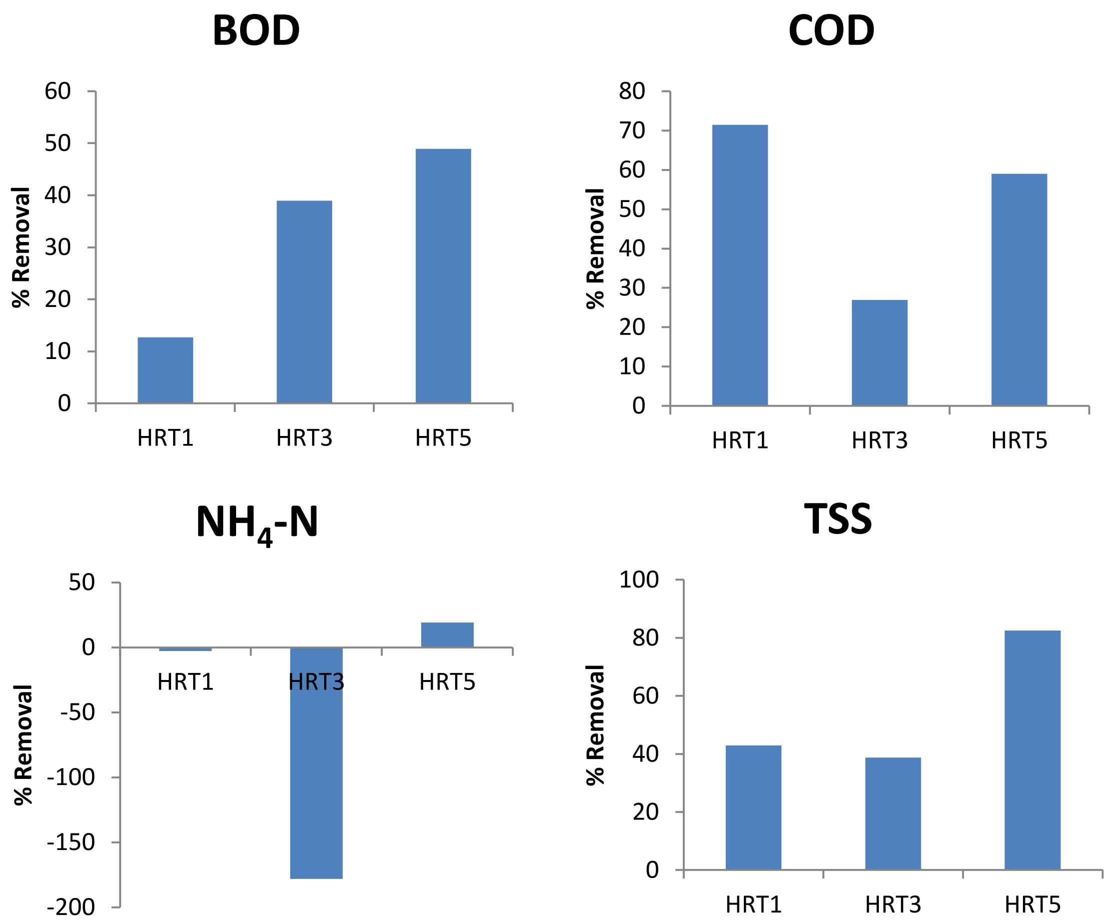

Figure 15: Pollutant removal efficiency by 2 mm sand at 0.65 m at HRT 1, 3 and 5.

4.5.2 Performance of 2 mm sand at 0.8 m depth in pollutant removal

The effect of retention time on removal efficiency of 2mm sand at 0.8 m depth was also assessed and found to be similar in performance to 0.65m mesocosms (Figure 15) for all but NH_4-N (Figure 16). Results indicated that retention time of 1 day produced significantly higher BOD_5 effluent than both 3 and 5 day retention experiments *(ANOVA, F = 259, d.f. = 2, p < 0.001)*. BOD removal for HRT 3 was not so different from HRT 5 *(ANOVA, F = 259, d.f. = 2,*

$p > 0.\ 1)$. One day retention outperformed both 3 and 5 day retention for COD removal *(ANOVA, $F = 32.34$, d.f. $= 2$, $p < 0.001$)*. NH_4-N reduction fluctuated with change in retention time. For HRT 3, NH_4-N was observed to increase rather than decrease by up to twice the influent concentration. HRT 5 gave the best treatment results compared to HRT 3 *(ANOVA, $F = 103.8$, d.f. $= 2$, $p < 0.001$)* and HRT 1 *(ANOVA, $F = 103.8$, d.f. $= 2$, $p < 0.001$)*. It was observed that performance in HRT 1 changed from NH_4 reduction as opposed to production noted for 0.65 mesocosms. TSS removal improved markedly at 5 day retention time when compared to 1 and 3 day retention *(ANOVA, $F = 10.39$, d.f. $= 2$, $p < 0.01$, $p < 0.001$)*. HRT 3 showed no difference in treatment from HRT 1 *(ANOVA, $F = 10.39$, d.f. $= 2$, $p > 0.1$)*.

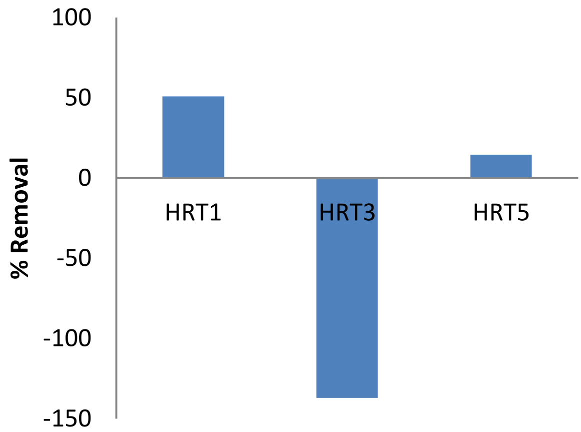

Figure 16: Removal efficiency of NH_4-N by 2 mm sand at 0.8 m at HRT 1, 3 and 5.

4.5.3 Performance of 8 mm quarry dust at 0.65 m depth in pollutant removal

The effect of retention time on removal efficiency of 8 mm quarry dust at 0.65 m depth was studied (Figure 17). Results indicated that both 3 and 5 day retention times produced significantly better BOD_5 effluent than 1 day retention time at p *(ANOVA, $F = 89.95$, d.f. $= 2$, $p < 0.000$, $p < 0.000$)*. It should be noted that $p < 0.000$ indicates interaction of other factors that affects the retention time, therefore HRT impact cannot be considered alone. COD removal in all the retention times differed significantly. One day retention gave the best treatment results *(ANOVA, $F = 27$, d.f. $= 2$, $p < 0.001$)* compared to 3 day and 5 day retention, which showed little difference in treatment *(ANOVA, $F = 27$, d.f. $= 2$, $p < 0.1$)*. Retention time at 3 and 1 days showed no difference in COD treatment *(ANOVA, $F = 27$, d.f. $= 2$, $p > 0.1$)*. NH_4-N removal was observed to be higher at 1 day retention compared to both 3 and 5 day retention *(ANOVA,* *F = 8.339, d.f. = 2, p < 0.05, p < 0.001)*. A different trend was observed for TSS removal in which 5 day retention performed better than both 1 and 3 day retention time *(ANOVA, F = 25.79, d.f. = 2, p < 0.000)*. The level of significance observed, indicated that there were other unknown factors that affected TSS removal aside from retention time.

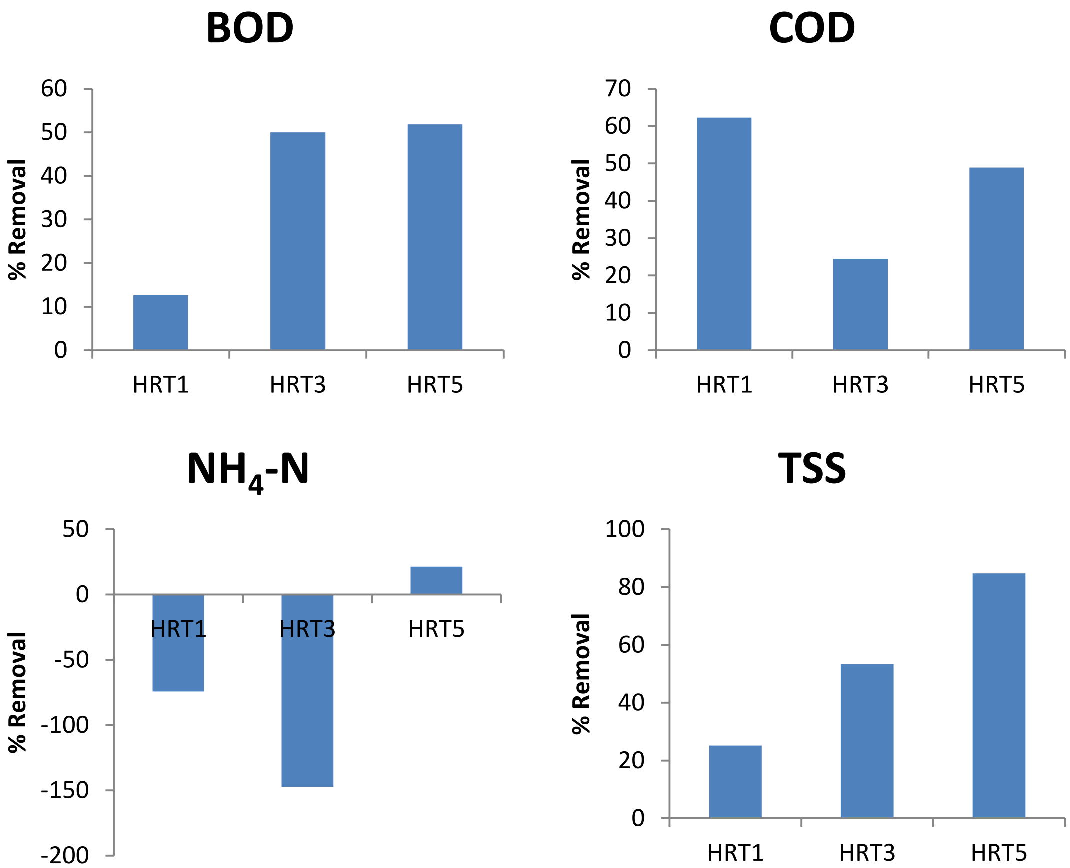

Figure 17: Pollutant removal efficiency by 8 mm Quarry dust at 0.65 m at HRT 1, 3 and 5.

4.5.4 Performance of 8 mm quarry dust at 0.8 m depth in pollutant removal

The effect of retention time on removal efficiency of 8 mm gravel at 0.8 m depth was studied. Performance was observed to follow a similar trend as in (Figure 17) except for BOD_5 removal which was negative at HRT 1 (Figure 18). Results indicated that both 3 and 5 day retention times performed similarly *(ANOVA, F = 59.85, d.f. = 2, p > 0.1)* and produced significantly better BOD_5 effluent than 1 day retention time, *(ANOVA, F = 59.85, d.f. = 2, p < 0.000)*. COD removal in all the retention times differed significantly in performance. One day retention gave the best treatment results in comparison to HRT 3 and HRT 5. NH_4-N concentration was observed to vary inconsistently for the different retention times studied. Five day retention produced the best treatment results compared to HRT 3 and 1 *(ANOVA, F = 25.79, d.f. = 2, p*

< 0.000). TSS removal was substantially better at 5 day retention compared to 3 retention at $p < 0.001$ and 1 day retention at $p < 0.01$.

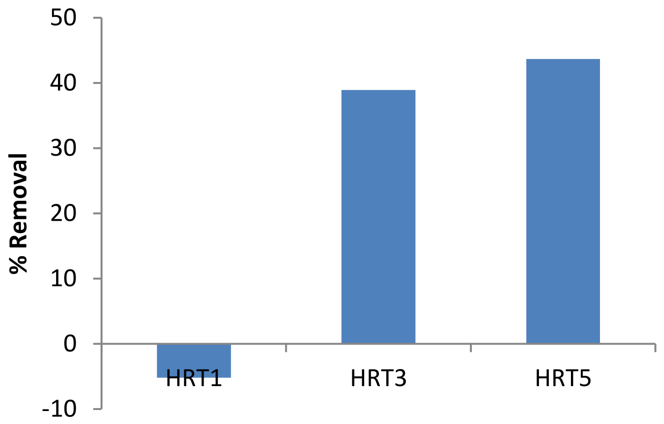

Figure 18: Removal efficiency of BOD_5 by 8 mm Quarry dust at 0.8 m at HRT 1, 3 and 5.

4.5.5 Performance of 16 mm gravel at 0.65 m depth in pollutant removal

Analysis of HRT effect on pollutant removal efficiency by 16 mm gravel at 0.65 m (Figure 19) revealed that 5 and 3 day retention gave twice as much BOD_5 treatment as 1 day retention time *(ANOVA, $F = 61.46$, d.f. $= 2$, $p < 0.000$)*. One day retention time was observed to produce much better effluent for COD than 3 and 5 day retention *(ANOVA, $F = 10.63$, d.f. $= 2$, $p < 0.001$)*. NH_4-N reduction was best at 5 day retention and differed significantly from both 3 and 1 day retention *(ANOVA, $F = 27.33$, d.f. $= 2$, $p = 0.001$, $p < 0.000$)*. At 3 and 1 day retention ammonia seemed to increase in the system rather than decrease. TSS removal was observed to differ between the tested retention times. Five day retention was observed to perform best compared to 3 day *(ANOVA, $F = 21.9$, d.f. $= 2$, $p < 0.001$)* and 1 day retention *(ANOVA, $F = 21.9$, d.f. $= 2$, $p < 0.01$)*. Performance at 3 days differed from that at 1 day *(ANOVA, $F = 21.9$, d.f. $= 2$, $p < 0.05$)*.

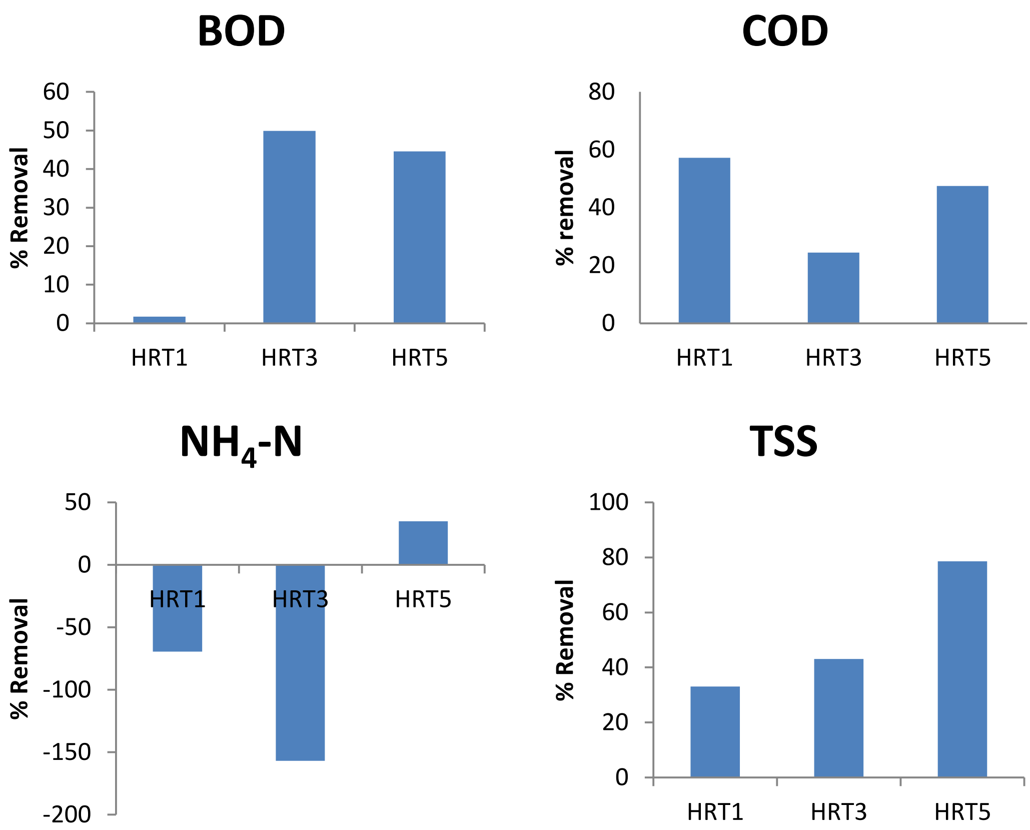

Figure 19: Pollutant removal efficiency by 16 mm Gravel at 0.65 m at HRT 1, 3 and 5.

4.5.6 Performance of 16 mm gravel at 0.8 m depth in pollutant removal

Effect of retention time on pollutant removal for 0.8 m mesocosms was assessed and found to be similar in trend to performance at 0.65 m (Figure 19) for all but NH_4-N (Figure 20). Results revealed that retention time affected treatment performance of all pollutants. After close inspection, it was observed that there was little difference in treatment performance between HRT 3 and 5 for BOD_5 *(ANOVA, $F = 230.6$, d.f. = 2, $p > 0.1$)*. Both retention times gave twice as much BOD_5 removal as 1 day retention *(ANOVA, $F = 230.6$, d.f. = 2, $p < 0.000$)*. One day retention gave best results for COD removal and was markedly different in performance from both 3 and 5 day retention times *(ANOVA, $F = 32.29$, d.f. = 2, $p < 0.000$)*. HRT 5 gave better results for NH_4-N treatment than either 3 or 1 day retention *(ANOVA, $F = 30.58$, d.f. = 2, $p = 0.1$, $p < 0.000$)*. For TSS removal, pollutant removal was in the order of HRT 5 to 1 with significantly poorer performance observed in both HRT 3 and 1 *(ANOVA, $F = 32.29$, d.f. = 2, $p < 0.000$)*.

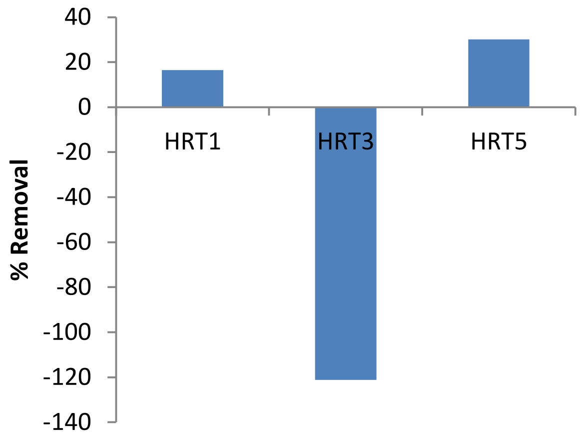

Figure 20: Removal efficiency of ammonium nitrogen by16 mm gravel at 0.8 m depth

Characteristics of the wastewater were highly variable. Substrate type and depth were observed to have little impact on overall pollutant removal hence, pointing to a stronger interplay between the microbial communities and *in-situ* parameters in form of biochemical processes in the wastewater. Furthermore, performance of individual substrates at both 0.65 m and 0.8 m depth for all retention times followed a similar trend. It should be noted that the 0.8 m mesocosms gave slightly better effluent concentration in all cases except in BOD where shallow quarry dust did better than the deeper mesocosm. This was also the case for COD where shallow sand mesocosm performed best, outperforming the deeper sand mesocosm. Retention time was observed to have a significant impact on organic matter reduction but at the expense of ammonia increase. A negative relationship between ammonia and organic matter breakdown was observed. It is worth noting that, as the BOD demand decreased, ammonia concentration continued to increase.

CHAPTER FIVE

DISCUSSION

5.1 Characteristics of the slaughterhouse wastewater and their temporal variation

According to Del Pozo, Tas, Hakan, Orhon and Diez, (2003), information on wastewater characteristics has been said to affect treatment plant design to a great extent. Previously, characterisation was based on modelling of processes within the treatment system. Recent studies indicate that there is a need to factor in the inter-relationship between biological, physical and chemical processes that steer pollutant removal processes (Del Pozo *et al.,* 2003; Metcalf and Eddy, 2003; Abdelhakeem *et al.,* 2015). This is because of the high variability of wastewater composition (Heger, n.d) and its biodegradability index, which have been proven to fluctuate highly and even affect treatment (Osorio, 2006). The wastewater in this study was characterised as being of high strength. Generally wastewater is classified as being "strong", where strength is based on concentrations above those of conventional ranges for strong domestic effluents. However, limited information exists on classification ranges (Heger, n.d).

The concentrations of some selected physico-chemical parameters observed in this study were higher than reported ranges for similar wastewater in other studies. Irshad *et al.* (2015); Sunder, G. and Satyanarayan, S. (2013), recorded a range of 11,000 mg/L-17,000 mg/L for BOD_5. This study found the BOD_5 to vary between 2,098 mg/L and 1,200 mg/L, which was much lower in comparison. COD, TSS and NH_4-N on the other hand, had concentration ranges falling within similar characterisation studies.

Temporal variations in influent wastewater parameters monitored over the study period showed statistical differences. This could be attributable to the wide range of ambient air temperature at the experimental site which ranged between lows of 10-12^oC at night and highs of 25-27^oC during the day. Biochemical processes are known to be subject to temperature changes (Kadlec and Wallace, 2009; Irshad *et al.,* 2015) and as such it is reasonable to relate the observed trend variations with temperature change. Similar results were also observed by (Zhao *et al.,* 2004: Abdelhakeem *et al.,* 2015). The variations in wastewater characteristics can also occur due to type and number of animals slaughtered, water used for washing of stomach contents, facility cleaning and efficiency of waste collection/separation.

The pollutant characteristics were observed to significantly change after the ten day detention period. A marked drop in concentration observed for COD, TSS, NH_4-N, DO, pH and EC in this study leads to the conclusion that the detention stage played a key role in pollutant reduction. This high reduction efficiency could be attributable to the rapid breakdown of easily biodegradable matter (Metcalf and Eddy, 2003) (cited by Sun *et al.*, [2003]), therefore pointing to the importance of a pre-treatment stage. Despite that the BOD_5 /COD biodegradability ratio was 0.1; the high COD reduction observed in this period could be an indication of presence of highly reducible COD fraction.

A look at the organic matter content in the TSS showed that it formed the larger fraction of solids in the raw effluent. The average percentage reduction of 79% achieved for COD in the current study further confirms this. Del Pozo *et al.* (2003) pointed to the importance of differentiating COD fractions into readily biodegradable, readily hydrolysed and inert. That greatly contributed to selection of an appropriate combination of pre (anaerobic) and post (aerobic) treatment stages that maximised pollutant reduction in his study.

5.2 Effect of substrate type and depth on pollutant removal efficiency

Substrate type was seen to have no impact on COD, BOD_5 and TSS removal in the wastewater at HRT 1 and 3. In contrast, significant differences were observed for HRT 5. It is possible that effects of depth, substrate characteristics and retention time are tied together. This supposition is supported by the better overall performance of the substrates in deeper mesocosms at 5 day retention.

With regard to the impact of substrate size on OM reduction, (García, Vivar, Aromir and Mujeriego, 2003) conducted a study on 3.5 mm and 10 mm substrates for 0.30 m and 0.46 m deep mesocosms. No marked difference was observed on the function of media size at both depths. Inconsistent patterns of treatment observed at the time contrast those of a similar study (Garcia and Mankin, 2002) (cited by Kadlec and Wallace, [2009]), in which fine media (19 mm) outperformed coarse media (38 mm). Despite that the current study used far smaller grain sizes (sand 2mm, quarry dust 8 mm and gravel 16 mm), the similarity of results coincide with those of (Garcia and Mankin, 2002) (cited by Kadlec and Wallace, [2009]). Caution should however be applied in consideration of this information due to a general knowledge gap on marked effects of media size in organic matter reduction. Further, longer term studies are necessary to verify observations noted in the current study.

Langergraber *et al.* (2007) indicated that majority of microbial biomass responsible for organic matter breakdown is located in the top 20 cm layer of a wetland. He further suggested that, this region has greater availability to hold diffuse oxygen and also that most particulate matter filtration occurs here. It has also been established that organic matter breakdown can occur under anaerobic conditions as well for high strength wastewaters (Sun *et al.*, 2003). The lack of marked differences in organic matter removal for all retention time can therefore be attributed to the insignificance of depth and microbial action in the top layer of a wetland.

Coleman *et al.* (2001) in a study on the importance of depth in OM removal compared shallow (45 cm) and deep (60 cm) beds. He found no difference in their performance at identical hydraulic loads thus emphasizing the insignificance of bed depth in treatment. It is possible therefore to conclude that depth makes no contribution to OM removal as observed in the current study. The insignificance of substrate-depth impact observed by (Coleman *et al.,* 2001) and the current study could have been due to the small difference (15 cm) between shallow and deep mesocosms. If so, then the results observed in the current study are in line with findings by (García, 2003 and Coleman *et al.*, 2001).

There was a significant difference observed for ammonia reductions between 0.8 m gravel with 0.8 m sand, in which the latter had higher ammonia reduction at a significance level of $\alpha < 0.05$. Based on this observation, it is presumable that ammonia removal increased with decrease in substrate size. Kadlec and Wallace, (2009) suggested that this behaviour could be due to the fact that coarse media bed have lesser surface area per unit volume thereby having limited attachment surface for ammonia oxidizing biofilms. However, the trend changed with increase in retention time, indicating interplay of substrate and time. Perhaps the effect of time on media action is due to action time required by microbial and bacterial groups responsible for OM breakdown, which adsorb/desorb ammonia.

5.3 Effect of HRT on pollutant removal efficiency

Hydraulic retention time is generally known to have a positive linear relationship with nutrient removal efficiency (Wu, Zhang, Li, Fan and Zou, 2013). The higher performance observed for overall pollutant removal at HRT 5 in this study is further supported by (Wu *et al.*, 2013). Perhaps the longer retention time allowed for increased contact between microbial communities and the wastewater. This consequently increased removal efficiency of organic matter. On the contrary, (Zhao *et al.*, 2003 and Molle *et al.*, 2006) found that the highest pollutant removal rates were achieved after a short saturation time. Bancolé *et al.* (2003) suggested that at higher feeding frequency of small volumes greatly enhanced both OM breakdown and nitrification. This would indicate that one day retention time was expected to achieve best results on organic matter reduction. The current study concurred with views by (Zhao *et al.*, 2003 and Bancolé *et al.*, 2003) only for COD removal. TSS and BOD which are also components of organic matter were found to perform better under a longer retention time of 5 days. Conversely, (Sultana, M., Mourti, C., Tatoulis, T., Akratos, C., Tekerlekopouloua, A. and Vayenasa, D., 2015) found COD reduction in cheese wastewater to be most efficient during a longer retention time of four days.

Nitrogen removal processes are generally known to be significantly influenced by temperature and dissolved oxygen rather than retention time (Bodin, 2013). Ammonia conversion is conventionally known to occur in three stages for biological treatment systems. These being: ammonification, nitrification and denitrification respectively. Organic nitrogen is first converted to ammonia form, after which it is nitrified and then oxidized to nitrite and/or to nitrate which are transformed to nitrogen gas in the last step (Metcalf and Eddy, 2003) (cited by Dallago, Gomes, Mees, Assis and Moreira, [n.d]). It is possible that the nitrification stage was inefficient due to the slow growth rate of nitrifying bacteria, namely, ammonia and nitrate chemoautotrophs, which are also known to have a low oxidation efficiency (Laanbroek, 2002 and Connolly *et al.*, 2004). So, despite the observed increase in DO recorded for 5 day retention time, low nitrification efficiency would result in the ammonia build up observed at HRT 1 and 3 as only the ammonification step would have been achieved. It would also explain the increase in ammonia observed during both 1 and 3 day HRT experiment cycles. Raised pH (> 9.5) could also have facilitated volatilization process.

It is possible that denitrification of whatever little nitrate/nitrite produced in this study was most likely by anoxic heterotrophs given sufficient carbon source. The carbon source was created by the high organic strength of the slaughterhouse wastewater; a requirement to facilitate anoxic denitrifying environment. Bodin, (2013) reported similar results under DO concentration of 1.5-0.3 mg/L for subsurface wetlands. Microbial consumption of CO_2 produced in turn raised pH to the observed ranges of 9-9.7.

Biomass production by OM degradation is known to immobilize ammonia by adsorption process (Molle *et al.,* 2006). Considering that an estimate of 0.6 g biomass is generated from breakdown of 1 g BOD_5 (Cannon *et al.*, 2000) (cited by Sun *et al.*, [2005]) and that 12.4 % of this biomass is nitrogen, it would be safe to infer that about 0.074 g nitrogen is immobilized for each gram of BOD_5 degraded. It is important to note that, the immobilized ammonia is quickly released back into water by ammonification when the biomass decomposes. Therefore, less than the theorised 0.074 g N would actually be produced. This phenomenon could help explain the better performance at five day retention. According to (Molle *et al.*, 2006) the adsorbed ammonia could be nitrified between batches given a longer feeding interval at HRT 5, thus stabilising nitrification. Also, Kadlec and Wallace, (2009) noted in a study on potato processing wastewater that, oxidised nitrogen levels were typically low to nil in wetlands with high BOD concentration. This observation concurs with the relatively low ammonia removal observed in the current study (14%-39 % NH_4-N removal). Sun, G., Zhao, Y., and Allen, S. (2005) indicated that significant nitrification could only be possible if BOD drops to 200 mg/l or less and there were frequent recirculation ratios of the wastewater. Van Oostrum, (1990) noted that sub surface flow systems in general had a low ammonia reduction capacity, therefore the low reduction efficiencies observed for ammonia are within expected results for such a system.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

From this study, the following conclusions were drawn:

1. Temporal variation in physico-chemical characteristics of slaughterhouse wastewater was found to differ over time. As such it is reasonable to conclude that slaughterhouse wastewater characteristics vary over time.

2. The small depth difference of 15 cm was insufficient to determine the significance of substrate at different depth in removal of pollutants from slaughterhouse wastewater. As such, an insignificant effect on removal efficiency of BOD and COD was observed. However, TSS and NH_4-N were noted to work best under a combination of small grain sized substrate and deeper mesocosms.

3. Variation of retention time was verified to have a significant influence on removal efficiency of organic matter. Five day retention achieved the best organic matter reduction overall. Ammonia removal on the contrary could not achieve significant reduction concentrations due to high organic load of the wastewater.

Although effluent concentrations were higher than the national effluent release regulations, the study sufficiently demonstrated the potential of vertical sub-surface flow constructed wetlands in treating slaughterhouse wastewater.

6.2 RECOMMENDATION

1. Smaller substrate sizes can be considered in CWs targeting organic matter. However, they would have a higher likelihood of faster clogging, thereby making a pre-treatment stage necessary. Alternatively, small, frequent batches of wastewater at short retention time could be applied to enhance substrate-water interactions for optimal treatment.
2. A further analysis step to be conducted using modelling. This will combine the significant factors identified for organic matter reduction into a prediction and management tool for slaughterhouse wastewater treatment.
3. Ammonium nitrogen could be treated in a later treatment step to achieve the overall pollutant reduction target.

Suggestions for further research

Effect of physico-chemical parameters on pollutant removal were identified in this study but could not be substantiated since that would be beyond the current study scope. Further investigation of physico-chemical characteristics of slaughterhouse wastewater would benefit understanding of pollutant removal processes. Further, modelling as a tool could be used to extract and quantify these relationships in order to obtain information on optimal targets of physical, chemical and biological processes in elimination of specific wastewater pollutants.

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APPENDICES

APPENDIX 1: Substrate preparation at preliminary setup stage. a and b show methods of substrate separation, c shows substrate washing stage and d shows final grade sizes and types of substrates used in the study.

APPENDIX 2: Inset on setup arrangement, wastewater input method and sampling technique applied during the study period

APPENDIX 3: 1 Way ANOVA output for effects of different substrates on pollutant removal efficiency.

	Differing mesocosms	**HRT1**	**HRT3**	**HRT5**
BOD	Quarry dust- Gravel	0.8998067	0.9881052	0.9720727
	sand-Gravel	0.7787666	0.9594913	0.0036932**
	sand- Quarry dust	0.5129826	0.9109184	0.0074955**
COD	Quarry dust- Gravel	0.0939131(.)	0.9713031	0.9882557
	sand-Gravel	0.1916319	0.916126	0.0333808*
	sand- Quarry dust	0.9714775	0.8127338	0.0481102*
NH_4-N	Quarry dust- Gravel	0.8067746	0.9526755	0.0722591
	sand-Gravel	0.0554086(.)	0.9020922	0.0000***
	sand- Quarry dust	0.0099172**	0.9887623	0.0002973***
TSS	Quarry dust- Gravel	0.904704	0.042**	0.0198986*
	sand-Gravel	0.2633643	0.042**	0.0024458**
	sand- Quarry dust	0.4638448	0.99	0.7367958

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '(.)' 0.1

APPENDIX 4: 1 Way ANOVA output for effect of depth at 0.65 m on pollutant removal efficiency

	Differing mesocosms	**HRT1**	**HRT3**	**HRT5**
BOD_5	Quarry dust- Gravel	0.5999007	0.9924798	0.7765427
	sand-Gravel	0.5506881	0.2407673	0.0847621(.)
	sand- Quarry dust	0.9999995	0.1978697	0.2902591
COD	Quarry dust- Gravel	0.4383152	0.9850965	0.970821
	sand-Gravel	0.1517524	0.7064506	0.0428552*
	sand- Quarry dust	0.8684689	0.7983274	0.0243896*
NH_4-N	Quarry dust- Gravel	0.9832761	0.5944788	0.002359**
	sand-Gravel	0.0594505(.)	0.8891749	0.0102457*
	sand- Quarry dust	0.0589809(.)	0.3545153	0.9587868
TSS	Quarry dust- Gravel	0.9832761	0.08(.)	0.0604588(.)
	sand-Gravel	0.0594505(.)	0.076(.)	0.2495908
	sand- Quarry dust	0.0589809(.)	0.98	0.8392028

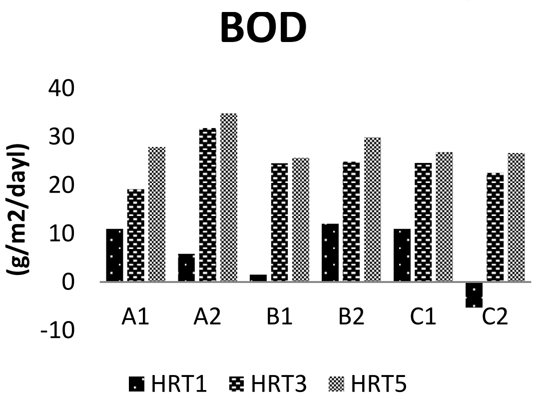
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '(.)' 0.1

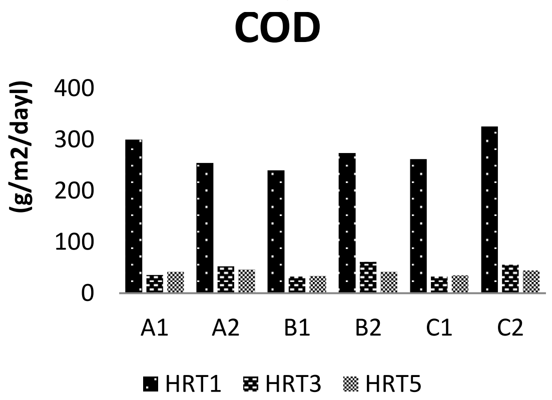
APPENDIX 5: 1 Way ANOVA output for effect of depth at 0.8 m on pollutant removal efficiency

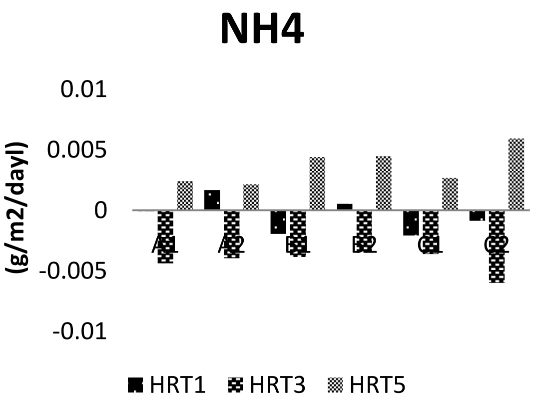
	Differing mesocosms	HRT1	HRT3	HRT5
BOD_5	Quarry dust- Gravel	0.1162452	0.042*	0.9210697
	sand-Gravel	0.8379682	0.042*	0.0469827*
	sand- Quarry dust	0.5249507	0.99	0.0170248*
COD	Quarry dust- Gravel	0.0842763	0.8720568	0.8979543
	sand-Gravel	0.8820935	0.9435648	0.4875642
	sand- Quarry dust	0.0572865(.)	0.9843517	0.7613752
NH_4-N	Quarry dust- Gravel	0.0606821(.)	0.2099156	0.6238795
	sand-Gravel	0.3186596	0.9716084	0.0000***
	sand- Quarry dust	0.0023095**	0.3290067	0.0000***
TSS	Quarry dust- Gravel	0.3104621	0.8212347	0.2991961
	sand-Gravel	0.155778	0.8344178	0.00026***
	sand- Quarry dust	0.7182578	0.6899	0.0304334*

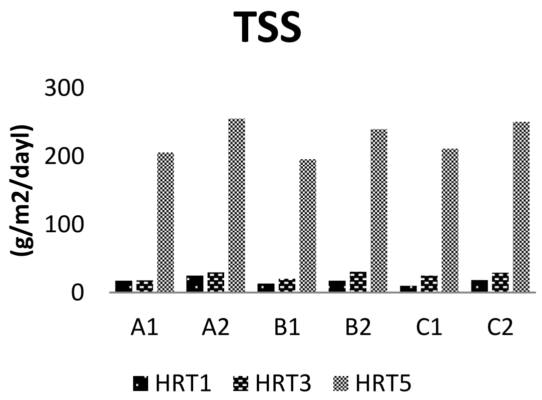
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '(.)' 0.1

APPENDIX 6: Mass removal rates of pollutants studied. Presented as the difference between influent and effluent concentrations in grams per meter squared per day









APPENDIX 7: Effect of retention time on pollutant removal efficiency of the different substrate

	Location	**Sand 0.65 m**	**Sand 0.8 m**	**Quarry dust 0.65 m**	**Quarry dust 0.8 m**	**Gravel 0.65 m**	**Gravel 0.8 m**
BOD_5	HRT3-HRT1	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***
	HRT5-HRT1	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***
	HRT5-HRT3	0.4830894	0.9084584	0.8758559	0.6990864	0.9349858	0.4235257
COD	HRT3-HRT1	0.0571218(.)	0.0000001***	0.1574194	0.0000***	0.0000864***	0.0021247**
	HRT5-HRT1	0.0000***	0.0000***	0.0000***	0.0000***	0.0000005***	0.0000***
	HRT5-HRT3	0.0000***	0.0000003***	0.0000152***	0.0000004***	0.1574194	0.0000015***
NH_4-N	HRT3-HRT1	0.0000004***	0.0000009***	0.0416744*	0.0000109***	0.3866717	0.0000048***
	HRT5-HRT1	0.0000***	0.0000***	0.0002542***	0.0000***	0.0000***	0.0000***
	HRT5-HRT3	0.0000***	0.0000***	0.1999965	0.0000004***	0.0000001***	0.0532615(.)
TSS	HRT3-HRT1	0.6874354	0.9483771	0.9989874	0.7246976	0.0384916*	0.9931863
	HRT5-HRT1	0.0307436*	0.0114129*	0.0002661***	0.0023559**	0.00406705**	0.0000***
	HRT5-HRT3	0.1923599	0.0013198**	0.0000882***	0.0000065***	0.0000003***	0.0000***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘(.)’ 0.1

During the course of the three month study, *in-situ* parameters of the slaughterhouse effluent wastewater were monitored in addition to the parameters studied. The results were grouped according to retention times in the order of HRT 1, HRT 3 and HRT 5. The treatments A1, A2, B1, B2, C1 and C2 represented sand at 65 and 80 cm, 16mm gravel at 65 and 80 cm and 8mm gravel at 65 and 80 cm respectively.

APPENDIX 8: Temporal variation of *in-situ* parameters for HRT 1.

Treatment	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**
Parameter						
DO	0.11 ±0.04	0.07±0.04	0.11±0.04	0.12±0.06	0.12±0.05	0.11±0.04
Temperature	20.74±0.16	20.20±0.22	20.80±0.16	20.99±0.20	20.97±0.18	20.92±0.17
EC	7.55±0.25	7.62±0.42	7.52±0.25	7.54±0.30	7.52±0.28	7.60±0.24
pH	8.72– 8.79	8.51– 8.58	8.75– 8.83	8.80–8.88	8.79-8.87	8.79-8.86

Means and standard deviations of each treatment replicates were calculated for n = 155. Influent concentrations at the beginning of the experiment cycle were 0.86±0.21 mg/l DO, 15.5±3.87 ^{o}C Temperature, 7.58±1.89 mS EC and 8.74±2.18 pH.

APPENDIX 9: Temporal variation of *in-situ* parameters for HRT 3.

Treatment	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**
Parameter						
DO	0.17±0.05	0.19±0.05	0.17±0.04	0.18±0.05	0.17±0.04	0.19±0.05
Temperature	19.95±0.60	21.74±0.24	20.16±0.52	21.35±0.38	20.54±0.48	20.61±0.57
EC	7.91±0.28	8.41±0.24	7.93±0.25	8.18±0.23	8.01±0.24	7.93±0.26
pH	8.36-8.59	8.04-8.26	8.36-8.56	8.24-8.45	8.31-8.51	8.44-8.67

Means and standard deviations of each treatment replicates were calculated for n = 215. Influent concentrations at the beginning of the experiment cycle were 0.06±0.004 mg/l DO, 18.45±0.11^{o}C Temperature, 6.78±0.002 m*S* EC and 9.81±0.014 pH.

APPENDIX 10: Temporal variation of *in-situ* parameters for HRT 5.

Treatment Parameter	A1	A2	B1	B2	C1	C2
DO	0.27±0.06	0.29±0.06	0.27±0.06	0.29±0.06	0.28±0.06	0.28±0.06
Temperature	19.17±0.63	19.56±0.58	19.28±0.57	19.71±0.59	19.59±0.59	19.68±0.59
EC	6.69±0.12	6.71±0.11	6.69±0.11	6.72±0.11	6.72±0.11	6.73±0.11
pH	9.62-9.76	9.59-9.76	9.62-9.74	9.59-9.72	9.62-9.75	9.57-9.69

Means and standard deviations of each treatment replicates were calculated for n = 233. Influent concentrations at the beginning of the experiment cycle were 0.06±0.007 mg/l DO, 21^{o}C Temperature, 7.71±0.0037 mS EC and 8.79±0.01 pH.

Appendices 8, 9 and 10 above summarize the changes of *in-situ* parameters measured for the different treatments over the retention times studied. ANOVA function was used to determine whether there were any significant differences between treatments in HRT 1, 3 and 5. DO and Temperature varied significantly between HRT 1 and 3 at $p < 0.001$.

Generally, DO was observed to increase with increase in retention time from an average of 0.10 at HRT 1, to 0.17 at HRT 3 and 0.27 at HRT 5. pH was observed to be highest (> 9.5) for HRT 5 as compared to HRT 1 and 3, perhaps due to salt by-products from microbial activity (Kadlec and Wallace, 2009). Temperature decreased with increase in retention time, possibly due to stability of conditions within the mesocosms from less frequent recirculation disturbance. Significance in differences observed for trends in pH, temperature and EC could not be determined due to possible interaction of other factors not included in the factorial analysis. The use of mixed models in statistical analysis could better explain interactions observed between physic-chemical parameters and pollutant removal.