UNIVERSITY OF NAIROBI

DEPARTMENT OF CIVIL AND CONSTRUCTION ENGINEERING

USE OF ENZYMES IN ANAEROBIC SEQUENCING BATCH REACTOR (ASBR) TREATMENT OF SLAUGHTERHOUSE WASTEWATER

BY

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Declaration /Approval

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

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Abstract

Slaughterhouse wastewater if not adequately treated has potential for environmental degradation including contamination of groundwater, deoxygenation of rivers and clogging of soil pores. In Kenya, treatment of slaughterhouse wastewaters to meet standards for discharge into public sewers faces several challenges such as high organic load in excess of 11,500 mg/L COD, lack of funding for conventional treatment methods and inadequate land for construction of waste stabilization ponds.

Anaerobic sequencing batch reactors (ASBRs) provide effective and economical alternative for the treatment of organic loads that are released intermittently. The reactors accomplish treatment of wastewater in four phases namely, feed, react, settle and draw, sequentially in a single reactor. However, conventional ASBRs operate with sophisticated control systems for monitoring and adjustment of the system to optimum operating conditions. Omission of controls owing to their high costs and skill requirements would result in unsatisfactory effluents. Therefore, there is need for improvement of the ASBR operated without control system to allow effluent discharge to public sewers. An effective improvement of ASBR performance can be achieved by the use of enzymes which have been widely used to aid wastewater treatment processes. Enzymes accelerate biochemical reactions in cells by lowering their activation energy.

This study evaluated the viability of proprietary enzyme secreting bacteria culture, Ecotreat®, in ASBR treatment of slaughterhouse wastewater from

Dagoretti slaughterhouses in Nairobi. The study was carried out using three bench scale reactors with Ecotreat® bacterial culture applied at 0 (control), 0.5 and 1.0% of slaughterhouse effluent and a volume exchange ratio (VER) of 40%. The enzymatic assisted ASBR treatment achieved up to 91 and 50% reduction of COD and TSS, respectively, within 8-hour reaction time. Application of the Ecotreat® bacterial culture at 1% concentration enhanced the ASBR reduction of COD by 14%. The treatment met the EMCR (2006) requirements for discharge into public sewers of less than 1,000 mg/L COD after 16 days of operation and therefore would allow discharge without recirculation. The ASBR effluents had BOD_5/COD ratio of 0.52 to 0.59 indicating they were readily biodegradable and, therefore, amenable to biological treatment in municipal wastewater treatment plants. The study recommends further investigations of enzymatic assisted ASBR treatment to establish the steady state performance.

Dedication

This thesis is dedicated to my dear wife Caroline and son Adrian. Through your prayers, support and co-operation, I attained the resolve to undertake and accomplish this study.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Slaughterhouse wastewater has elevated concentration of organic matter of 6,908 to 11,500 mg/L COD derived from blood, fat and suspended solids (Massé and Masse, 2000). The wastewater may also contain pathogens, including salmonella and shigella bacteria, parasite eggs, and anaerobic cysts (Mijinyawa and Lawal, 2008). The pollution potential of meat-processing and slaughterhouse plants has been estimated as over one million population equivalent in the Netherlands (Sayed, 1987) and three million in France (Festino and Aubart, 1986).

Discharge of untreated slaughterhouse wastewater into the environment can cause deoxygenation of rivers and contamination of groundwater. Similarly, discharge of inadequately treated slaughterhouse wastewater into sewers is undesirable as it results in overloading of the municipal treatment facilities. Land application of wastewater causes surface and ground water contamination, odour problems and soil pore clogging from excessive fat content. Therefore, slaughterhouse effluent should be treated before discharge to reduce adverse environmental impacts. Treatment methods adopted usually involve biological processes to reduce organic and pathogenic loads, under aerobic or anaerobic conditions.

Selection of treatment methods should consider both the elevated organic load and the intermittent nature of release of slaughterhouse wastewater. Conventional treatments methods such as activated sludge are usually too expensive for communities in the developing countries. Additionally, they produce large volumes of putrefactive and bulky sludge that require special handling and further treatment (Johns, 1995). On the other hand, waste stabilization ponds, which are popular in the tropics, require large tracts of land that are unavailable within the urban settings of most slaughterhouses.

Anaerobic waste treatments provide reliable treatment method with short retention times (Yiu et al., 2001). They have several advantages over aerobic processes including lower electricity costs, high efficiencies, low construction and operation costs, low rates of sludge production, high organic loading rates and production of useable biogas. Additionally, it is not necessary to feed anaerobic biomass continuously because anaerobic metabolism is a slow process and the viable sludge can remain inactive for several months. These characteristic make anaerobic treatment ideal for treating seasonal and intermittently released wastewaters such as slaughterhouse effluent (Omil et al., 1996).

The ASBR is a modified form of activated sludge system, which utilizes a single batch reactor to treat wastewater under anaerobic conditions. Equalization, reaction and clarification are all accomplished sequentially in a single batch reactor, thus reducing treatment costs. However, satisfactory performance of the reactors requires automated controls (Pat et al., 2011). The

performance of the reactor can also be improved using a variety of design changes including mixing, heating and attached growth processes. However, these changes increase operation costs.

An innovative improvement to ASBR performance is the introduction of biological additives such as enzymes to enhance the wastewater treatment. Enzymes are proteins produced by living cells that act as biological catalysts. They occur inside cells or they may be secreted by cells. Enzymes enhance wastewater treatment process by increasing the metabolic activity and digestion rate and maintaining a healthy microbial population. Addition of enzymes into anaerobic digestion processes cuts down digestion time, improves sludge digestibility and reduces disposal costs (e.g. Wawrzynczyk et al., 2008; Ronja, 2008). The enzyme aided treatment process is easy to control and its products harmless to the environment (Ahuja et al., 2004).

This study evaluated viability of using enzymes in ASBR treatment of slaughterhouse wastewater to meet effluent standards for discharge into public sewers.

1.2 Problem Statement

Slaughterhouses in Kenya are located within urban areas for proximity to meat markets. Improper disposal of untreated slaughterhouse effluent has resulted in serious detrimental effects on the environment including surface and groundwater pollution, unsightly ponding, odour release and reduction in productivity of arable land. Direct discharge of slaughterhouse wastewater into public sewer attracts surcharge by the municipalities because of its high

organic contents. Conventional treatment of wastewater has prohibitive capital and operation costs. Moreover, scarcity of land in urban areas where most Slaughterhouses are located limits use of waste stabilization ponds. Therefore, there is need to investigate alternative treatment methods with minimal land and cost requirements. The ASBR, by accomplishing four treatment phases feed, react, settle and draw, sequentially in a single reactor, can reduce the cost of slaughterhouse wastewater treatment. The batch-wise treatment using ASBR is also suitable for slaughterhouse wastewater-streams that have intermittent flows. However, for satisfactory performance the reactors require automated controls that are unaffordable by communities in developing countries (Pat et al., 2011). On the other hand, introduction of enzymes into the waste to improve the biological treatment can reduce the cost of treatment. Therefore, there is need to investigate the viability of using enzymes in the ASBR for improvement of the treatment of slaughterhouse wastewater.

1.3 Objective

The overall objective of this study was to evaluate viability of using enzymes in ASBR for improvement of the treatment of slaughterhouse wastewater.

The specific objectives were to:

1. Evaluate variation of COD with time during ASBR treatment of slaughterhouse wastewater
2. Establish effect of enzyme application rate on reduction of COD and TSS in ASBR treatment of slaughterhouse wastewater

3. Establish the biodegradability of enzyme assisted ASBR treatment effluent for biological degradation in municipal wastewater treatment plants

1.4 Scope of the Study

This study involved investigating the treatment of slaughterhouse wastewater from Dagoretti Slaughterhouse Company Ltd using enzymes in anaerobic sequencing batch reactor (ASBR). The wastewater was inoculated with activated sludge from anaerobic pond at the slaughterhouse for acclimatization. Enzymes were obtained from enzyme secreting bacterial product, Ecotreat®, which is supplied by Ecosave Africa Limited. The parameters evaluated included COD, TSS and BOD_5, reaction time and enzyme application rate.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Characteristics and Pollution Potential of Slaughterhouse Wastewater

In the Netherlands, the pollution potential of meat-processing and slaughterhouse plants exceeds one million population equivalent (Ten Have, 1976). Slaughterhouses generally produce lower effluent and pollutant quantities per ton of livestock weight killed (LWK) than meat packing plants, which perform more meat handling operations (Table 2.1). Wastewater from slaughterhouse varies widely in composition, strength and flow. Their differences in terms of characteristics and quantity can be primarily attributed to differences in processing activities, animal species, employee habits and wastewater management.

Table 2.1: Slaughterhouse Capacity and Pollution Equivalent of their Wastewater in the Netherlands (Ten Have, 1976)

Source	LWK[a] (Tons per year)	P.E.[b] (Per ton LWK)	Total P.E.[b]
Cattle	500,000	0.5	250,000
Poultry	370,000	0.7	259,000
Pigs	116,400	0.3	349,000
Calves	110,000	0.2	22,000
Total			1,027,000

[a]: LWK – Livestock weight slaughtered
[b]: PE – Organic population equivalents

Slaughterhouse wastewater contains diluted blood, fats and suspended solids. The wastewater is characterized by high organic strength and total suspended solids (Table 2.2).

Table 2.2: Characteristics of Screened and Settled Slaughterhouse Wastewater (Yiu et al., 2001)

Pollutant	Concentration range (mg/L)	Pollutant	Concentration range (mg/L)
COD	2,000-6,000	Fat, oil and grease	10-15
Soluble COD	1,200-3,600	Total nitrogen	15-50
BOD_5	1,000-3,000	Total phosphorus	0.5-2
TSS	200-2,000	Fecal coli-forms	10^7-10^{8a}

[a] Measured in counts per 100ml

Bovine blood, one of the constituents of slaughterhouse wastewater, has COD of about 300,000 mg/L and therefore, its proportion in slaughterhouse wastewater determines largely the overall concentration of organic matter (Yiu et al., 2001). Suspended solids in the wastewater consist of fat, grease, hair, flesh, manure, grit and undigested feed (Tritt and Schuchardt, 1992). About 40 - 50% of slaughterhouse waste pollutant originates from the lipid and protein materials and may also include lignocellulosic substances and bacterial cell walls if manure is part of the wastewater (Sayed, 1987).The high fat, oil and grease concentration in wastewater results in slowly biodegradable polymeric substrate that must first undergo hydrolysis (liquefaction) before biological decomposition. Fibrous proteins from hair, skin, nails, bones, etc. are less susceptible to hydrolysis (Varel et. al., 1977). Slaughterhouse wastewater is thus comparable to other complex wastewaters like municipal wastewater and dilute manure effluent.

An important consequence of treating complex wastewaters that is partially insoluble is the significant decrease in the methanogenic capacity of the treatment system. The reduction of methanogenic capacity result from

entrapment of non-biomass coarse suspended solids in the sludge. Entrapment solids result in a dilution effect of the active biomass and ultimately in severe decrease in the methanogenic bacteria concentration in the sludge (de Man, 1986).

Discharge of slaughterhouse wastewater into the environment without proper treatment contributes to degradation of aquatic environment (Seif and Moursy, 1992). Leaching of slaughterhouse wastewater into groundwater is of concern especially because of the recalcitrant constituents such as slowly biodegradable manure (Sayed, 1987). The Environmental Protection Agency (EPA) classifies slaughterhouse wastewater as one of the most harmful wastewaters for the environment (Walter et al., 1974). Therefore slaughterhouse wastes require sufficient treatment to achieve standards for discharge into public sewers (Table 2.3).

Table 2.3: Effluent Requirements for Discharge to a Public Sewer (EMCR, 2006)

Pollutant	Limiting Concentration (mg/L)	Pollutant	Limiting Concentration (mg/L)
COD	1,000	Nitrates	20
BOD_5	500	Ammonia – Nitrogen	20
Fat, oil and grease	5	Phosphates	30

The composition of the slaughterhouse wastewater depends considerably on the production process and the type of animals slaughtered. The major waste load originates from the slaughtering process, a one-shift operation for most slaughterhouses in Kenya.

Slaughterhouse processes and waste generated are presented in Table 2.4.

Table 2.4: Slaughterhouse processes and waste generated

Process	Waste Generated
Lairage	Dung (manure)
Slaughter	Blood and fluids
Skinning and dressing	Horns, hide and wolves.
Evisceration	Gut fill, blood and fresh trimmings
Washing	Trimmings, blood, fats and grease

The bulk of wastewater is generated during regular floor washing carried out at the closing stages of the entire slaughtering process. It comprises of significant proportions of blood, innings, bits of carcasses and animal wastes.

2.2 Slaughterhouse Wastewater Treatment Methods

2.2.1 Conventional Primary and Secondary Treatment

Primary treatment by physical and chemical methods can be applied for slaughterhouse wastewater treatment to comply with water pollution control standards and to reduce costs on sewer surcharges. It involves a combination of screening with static and vibrating screens, centrifugation, hydrocyclones, sedimentation, flocculation, precipitation and air flotation for grease recovery (Witherow & Lammers, 1976).

Secondary treatment for slaughterhouse wastewater involves bacterial decomposition of the organic pollutants and nitrogen removal. The biological processes include the conventional anaerobic processes, anaerobic contact

process (Schroepfer et al., 1955), anaerobic ponds (Rollag and Dornbush, 1966; Oswald, 1964) and aerobic ponds (Steffen, 1961). Combinations of these systems can be required in cases where effluent discharge to surface water is desired. Even for discharge into public sewers, single secondary treatment processes rarely provide permissible effluent quality.

Most secondary treatment methods are low rate treatment systems that result in large land requirement. Their investment and operating costs are usually high and odour nuisance problems are unavoidable, particularly with the anaerobic ponds.

2.2.2 High Rate Anaerobic Systems

Modern high rate anaerobic treatment systems have been developed in response to the short-comings of the conventional low-rate anaerobic systems. These systems accelerate treatment and therefore, reduce area requirements. They include the anaerobic filter (Young & McCarty, 1969), the down-flow stationary fixed film reactor (Van den Berg and Lentz, 1979), the anaerobic attached film expanded bed (Switzenbaum and Jewell, 1980), the fluidized bed reactor (Heijnen, 1983) and Anaerobic Sequencing Batch Reactors (ASBR), which was developed by Agriculture and Agri-Food Canada (Massé and Masse, 2000).

High rate anaerobic treatment processes are based on the achievement of a high retention of viable biomass and significant contact between incoming wastewater with the sludge. They employ carrier materials for preventing

biomass washout, through use of bacterial attachment or entrapment of bacterial aggregates in the packing materials.

Some of the merits of high rate anaerobic treatment systems in application to wastewater treatment include;

1. Large organic loading rates can be applied at optimal temperatures and for mainly soluble wastewaters, consequently small reactor volumes suffice;
2. High stability of high rate systems to sub-optimal conditions (lower temperatures, shock loads, presence of inhibitory compounds) except when designed at their maximum loading potentials,
3. Anaerobic treatment is economically feasible as no aeration mechanism is required and associated costs avoided.

2.3 Anaerobic Digestion Systems

Anaerobic digestion as secondary treatment process has numerous advantages over conventional operations and processes in the treatment of high organic load wastewater. It achieves high COD and suspended solids (SS) removal while generating very low quantity of sludge. It does not require aeration or chemical pretreatment. The anaerobic bacteria can survive unfed for long periods of time, an important feature for occasions of close down. Anaerobic digestion also produces methane gas which is a source of energy.

Anaerobic digestion of organic material is a complex microbiological process involving the combined activity of several groups of microorganisms with different metabolic capacities (Zinder, 1984). The process generally involves multiple bacterial and archaea species which convert organic matter into

volatile fatty acids and finally into methane and carbon dioxide under anaerobic conditions. It consists of four distinct stages; namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis. These stages are discussed in the following sub-sections.

2.3.1 Hydrolysis

Bacteria generally are unable to break-down particulate organic material. These organic pollutants first have to be liquefied into soluble polymers or monomers with low molecular weight that can cross bacterial cell barrier. Thus, liquefaction is the first step required for microbial utilization of complex biopolymers. With the aid of exo-enzymes of hydrolytic bacteria, complex organic matters such as carbohydrates, albumins, and fats, are broken down to water-soluble simple organic structures including amino acids, sugars and fatty acids.

The rate of liquefaction is determined by the biodegradability and physical nature of the substrate (Lin et al., 1985). The size and porosity of the separate particles in substrate determine penetration depths of the enzymes. Environmental factors such as temperature are limiting factors (Pfeffer, 1974).

In the digestion of complex wastes containing high amounts of insoluble substrate, such as slaughterhouse wastewater, liquefaction step frequently has been found to be the rate limiting step in the overall process (e.g. Schomaker et al., 1986; van Velsen, 1981; Gijzen, 1987).

2.3.2 Acidogenesis

In acidogenesis, the products of the hydrolysis that include long-chain fatty acids, amino acids, sugars and alcohols are metabolized by hydrolytic and non-hydrolytic bacteria. The end-products of acidogenesis are low molecular weight organic acids, hydrogen, and carbon dioxide. However, these end-products formed vary with the types of bacteria as well as environmental conditions. Minor amounts of formate, lactate, valerate, methanol, ethanol, butanediol or acetone may be produced by fermentative bacteria. Because volatile fatty acids (VFA) are the main products of the bacteria, they are usually designated as acidifying or acidogenic bacteria. These bacteria are resistant to low pH values and formation of acids can proceed at pH values as low as pH 4 (Sayed, 1987).

2.3.3 Acetogenesis

The hydrogen-producing acetogenic bacteria are responsible for the anaerobic oxidation of the acidogenic stage products to substrates suitable for methanogenesis (Bryant, 1979). The oxidation reactions of the hydrogen producing acetogenic bacteria are thermodynamically unfavorable unless the partial pressure of hydrogen is kept below 10^{-3}atmospheric pressure (Gujer & Zehnder, 1983). As a result, these bacteria are obligatorily coupled to hydrogen-utilizing bacteria such as methanogens and sulphate reducing bacteria (Mclnerney et al., 1981). Mclnerney et al. (1981) found interspecies hydrogen transfer reactions in formation of methane from propionate and long-chain fatty acids.

2.3.4 Methanogenesis

Methanogenesis, the final stage in the overall anaerobic conversion of organic materials into methane and CO_2 is catalyzed by methanogenic bacteria. Methanogens utilize only a limited number of simple substrates such as acetate or the C_1-compounds CO_2/H_2, formate methanol and CO. Methanogens are classified into two major groups: the acetate converting (also called acetoclastic) and the hydrogen utilizing (hydrogenotrophic) bacteria. The growth rates of the acetoclastic bacteria are low which explains the need for high biomass retention time in anaerobic treatment systems.

Generally, 70 - 80 % of the methane formed from the organic materials originates from acetate. The rest is mainly derived from H_2 and CO_2. Hydrogenotrophic bacteria have a much higher maximum growth rate than the acetoclastic bacteria. Therefore, the hydrogenotrophic bacteria are presumably not a critical group. However, the ability of these bacteria to maintain very low pH forms the basis for thermodynamically favourable conditions for the preceding essential pre-methanogenesis substrate conversion steps.

A simplified schematic of anaerobic digestion is shown in Figure 2.1

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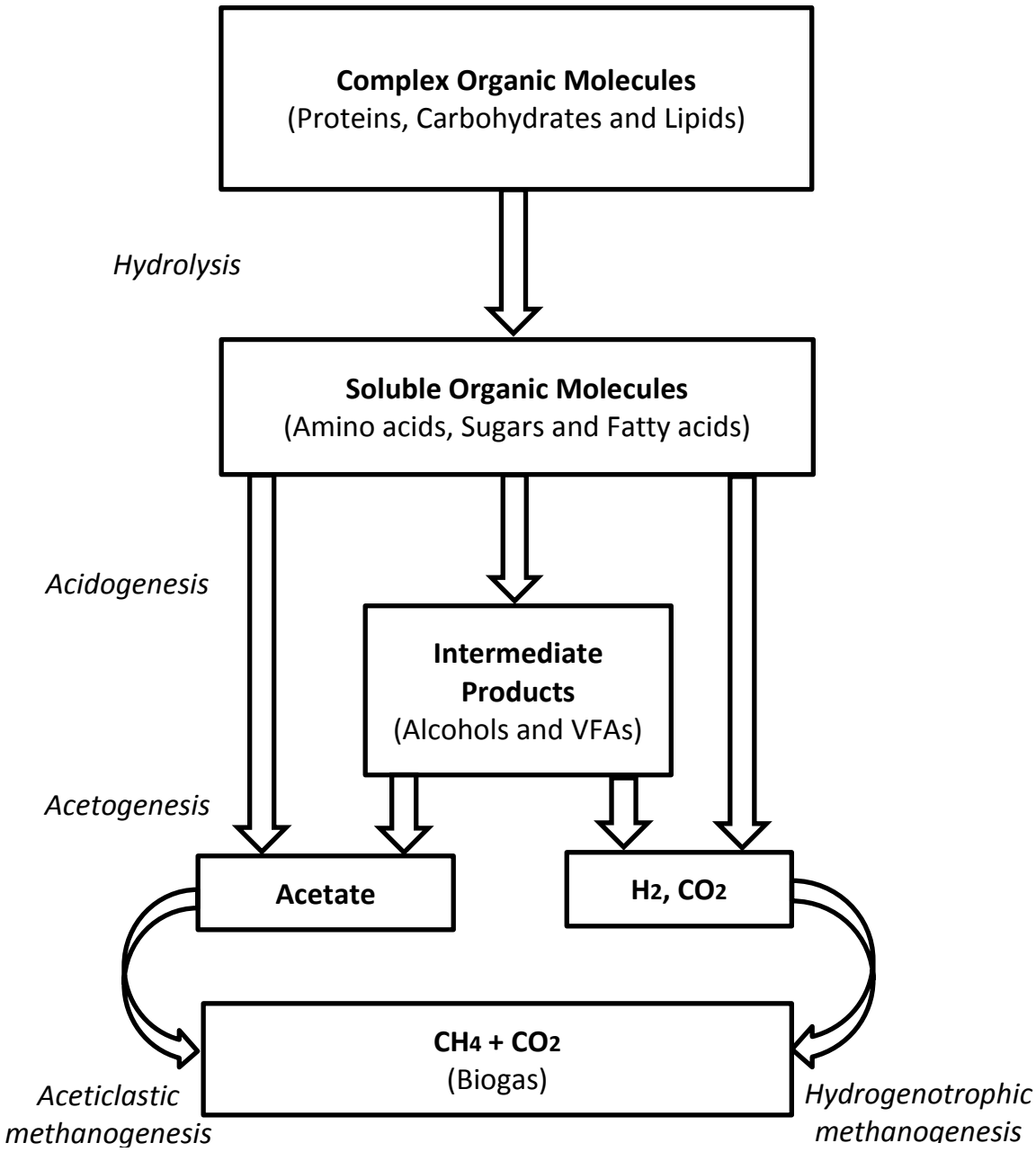


Figure 2.1: Schematic of the degradation steps of carbon in Anaerobic Digestion process (McCarthy, 1964)).

2.4 Anaerobic Sequencing Batch Reactor (ASBR)

The ASBR is one of the designs of high-rate anaerobic systems. The ASBR accomplishes, four treatment phases; namely, feed, react, settle and draw sequentially in one vessel (Figure 2.2) as described below.

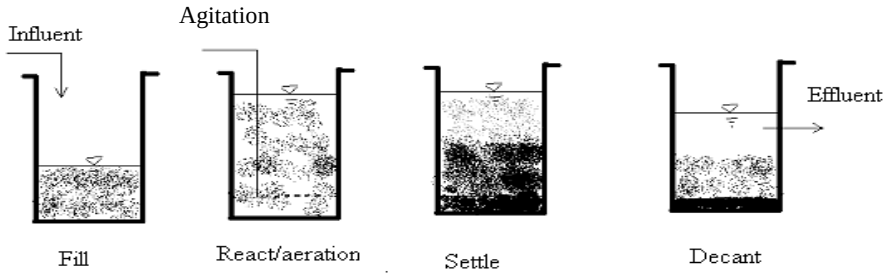


Figure 2.2: Typical ASBR Process Cycle for BOD Removal and Nitrification (Metcalf & Eddy, 2003)

(a) Fill

During the fill period, raw wastewater is allowed into a vessel containing sludge or biomass retained from the previous cycle. The volume of wastewater added to the reactor is based on the desired hydraulic residence time (HRT), organic loading rate (OLR) and expected settling characteristics of the sludge (Elizabeth et al., 2006). The fill period ends either when the tank is full or when a maximum time for filling is reached and the wastewater flow is directed to another reactor (Arora et al., 1985; Irvine and Bush, 1979; Dennis and Irvine, 1979). The reactor vessel contents are gently mixed continuously or intermittently to promote biological reactions (Metcalf & Eddy, 2003).

(b) React Stage

In the react stage, the level of reactor contents is maintained while mixing to ensure distribution of the substrate and improve the overall performance of the

reactor. The mixing should be short and gentle to avoid destroying the anaerobic bioflocs which would result in poor settling sludge. Sung and Dague (1995) found that intermittent mixing improves biomass settling and reactor performance compared with continuous mixing. The length of reaction stage is determined by the time required to achieve the desired effluent quality (Irvine and Bush, 1979). Generally, large concentrations of suspended solids require more contact time between bacteria and substrate for complete hydrolysis of the particulate.

At the beginning of the react stage, food to micro-organism (F/M) ratio is high and organic conversion is at its maximum (Sung and Dague, 1995). The biomass consumes the substrate under controlled environmental conditions after which a famine stage starts. Some microorganisms also undergo endogenous decay which helps reduce the volume of the settling sludge. The suppressed F/M ratio at the end of the react stage favors biomass flocculation and settling.

(c) Settle Stage

During settling solids-liquid separation takes place under quiescent conditions in the reaction vessel. Because the partial pressure of the generated biogas within the reactor remains constant, release of CO_2 that would cause biomass solids to float is greatly minimized. This quiescent condition results in faster solids settling and increased ability to process large liquid volumes while maintaining long solids retention times. Solids separation takes place leaving clear supernatant above a sludge blanket.

The settling time varies typically from 10 minutes to one hour depending on the concentration of biomass solids in the reactor and their settleability. The settling time must be short enough to wash out the poorly settling biomass, but not so short to allow flocculent biomass to wash out of the reactor. Typical range of 0.7 to 1.0 hour is usually recommended to ensure adequate settling of the sludge blanket (Alleman and Irvine, 1980; Irvine et al., 1983).

(d) Decant Stage

Once sufficient solid separation has occurred, the supernatant is decanted from a fixed port at a pre-determined level. Alternatively, a floating weir can be used to decant the supernatant at a fixed depth below the liquid surface. Decanting should be carried out without disturbing the settled sludge by using floating or adjustable weirs (Metcalf & Eddy, 2003). Norcross (1992) proposes a decanting level of 0.4 m below the scum.

The total volume of supernatant decanted is based on the volume of the reactor and the hydraulic residence time (HRT). It is usually equal to the volume that was fed in the fill stage. During effluent draw-down, microorganisms with poor settling characteristics are also removed from the reactor, leaving behind the heavier bacterial flocs (Sung and Dague, 1995).

(e) Idle Stage

The period between decanting and the new cycle is referred to as idle time. This stage can be used to waste sludge or perform backwashing of the jet aerator. Sludge wasting is preferably carried out during the idle stage to provide the highest concentration of mixed liquor suspended solids (MLSS).

The frequency of sludge wasting ranges between once each cycle to once every two to three months depending upon system design. No set time period within the cycle is dedicated to sludge wasting (Arora et al., 1985). However, Metcalf & Eddy (2003) recommends that sludge wasting be carried out during the reaction phase for discharge of uniform solids including both fine material and large floc particles. The length of the idle mode may be adjusted or eliminated depending on requirements of the treatment system.

An important feature of ASBR process is the gradual conversion of flocculent biomass into a well-settling and highly active granular biomass. This process, which is referred to as granulation can be noticed as the anaerobic microorganisms tend to adhere to one another as well as to inorganic and/or organic support particles to form firm dense granules. The ASBR tends to promote granulation process by imposing a selection pressure during the decant cycle. The decanting process washes out the poorly settling flocs and dispersed organisms and selects for the heavier, more rapidly settling aggregates. Thus, over time, granular biomass becomes dominant and leads to a rapidly settling sludge and a highly stable reactor system.

The ASBR offers several advantages over the current anaerobic technologies applying continuous-flow principles. Because the reactor is batch-fed, there is no short-circuiting; therefore, there is no need for an extensive feed distribution system in the bottom of the reactor as required for the up-flow anaerobic sludge blanket (UASB) reactor and up-flow anaerobic biofilters. In addition, batch feeding offers some significant kinetic advantages over

continuous-flow processes. The alternating feast and famine conditions in the reactor results in high rates of substrate removal during the react phase but also result in low levels of intermediate soluble organics in the reactor decant. The ASBR also provides a competitive advantage to methanogens that are capable of growing at low-volatile fatty acids (VFA) concentrations, which may explain the observed low concentration of VFA in the ASBR effluent (Sung and Dague, 1995).

Other advantages of the ASBR technology include low capital and operating costs and minimum daily maintenance. Additionally, the ASBR have flexibility in operation because of its ability to hold effluent until it meets specified requirements. Irvine (1985) also found the RNA content of the microorganisms in the SBR system was three to four times greater than would be expected from a conventional continuously-flow system. As such, the higher content of the intracellular machinery in the ASBR culture is capable of processing greater quantity of substrate at a greater rate than is possible in a conventional continuous-flow system.

2.5 Use of Enzymes in Wastewater Treatment

An enzyme is a molecule, which catalyzes biological reactions. The catalysis takes place at a particular site on the enzyme called the active site. Nearly all known enzymes are proteins (Bert et al., 2002).

During enzyme action, the substrate to be acted upon binds itself to a specific location on the enzyme known as active site to form enzyme-substrate complex. The fit between substrate and active site is precise as explained by

the “Lock and Key” hypothesis (Fischer, 1894). It is the shape of active site in the large enzyme molecules that allow them to function and delineate their specificity. However, another theory “Induced-fit hypothesis” suggests that active site is flexible and is not exactly complementary to the shape of the substrate (Vasella et al., 2002). It clarifies that during binding of substrate to the active site, there is induced slight change in shape of the active site to enclose the substrate making the fit more precise.

The activity and shape of the enzyme can be affected by substrate and enzyme concentration besides the environmental factors such as temperature and pH. The rate of enzymatic reaction can be improved by increasing enzyme concentration up to a certain point, beyond which it become constant. This results from depletion of the substrate molecules and upon which the reaction can only be improved by increasing the substrate concentration. Similarly, pH and temperature can become limiting factors to enzymatic reactions. Changes in pH or temperature beyond optimum range result in distortion of enzyme’s shape that reduce and ultimately destroy its effectiveness as a catalyst.

Microorganisms can express specific xenobiotic metabolizing enzyme that would degrade even the most recalcitrant industrial waste. However, the limiting capacity for this natural degradation is the considerable amount of biomass generated by the microorganisms and slow rate of substrate degradation. The individual bacteria may also be inhibited by the presence of other pollutants.

Enzymes were first proposed for the treatment of industrial wastes in the 1930's but it was in the 1990s that enzyme technology received much attention for the improvement in biological remediation for industrial effluents (Aitken, 1993; Whiteley and Lee, 2006). Efforts to advance the biological decomposition of organic matter in wastewater have resulted in the use of hydrolytic enzymes. These enzymes that include glycosidase, lipases and proteases achieve degradation of extracellular polymeric substances (proteins and polysaccharides) and other biological slimes in the organic matter (Roman et al., 2006) and as such are suitable for enzymatic treatment of slaughterhouse wastewater. Enzymes enhance wastewater treatment by increasing the metabolic activity and digestion rate and maintaining a healthy microbial population. Addition of enzymes into anaerobic digestion process cuts down digestion time, improves sludge digestibility and reduces disposal costs (Wawrzynczyk et al., 2008; Ronja, 2008). Moreover, enzyme aided treatment process is easy to control and its products harmless to environment (Ahuja et al., 2004).

Ecotreat® is a proprietary product that contains enzyme secreting microorganisms. The product has been used locally to assist in treatment of domestic and industrial wastewaters. This enzyme secreting bacteria product propagates rapidly to form viable cultures that create formidable reservoirs of enzymes necessary to breakdown organic matter. Ecotreat® has found wide application in municipal wastewater treatment works, oxidation ponds, biofilters, lagoons and septic tanks. Its application rate varies with the nature of the wastewater to be treated. For high organic content wastewaters such as

slaughterhouse wastewaters, enzyme concentration of 0.5 – 1.0 % of the wastewater is recommended. Domestic wastewaters of mild organic loads require an application rate of 0.2% (Wanjuki, personal communication, August 17, 2013).

2.6 Studies on ASBR and Enzymatic Wastewater Treatment

Morris et al. (1998) treated slaughterhouse wastewater in two 11.5 L ASBRs operated at 30 °C with reactor content mixed for 30 seconds every 10 minutes. COD reduction was observed to increase with hydraulic retention time. This probably reflected high suspended solids losses due to poor biomass settling at low hydraulic retention time. The soluble COD was reduced by over 90%at hydraulic retention time of 36 h.

Massé and Masse (2000) treated slaughterhouse wastewater in four 42-L ASBRs operated at 30 °C. Two ASBRs were seeded with anaerobic granular sludge from milk processing plant reactor while the other two received anaerobic non-granulated sludge from a municipal wastewater treatment plant. Influent total chemical oxygen demand (TCOD) ranged from 6,908 to 11,500 mg/L of which approximately 50% were in the form of suspended solids (SS). Total COD was reduced by 90 - 96% at organic loading rates ranging from 2.07 to 4.93 $kgm^{-3}d^{-1}$ and a hydraulic retention time of 2 days. Soluble COD was reduced by over 95% in most samples.

Kim et al. (2005) experimented on the efficiency of enzymatic pre-treatment on solubilisation of food waste, with commercial enzymes. The acidification efficiency and the volatile fatty acid (VFA) production potential of

enzymatically pretreated food waste were examined. An optimum enzyme dosage for solubilization of food waste was 0.1% with the enzyme mixture ratio of 1:2:1. In the acid fermentation of enzymatically pretreated food waste, the maximum VFA production and the highest VFA fraction in soluble COD (SCOD) were also achieved at 0.1% of the total enzyme dosage.

CHAPTER THREE

3.0 METHODOLOGY

This chapter describes the procedures adopted for evaluation of viability of using enzyme secreting bacteria product, Ecotreat®, to improve ASBRs treatment of slaughterhouse wastewater. The experimental set-up consisted of three reactors. Activated sludge was added to each of the reactors and culture of anaerobic micro-organism cultivated. The reactor contents were replaced gradually with slaughterhouse wastewater in daily increments until volume exchange ratio (VER) of 40% was achieved. Ecotreat® was dosed to the reactor contents of the first and second reactor at 0.5 and 1.0% respectively, which were within the range of concentration recommended by the supplier. The third reactor was used as control with no addition of Ecotreat®. Samples of the supernatant were collected at the end of the reactions for analysis of COD and TSS. Effluent or supernatant COD was compared with the ECMA (2006) standard of 1,000 mg/L for discharge into public sewers.

3.1 Sampling and Characterization of Slaughterhouse Wastewater

Samples of slaughterhouse wastewater were obtained from Dagoretti Slaughterhouse Company Ltd at Dagoretti Market in Nairobi. Sampling was carried out during the morning hours to include the streams from all slaughterhouse activities including slaughtering, washing of innings and floor washings. Grab samples were taken from the mixed stream after screening and grit removal but before biological treatment. Samples were collected in four 20L containers filled alternately to ensure homogeneity. The samples were

then transported to the Department of Biochemistry, Chiromo Campus, University of Nairobi, within one hour and stored in a cold room at 4 ^{o}C.

The raw wastewater was characterized in the Public Health Engineering Laboratory of the University of Nairobi for pH, alkalinity, COD and BOD_5 following Standard Methods (Eaton et al., 2005).

3.2 Treatment with Enzymes

Enzymes for use in the study were obtained from Ecotreat® bacterial product. Ecotreat® is a proprietary product consisting of a set of facultative natural bacteria capable of producing copious amounts of exo-enzymes through fermentation. These bacteria are non-pathogenic and pose little danger to animals and plants. They propagate rapidly to form viable cultures that create formidable reservoirs of enzymes necessary for breakdown of organic matter.

Ecotreat® bacteria were purchased from Ecosave Africa Limited. The product is used in Kenya for treatment of both industrial and domestic wastewaters with reports of success (Wanjuki, personal communication, August 17, 2013). Ecotreat® has a short shelf life of about 14 days and requires storage at temperatures range of 16 – 37 °C. In treatment of industrial wastewaters, Ecotreat® bacteria are usually introduced after preliminary treatment, at pH in the range of 4 – 8. For fresh wastewaters or those with pH 4 – 8, no acclimatization is required. However, for wastewaters with depressed pH, for example, because of considerable stay, conditioning to obtain favorable pH is necessary.

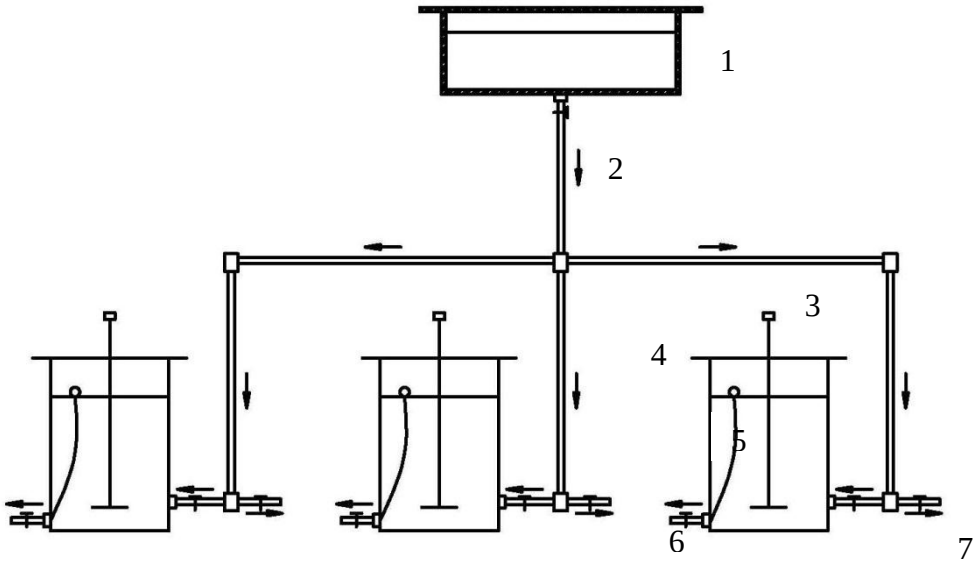
Ecotreat® bacteria were procured 3 days prior to experimental work and stored at room temperature at the Public Health Engineering Laboratory of the University of Nairobi.

3.3 Experimental Set-up

ASBR treatment was carried out at the Public Health Engineering Laboratory of the University of Nairobi. The treatment was carried out in three ASBR reactors fabricated from 6 mm thick Perspex-glass, each 7.5 L capacity (18 x 18 x 23 cm) with a working volume of 5 L (Figure 3.1).

Each reactor consisted of inlet pipe with regulating valve connected to overhead tank and an outlet for the treated effluent. A float decanting mechanism consisting of a flexible pipe suspended by a floater 25 mm below the water surface was used for decanting the supernatant. The floater mechanism allowed decanting of the effluent at a fixed depth below the water surface. A desludging pipe fitted with sludge wasting valve was connected at the bottom of the reactor. A mixer positioned above the reactor agitated the reactor contents at a speed of 50 rpm.

A space of about two litres above the 5 L capacity was provided at the top of each reactor to facilitate methane gas accumulation and evacuation. Covers were fitted on the reactors to create anaerobic conditions.



Legend: 1.Raised feed tank with removable lid, 2. Influent feed control valve, 3. Mixer fitted with a blade, 4.Removable lid, 5.Floating mechanism for decanting control, 6.Decanting valve, 7. Sludge wasting valve

Figure 3.1: Schematic of ASBR Set-up

3.4 Volume Exchange Ratio (VER)

The ASBR operation requires replacement of a portion of reactor content after the reaction stage with an equivalent amount of fresh wastewater. The ratio of the volume decanted and the sludge wasted to the working volume is referred to as the volume exchange ratio (VER). Kariuki (2014) found VER in the range of 30 – 50% were not a significant factor in the performance of sequential batch reactors. Therefore, a VER of 40% was adopted in this study. To achieve 40% VER using the 5 L capacity reactors, 2000 mL of the reactor contents was withdrawn through sludge wasting at the end of reaction stage while agitating, and decanting of the supernatant after settling. The withdrawn contents were replaced with fresh raw wastewater.

3.5 Test Procedure

The following three ASBR test procedures were carried out;

(i) Acclimatization of ASBR

(ii) Reaction time test

(iii) Treatment test

These tests are described in detail below.

3.5.1 Acclimatization of ASBR

Acclimatization process was carried out to enhance the growth of species that act on slaughterhouse wastewater as their substrate and, therefore, biodegrade it. Activated sludge obtained from anaerobic pond at the treatment plant of Dagoretti Slaughterhouse Company Ltd was used to startup the digestion process in the ASBR. The sludge was collected in a 20 L container and preserved in the laboratory at 4 ^{o}C prior to use. This sludge was used for inoculating the slaughterhouse wastewater for about 6 days.

The acclimatization process was carried out by filling each of the three ASBR reactors ASBR1, 2 and 3 with 5,000 ml of activated sludge. The contents were covered for 3 days to cultivate a culture of anaerobic micro-organisms. At the end of the third day untreated slaughterhouse wastewater was added gradually, in daily increments of 0.5 L up to 2.0 L, to prevent shock loading. The enzyme bacterial culture Ecotreat® of 25 and 50 mL was added to ASBR1 and ASBR2 on the fourth day, respectively, to achieve the enzyme application rate of 0.5% and 1%, respectively, as recommended by the supplier. Reactor, ASBR3 was the control and therefore without addition of Ecotreat®. The reactor contents were taken through the ASBR treatment cycle. In each cycle,

supernatant was decanted from each reactor in increments of 0.5 L up to 2.0 L per day and replaced with equal amount of slaughterhouse wastewater at the start of the subsequent treatment cycle until a VER of 40% was achieved on the seventh day. The acclimatization process was continued for further two days while replacing 2.0 L of the supernatant with equal amount of raw slaughterhouse wastewater and operating the reactors on the complete ASBR treatment cycle.

The pH and COD of the supernatant were monitored daily. During decanting stage, 100 mL-samples of supernatants was collected for COD analysis. A volume of 500 ml or 10% was also wasted from each reactor at the end of reaction stage while mixing, to maintain a sludge retention time (SRT) of 10 days.

3.5.2 Reaction Time Tests

Reaction time tests were carried out prior to the experimental treatment cycle to establish the suitable reaction time for the ASBR treatment. The test was carried out for an overall reaction time of 8 hours which was considered the maximum available time for react stage of the treatment cycle.

2 L of raw slaughterhouse wastewater were added to each of the reactors containing 3 L of dense culture obtained from acclimatization process over a period of 20 minutes. Reactor contents were mixed during the reaction stage by stirring at 50 rpm for15 minutes every hour. After four hours of reaction time, 200 mL of each reactor content were extracted and settled for 45

minutes. Further samples were extracted every hour. The sludge was returned into the reactors while the supernatant samples were tested for COD. The COD values were plotted against time to establish suitable reaction time.

3.5.3 Treatment

ASBR treatment procedure consisted of the four typical ASBR phases; namely, fill, reaction, settling and decanting (Figure 2.1). The 5 L reactor contents containing acclimatized microorganisms were allowed to settle for 45 minutes to obtain a dense culture of microorganisms. 2 L of clear supernatant was decanted and replaced with 2L of raw slaughterhouse wastewater over a period of 20 minutes. The contents were mixed intermittently at 50 rpm for 15 minutes every hour to ensure good distribution of the substrate. This rate of mixing was considered short and gentle.

During reaction phase, a period of time selected from reaction time test was adopted and mixing carried out at 50 rpm for 15 minutes every hour. At the end of the reaction time, sludge wasting was done while mixing to discharge uniform solids including both fine material and large floc particles.The reactor contents were then allowed to settle for about 45 minutes. 200 mL of supernatant was decantedthrough a floating decanter maintained about 25mm below the scum by a float.

3.5.4 Monitoring Process

The reactors were operated at room temperature (22 – 25 ^{o}C) with no temperature control. Continuous monitoring of the reactor contents pH was

carried out during ASBR treatment process and alkaline buffer used as necessary to ensure stability of the system.

3.6 Analytical Methods

Supernatant samples from all the tests were analyzed for pH, alkalinity, and COD following Standard Methods (Eaton et al., 2005). The BOD_5 test was conducted for the raw slaughterhouse wastewater and for some ASBR treated effluents for assessment of biodegradability in conjunction with the COD test.

3.7 Statistical Methods

The viability of using enzymes in ASBR for treatment of slaughterhouse wastewater was evaluated using analysis of variance (ANOVA) of the set of obtained results. A p-value, which measures the probability of falsely rejecting the null hypothesis, was calculated. The null hypothesis formulated stated that there was no significant difference in effluent COD between the different concentrations of enzymes feed and control.

CHAPTER FOUR

4.0: RESULTS AND DISCUSSIONS

This study involved investigation of the viability of using enzymes in the ASBR to improve treatment of slaughterhouse wastewater.

Ecotreat® enzyme secreting bacteria were added to slaughterhouse wastewater in three reactors at 0 (control), 0.5 and 1.0% respectively. The reactor contents were taken through full ASBR treatment cycles of feeding, reaction, settling, decanting and idle phases. Tests were carried out at VER of 40%. Effluent wastewater samples were analyzed for COD, TSS and BOD_5.

The results and discussion are presented in subsequent sections.

4.1 Raw Slaughterhouse Wastewater Characterization

The raw slaughterhouse wastewater from Dagoretti Slaughterhouse Company Ltd was collected after screening of innings, fragments of bones, hooves and other coarse solids but before biological treatment. The wastewater was characterized as follows (Table 4.1).

Table 4.1: Characterization of Raw Slaughterhouse Wastewater from Dagoretti Slaughterhouse Company Limited

Parameter	**Range (mg/L)**			**Mean (mg/L)**	**SD**	Number of samples, **n**
COD	10,560	-	10,720	10,640	±63	4
BOD_5	7,020	-	7,200	7,120	±75	3
TSS	200	-	200	200	±0	3
pH (No units)	6.7	-	6.8	6.75	± 0.05	3

The slaughterhouse wastewater had greater organic load than the typical range for slaughterhouse wastewater indicated by Yiu et al. (2001) (Table2.2). In

addition, it comprised of highly soluble organics with TSS accounting for less than 2% of the measured COD. This is below the TSS/COD ranges of 10 – 33% and 27 – 67% obtained by Yiu et al. (2001) and Massé and Masse (2000) respectively. The difference between these characteristics and the typical values in literature may be attributed to sampling of time of the slaughterhouse wastewater which was carried out during the morning slaughterhouse operations and before floor washing and rinsing were undertaken. The wastewater stream therefore represented peak concentration and did not include water from the afternoon washing, which would probably dilute the wastewater. High ratio of bovine blood to the total volume of wastewater stream in the slaughterhouse could be the cause of high solubility of the organic polluting load of the slaughterhouse wastewater.

The raw slaughterhouse wastewater did not meet EMCR (2006) standards for disposal into public sewers (Table 2.3); therefore, treatment was required before discharge to the environment to prevent its detrimental effects. The BOD_5/COD ratio of the slaughterhouse wastewater was 0.67 which is within 0.5 – 0.67 range for readily biodegradable wastewaters. This is comparable to the typical BOD_5/COD ratio for untreated domestic wastewater of 0.5 – 0.8 which are discharged into public sewers for treatment. Therefore, the wastewater may be classified readily biodegradable (Metcalf & Eddy, 2003) and biological treatment processes may be appropriate.

4.2 Activated Sludge for Acclimatization characteristics

The activated sludge used as inoculant to start-up the digestion process in ASBR was obtained from anaerobic pond at Dagoretti Slaughterhouse Wastewater Treatment Plant. Scum and floating materials in the anaerobic pond were skimmed and settled sludge and wastewater stirred before collection to ensure uniformity. Its main characteristics were as shown in Table 4.2.

Table 4.2: Characterization of Activated Sludge from Anaerobic pond at Dagoretti Slaughterhouse Wastewater Treatment Plant

Pollutant	Range (mg/L)	Mean (mg/L)	SD	N
COD	920-960	947	±23	3
BOD_5	600-640	620	±20	3
TSS	140-180	160	±20	3
pH (No units)	7.10- 7.40	7.23	± 0.15	3

The activated sludge had less organic load compared to raw slaughterhouse wastewater. This is exhibited by COD which was less than 10% of raw slaughterhouse wastewater at comparative mean TSS concentration of 160 and 200 mg/L for activated sludge and raw slaughterhouse wastewater respectively. The presence of active biomass within the activated sludge in the anaerobic pond assists in the biodegradation of slaughterhouse wastewater in the treatment process. The activated sludge contributes to the observed over 90% COD reduction from 10,640 mg/L at the inlet works to 947 mg/L in the anaerobic pond of the Treatment Works.

4.3 Sludge Acclimatization

A culture of anaerobic micro-organisms from the activated sludge was cultivated in the reactors for 3 days. From the fourth day, slaughterhouse wastewater was introduced gradually added in daily increments of 0.5 L for acclimatization to enhance growth of species that biodegrade slaughterhouse wastewater. Ecotreat® was also added to two reactors (ASBR1 and ASBR2) at 1 and 0.5% concentrations respectively on day four of acclimatization.

Settled effluent COD concentration at the end of each treatment cycle was used to evaluate acclimatization of the micro-organisms for different enzyme concentrations (Figure 4.1).

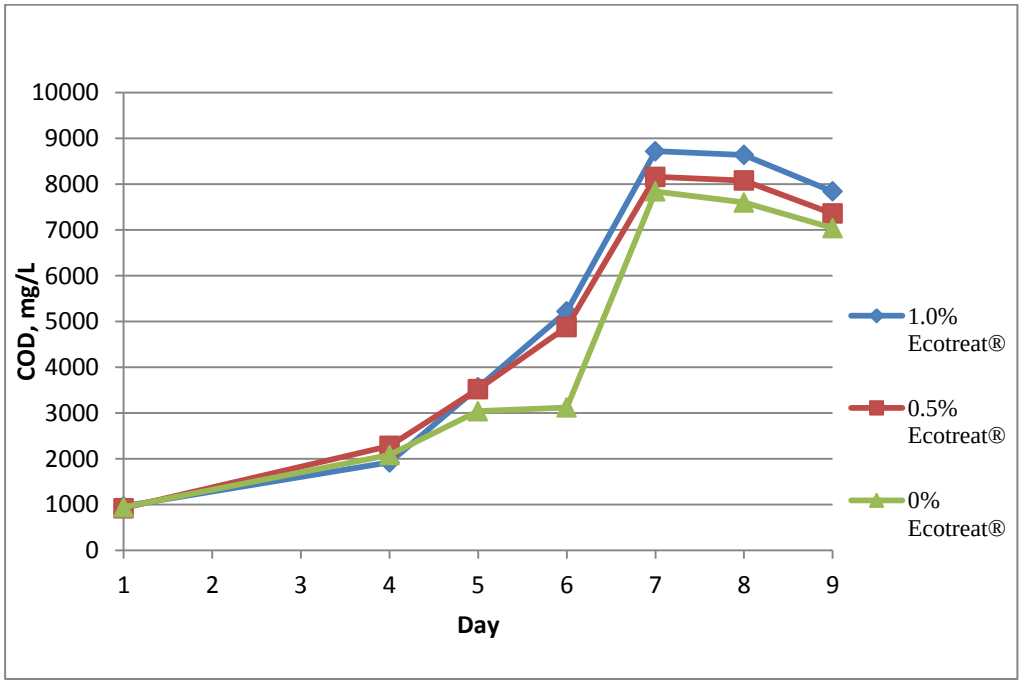


Figure 4.1: COD for ASBR Sludge Acclimatization Stage

In the first 3 days of acclimatization stage, activated sludge was covered in the reactors and samples were not collected for COD testing. The sharp increase

in effluent COD between day four and seven of acclimatization is as a result of the replacement of activated sludge with slaughterhouse wastewater in daily increments. The rate of increase in substrate (raw slaughterhouse wastewater) in the reactor apparently exceeds the rate of break-down in the anaerobic digestion process. Acclimatization of bacteria in the reactors appears to have stabilized on day seven. This is shown by the gradual drop in effluent COD between days 7 and 9 at constant VER of 40%. The result was interpreted as acclimatization of anaerobic bacteria. Therefore, treatment tests were commenced.

It was observed that the control reactor produced effluents of the lowest COD throughout acclimatization period. Overall COD reduction during this period for control, 0.5 and 1% enzyme concentration reactors are 34, 31 and 26% respectively. The achievement of lower COD reduction with increased enzyme concentration in the reactors during acclimatization phase could be as a result of continued competition between the micro-organisms species in slaughterhouse wastewater and Ecotreat®. The micro-organism species from different environments (niche) would compete for the substrate especially at the famine stage. However, the competition is expected to subside with subsequent ASBR treatment cycles as steady state sets in the reactor system and enzyme action dominates the biochemical reaction.

Statistical analysis of the effluent COD values at this stage using ANOVA shows lack of significant difference between the three ASBR reactors ($p = 0.85 > 0.05$, Appendix B). This confirms that addition of enzymes in the

reactors was not significant to the treatment process during the acclimatization period

4.4 Reaction Time Test

Reaction time tests were carried out prior to the experimental treatment cycle to determine the appropriate reaction time for adoption in the ASBR treatment tests. The typical ASBR treatment cycle was followed at VER of 40% for the three reactors. After four hours of reaction time, 200 mL of the contents was extracted every hour, settled for 45 minutes and the sludge returned into the reactor while the supernatant was tested for COD.

Results of reaction time test are shown in Figure 4.2.

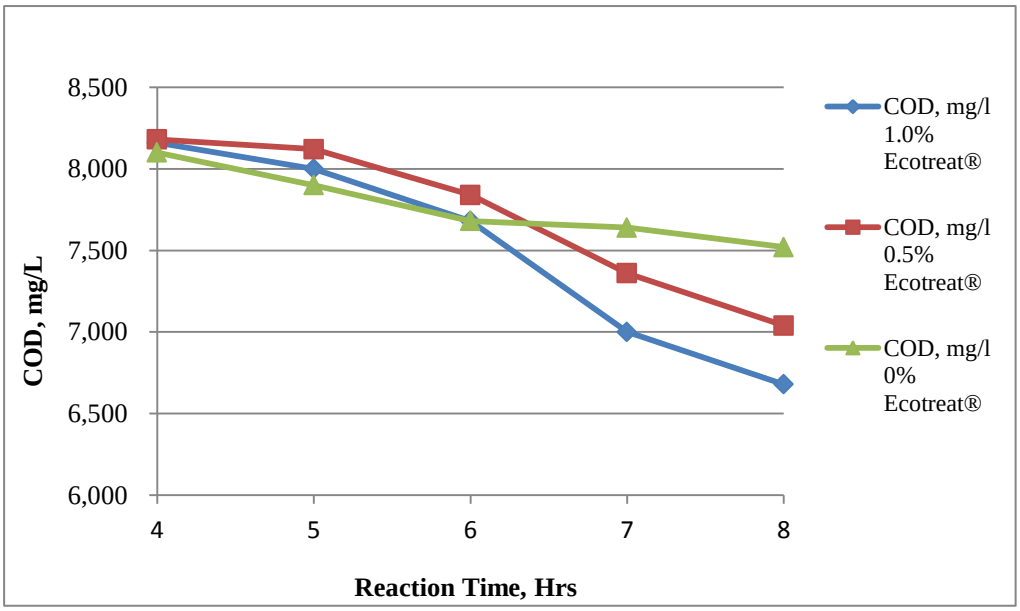


Figure 4.2: Variation of Effluent COD with ASBR Reaction Time Test

COD reduction in all the reactors increased with time for the first 7 hours for the enzyme fed reactors and 6 hours for the control (Figure 4.2). Thereafter, the reduction declines. The highest COD reduction recorded within the 8-hour

period was for 8-hour reaction time at 37, 33 and 29% for reactors with 1, 0.5 and 0% (control) enzyme concentrations, respectively. For all reactors, reaction time of 8 hours produced most improved effluent as compared to all other different reaction time ($p = 3.09E-05 << 0.05$). This confirms that reaction time affects the effluent quality of ASBR provided the substrate is not exhausted and biodegradable bacteria are still active. More time is required for complete breakdown of the substrate to final products of anaerobic digestion and the maximum available reaction time of 8-hours was thus adopted for the treatment stage.

The reactor with the highest enzyme concentration, 1%, produced effluents of lowest COD at reaction times greater than 6 hours. Over the same period, the control produced effluent with highest COD. This indicated that the enzymes from the Ecotreat® bacteria were assisting reduction of COD.

4.5 Treatment Stage

ASBR treatment involves feeding, reaction, settling, decanting and idle stages. Treatment of wastewater collected from Dagoretti Slaughterhouse in ASBR with the aid of Ecotreat® was carried out from day eight for 7 days at reaction time of 8 hours. Performance of enzyme in the ASBR treatment was assessed by analyzing organic load of the respective ASBR effluents through COD and TSS tests. The results are presented and discussed in the following sub-sections.

4.5.1 COD Removal

Variation of COD during the treatment stage with daily treatment cycles is shown in Figure 4.3 for the entire experimental work duration.

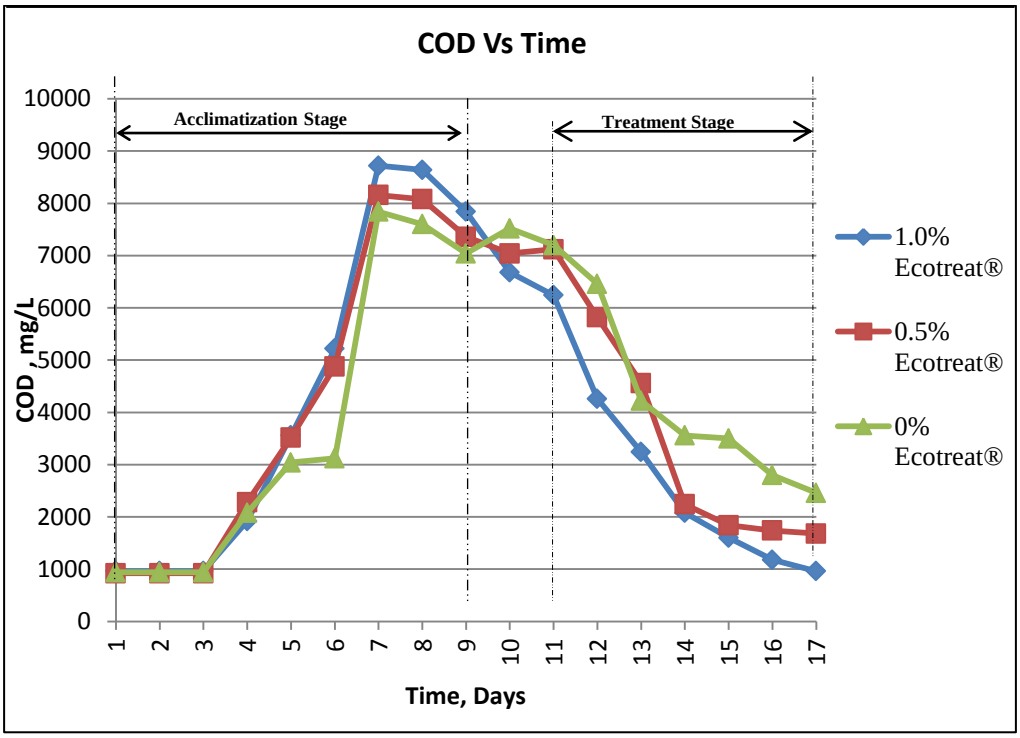


Figure 4.3: Effluent COD variation with time during ASBR Treatment

The effluent quality improved with time for all the three reactors. This can be explained by the improved ASBR performance over time as a result of granular biomass formation and consequential progress towards attainment of stable reactor system (Mass and Masse, 2000). ASBR tends to promote granulation process by imposing a selection pressure during the decant cycle. The decanting process washes out the poorly settling flocs and dispersed organisms and selects for the heavier, more rapidly settling aggregates. As a result, every other decanting cycle leads to enhanced treatment of wastewater.

However, ASBR treatment was stopped after 7 day period as the COD trend between day 14 and 17 was observed to have entered lag phase and stable reactor system assumed to have been attained in the ASBRs. Statistical analysis using ANOVA (Appendix B) also shows higher variance in effluent COD values between day 11 and 14 than the subsequent period between days 14 to 17. (p_{max} = 0.0467< 0.05). ASBR treatment for any additional day is thus expected to produce effluent of similar quality.

On the 17th day of the experimental work after attainment of stable reactor system, the reactor with highest enzyme concentration (1%) resulted in the maximum COD reduction of 91% compared to 84% and 77% COD reduction for 0.5% and 0% enzyme concentrations respectively with 8-hour reaction time. The trend in effluent quality is as shown in Figure 4.4.

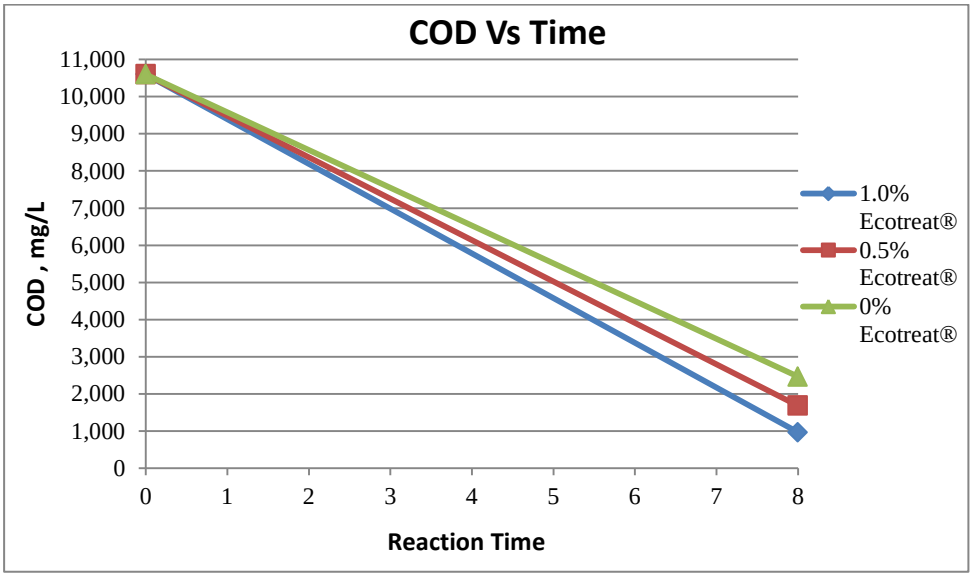


Figure 4.4: ASBR effluent COD Trend for Stable Reactor System

COD removal of 91% in the reactor with 1% enzyme concentration in the stable reactor system is comparable to 90 – 96 % COD reduction achieved by

Massé and Masse (2000) in ASBR with retention time of 2 days and influent with approximately 50% organic loading in the form of suspended solids (SS). Enzymes enhance wastewater treatment process by increasing the metabolic activity and digestion rate and maintaining a healthy microbial population. The attainment of high COD reduction at shorter hydraulic time in this study is an indication of the catalytic effect of the enzyme in the anaerobic digestion of the wastewater which is proportionate to the enzyme concentration applied. Ecotreat bacteria propagate with time and as such the rate of enzymatic reaction in the reactors in which it was applied improved over the treatment period beyond the ability of stable reactor system as in the control. However, the enzyme concentration of 1% giving the high COD reduction is higher compared to 0.1% enzyme dosage applied by Kim et al. (2005) in experimenting on the efficiency of enzymatic pre-treatment on solubilisation of food waste with commercial enzymes. This could be attributed to the complex nature of slaughterhouse wastewater. The fibrous proteins contained in the wastewater originating from hair, skin, nails and bones are tough and less susceptible to hydrolysis (Varel et. al., 1977).

4.5.2 Total Suspended Solids (TSS) Removal

The ASBR effluents were tested for TSS after each treatment cycle. The trend in effluent TSS is shown in Figure 4.5.

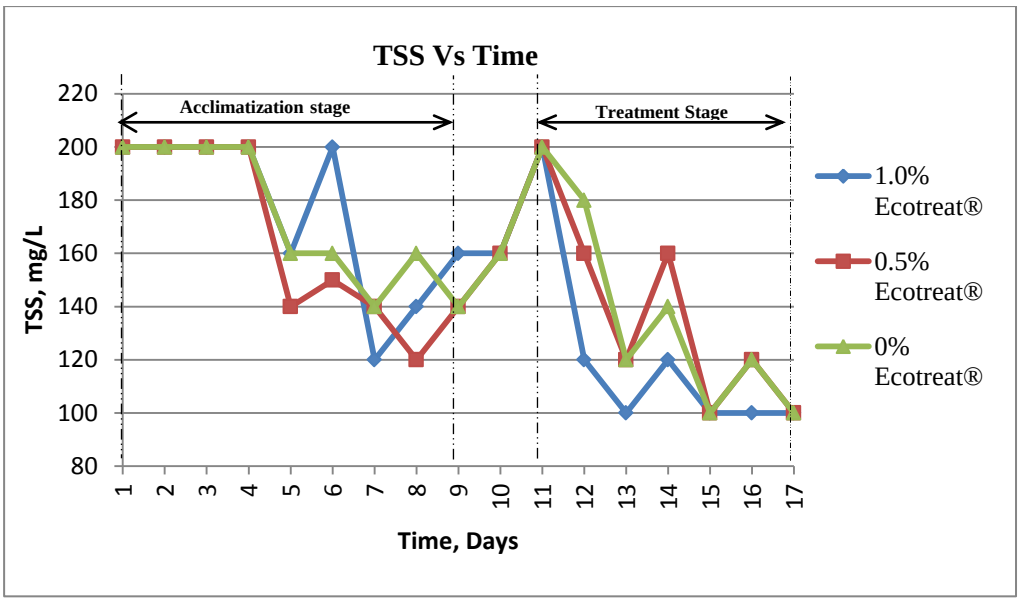


Figure 4.5: Effluent TSS Trend during ASBR Treatment

A rapid drop in effluent TSS occurred between days 4 and 5 followed by gradual TSS reduction during the acclimatization stage. This is reflected by TSS reduction of 20, 30 and 20% between days 4 and 5 compared to 13, 14 and 13% in the remaining period of acclimatization stage for the reactors with enzyme concentration of 1, 0.5 and 0%, respectively. During the treatment stage, effluent TSS decreased from 200 to 100 mg/L on day 15 and remained fairly stable for all the reactors irrespective of applied enzyme concentration, representing 50% reduction. This could be as a result of formation of granular biomass in the reactors and rapidly settling sludge, and achievement of stable reactor system which is independent of the enzyme concentration.

Decreasing effluent TSS in the reactors with time indicates improved settling in the reactors as a result of formation of granular biomass. As expounded in section 4.5.1, decanting process tends to wash out poorly settling flocs and dispersed organisms and selects for the heavier, more rapidly settling

aggregates. As a result, every subsequent decanting in ASBR results in less suspended solids in the reactor effluent until stable reactor system is attained when effluent TSS is expected to remain almost constant. In this study, stability appears to have been achieved 15 days from the start of the tests.

The overall TSS removal attained in the reactors of 50% is less compared to 87% TSS reduction obtained by Massé and Masse (2000) on influent wastewater of 2,500 mg/L TSS. The smaller removal rate in the current study may be attributed to the smaller influent TSS concentration of 200 mg/L of the slaughterhouse wastewater, which is at the lower limit of typical values for slaughterhouse wastewater (Table 2.2). It was previously reported that the raw slaughterhouse wastewater used in this study was collected after screening of coarse and floating objects. On the other hand, supernatant TSS concentration is a factor of settlement time; for a given settling period of granulated solids, particles with size greater than a certain threshold will settle out while the rest will remain in suspension.

4.5.3 Biodegradability of the ASBR Treated Effluent

The objective of this study was to evaluate the viability of using enzymes in the treatment of slaughterhouse wastewater in ASBR with aim of discharging into public sewers. The public sewers however convey the collected wastewater to sewerage treatment works, which in most cases adopt biological treatment processes. Therefore the final effluent of ASBR treatment in each reactor was assessed for biodegradability (Table 4.3).

Table 4.3: BOD_5/COD ASBR Effluent

Ecotreat® Concentration	ASBR1 1%	ASBR2 0.5%	ASBR3 0%
BOD_5, mg/L	500	960	1440
COD, mg/L	960	1,680	2,460
BOD_5 / COD ratio	0.521	0.571	0.585

The BOD_5/COD ratios of the effluents from all the reactors are above 0.5, which indicates easily biodegradable wastewater (Metcalf & Eddy, 2003). Therefore, the effluent can be treated using biological processes. If discharged into public sewer, these effluents would undergo treatment with neither need for acclimatization nor interference with the operations of the municipal wastewater treatment plants. The results are comparable to the typical BOD_5/COD ratio for untreated domestic wastewater of 0.5 – 0.8 which are discharged into public sewers for treatment.

4.6 General Discussions

Treated effluent COD of 960, 1680 and 2460 mg/L were achieved for 8-hour reaction time in reactors with 1, 0.5 and 0% enzyme concentrations respectively, representing respective removal rates of 91, 84 and 77%. The COD reduction of 77% by the control reactor within 8 hours shows the efficacy of ASBR treatment. Addition of 1% enzyme concentration to the reactor resulted in further 14% reduction in COD.

The organic load in the wastewater effluent for ASBR has also been observed to decrease with time even at the same VER. This is as a result of gradual

formation of granular biomass in ASBR which ultimately lead to the attainment of stable reactor systems.

The effluent COD of the reactor with 1% enzyme concentration was 960 mg/L COD. Therefore, the enzyme dosage produced effluent that complied with EMCR (2006) requirement of less than 1,000 mg/L COD for discharge to public sewers. Further reduction of COD may be obtained if the system is operated beyond the 16 days used in this study, to obtain steady state conditions. The effluents BOD_5/COD ratio indicated that the pre-treated wastewater can be treated using biological means in the municipal wastewater treatment plants, which frequently adopt waste stabilization ponds and other biological processes for treatment.

The enzyme secreting bacteria was added to the wastewater at a rate of 0.5 to 1.0%. However, because the bacteria are reproductive, it is not necessary to dose them continuously unlike other additives in wastewater treatment whether chemical or natural. The bacteria only need to be replenished only after a period of six months (Wanjuki, personal communication, August 17, 2013). Consequently, use of bacteria enzymes incurs insignificant costs while aiding in treatment of the waste.

CHAPTER FIVE

5.0: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study evaluated enzymatic treatment of slaughterhouse wastewater in ASBR. The conclusions of the study are:

1. Enzyme assisted ASBR for treatment of slaughterhouse wastewater can achieve over 80% and 50% reduction of COD and TSS respectively within 8-hour reaction time after adequate acclimatization to attain a stable reactor system compared to ASBR treatment without enzyme aid.
2. Use of 1% enzyme concentration in ASBR treatment enhanced the COD reduction by14 %.
3. Enzyme concentration of 1% in 8 – hour ASBR treatment of slaughterhouse wastewater meets EMCR (2006) requirements for discharge into public sewers of COD less than 1,000 mg/L after 15 days of operation; before the end of the 15 days period, the effluent should be recirculated.
4. The effluents from enzymatic treatment of slaughterhouse wastewater in ASBR are biodegradable with minimum BOD_5/COD ratio of 0.52 and, therefore, can be further treated using biological processes in public sewage treatment plant.

5.2 Recommendations

The recommendations of the study are:

1. This study found 91 % reduction of COD within 8 hours for enzyme dosage of 1%. Further tests should be carried out to evaluate potential of enzyme application rate greater than 1% to reduce on reaction time and therefore, treatment costs.

2. Treatment of the slaughterhouse waste should be carried out beyond the 16 days evaluated in this study to establish the duration of stable performance of the enzyme assisted ASBR systems.

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APPENDICES

APPENDIX A: EXPERIMENTAL WORK RESULTS

a) Raw Slaughterhouse Wastewater Characteristics

Parameter	1	2	3	4	Average	Std Dev.
COD, mg/l	10,720	10,680	10,600	10,560	10,640	±73.0
BOD, mg/l	7,200	-	7,140	7,020	7,120	±91.7
pH	6.7	-	-	6.8	6.75	±0.1
Alkalinity, mg/L as $CaCO_3$	800	-	-	780	790	±14.1
TSS, mg/l	200	200	200	200	200	±0.0

b) Characteristics of Activated Sludge for Acclimatization

Parameter	1	2	3	Average	Std Dev.
COD, mg/l	920	960	960	946.7	±23
BOD, mg/l	640	620	600	620.0	±20
pH	7.1	7.2	7.4	7.23	±0.15
TSS, mg/l	140	160	180	160.0	±20

c) Acclimatization Stage: COD, mg/L(4 Hour Reaction time)

Day	ASBR1 (1.0% Ecotreat®)	ASBR2 (0.5% Ecotreat®)	ASBR3 (0% Ecotreat®)	Remarks
1	960	920	940	No Replacement
4	1920	2280	2080	0.5 L Replacement
5	3560	3520	3040	1.0 L Replacement
6	5220	4880	3120	1.5 L Replacement
7	8720	8160	7840	2.0 L Replacement
8	8640	8080	7600	2.0 L Replacement
9	7840	7360	7040	2.0 L Replacement

d) Acclimatization Stage: TSS, mg/L

Day	ASBR1 (1.0% Ecotreat®)	ASBR2 (0.5% Ecotreat®)	ASBR3 (0% Ecotreat®)	Remarks
1	200	200	200	No Replacement
4	200	200	200	0.5 L Replacement
5	160	140	160	1.0 L Replacement
6	200	150	160	1.5 L Replacement
7	120	140	140	2.0 L Replacement
8	140	120	160	2.0 L Replacement
9	160	140	140	2.0 L Replacement

e) Reaction Time Test: COD, mg/L

Hour	ASBR1 (1.0% Ecotreat®)	ASBR2 (0.5% Ecotreat®)	ASBR3 (0% Ecotreat®)
4	8,160	8,180	8,100
5	8,000	8,120	7,900
6	7,680	7,840	7,680
7	7,000	7,360	7,640
8	6,680	7,040	7,520

f) Treatment Stage: COD, mg/L

Day	ASBR1 (1.0% Ecotreat®)	ASBR2 (0.5% Ecotreat®)	ASBR3 (0% Ecotreat®)	Remarks
11	6,240	7,120	7,200	2.0 L Replacement
12	4,260	5,820	6,460	2.0 L Replacement
13	3,240	4,560	4,220	2.0 L Replacement
14	2,080	2,240	3,560	2.0 L Replacement
15	1,600	1,840	3,500	2.0 L Replacement
16	1,180	1,740	2,800	2.0 L Replacement
17	960	1,680	2,460	2.0 L Replacement

g) Treatment Stage: TSS, mg/L

Day	ASBR1 (1.0% Ecotreat®)	ASBR2 (0.5% Ecotreat®)	ASBR3 (0% Ecotreat®)	Remarks
11	200	200	200	2.0 L Replacement
12	120	160	180	2.0 L Replacement
13	100	120	120	2.0 L Replacement
14	120	100	140	2.0 L Replacement
15	100	100	100	2.0 L Replacement
16	100	120	100	2.0 L Replacement
17	100	100	100	2.0 L Replacement

APPENDIX B: ANALYSIS OF VARIANCE (ANOVA)

- ACCLIMATIZATION – ANALYSIS OF COD VALUES:

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	6	35900	5983.333	8192067
Column 2	6	34280	5713.333	6318347
Column 3	6	30720	5120	6960640

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2340577.778	2	1170289	0.163516	0.850646	3.68232
Within Groups	107355266.7	15	7157018			
Total	109695844.4	17				

- REACTION TIME – ANALYSIS OF COD VALUES:

(a) 4 Hr Vs 5 Hr Reaction Times

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	16160	8080	12800
Column 2	2	16300	8150	1800
Column 3	2	16000	8000	20000

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22533.33	2	11266.67	0.976879	0.471281	9.552094
Within Groups	34600	3	11533.33			
Total	57133.33	5				

(b) 5 Hr Vs 6 Hr Reaction Times

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	15680	7840	51200
Column 2	2	15960	7980	39200
Column 3	2	15580	7790	24200

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	38800	2	19400	0.507853	0.645712	9.552094
Within Groups	114600	3	38200			
Total	153400	5				

(c) 6 Hr Vs 7 Hr Reaction Times

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	13	6.5	0.5
Column 2	2	14680	7340	231200
Column 3	2	15200	7600	115200
Column 4	2	15320	7660	800

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	85095563	3	28365188	326.7874	3.09E-05	6.591382
Within Groups	347200.5	4	86800.13			
Total	85442764	7				

(d) 7 Hr Vs 8 Hr Reaction Times

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	15	7.5	0.5
Column 2	2	13680	6840	51200
Column 3	2	14400	7200	51200
Column 4	2	15160	7580	7200

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	78289734	3	26096578	952.4255	3.66E-06	6.591382
Within Groups	109600.5	4	27400.13			
Total	78399335	7				

(e) Overall ASBR reaction rates

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	
Column 1	5	37520	7504	410080	410080
Column 2	5	38540	7708	244320	
Column 3	5	38840	7768	53320	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	191520	2	95760	0.405923	0.675176	3.885294
Within Groups	2830880	12	235906.7			
Total	3022400	14				

- TREATMENT STAGE – ANALYSIS OF COD VALUES:

(a) Variation of COD for 1% Enzyme Concentration Reactor– ASBR1

Anova: Single Factor

COD Test Results

6,240	2,080
4,260	1,600
3,240	1,180
2,080	960

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	15820	3955	3113700
Column 2	4	5820	1455	244100

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12500000	1	12500000	7.445351	0.034249	5.987378
Within Groups	10073400	6	1678900			
Total	22573400	7				

(b) Variation of COD for 0.5% Enzyme Concentration Reactor – ASBR2

Anova: Single Factor

COD Test Results	
7,120	2,240
5,820	1,840
4,560	1,740
2,240	1,680

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	19740	4935	4320367
Column 2	4	7500	1875	63566.67

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18727200	1	18727200	8.543561	0.02653	5.987378
Within Groups	13151800	6	2191967			
Total	31879000	7				

(c) Variation of COD for Control (0% Enzyme Concentration) Reactor – ASBR3

Anova: Single Factor

COD Test Results

7,200	3,560
6,460	3,500
4,220	2,800
3,560	2,460

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	21440	5360	3045067
Column 2	4	12320	3080	289866.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10396800	1	10396800	6.235087	0.046716	5.987378
Within Groups	10004800	6	1667467			
Total	20401600	7				

(d) Variation of COD for 3 Reactors: Day 1 – Day 4 of ASBR Treatment

Anova: Single Factor

ASBR1	***ASBR2***	***ASBR3***
6,240	7,120	7,200
4,260	5,820	6,460
3,240	4,560	4,220
2,080	2,240	3,560

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	15820	3955	3113700
Column 2	4	19740	4935	4320367
Column 3	4	21440	5360	3045067

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4153400	2	2076700	0.594524	0.572124	4.256495
Within Groups	31437400	9	3493044			
Total	35590800	11				

(e) Variation of COD for 3 Reactors: Day 4 – Day 7 of ASBR Treatment

Anova: Single Factor

ASBR1	***ASBR2***	***ASBR3***
2,080	2,240	3,560
1,600	1,840	3,500
1,180	1,740	2,800
960	1,680	2,400

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	5820	1455	244100
Column 2	4	7500	1875	63566.7
Column 3	4	12320	3080	289866.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5692066.7	2	2846033	14.28891	0.00161	4.256495
Within Groups	1792600	9	199177.8			
Total	7484666.7	11				

APPENDIX C: PHOTOGRAPHS

Plate 1: Raw Slaughterhouse Sampling Point (After screening) – Dagoretti Slaughterhouse, Nairobi

Plate 2: Activated Sludge Sampling (Anaerobic Pond) – Dagoretti Slaughterhouse Wastewater Treatment Plant, Nairobi

Plate 3: Maturation Pond at Dagoretti Slaughterhouse Wastewater Treatment Plant, Nairobi

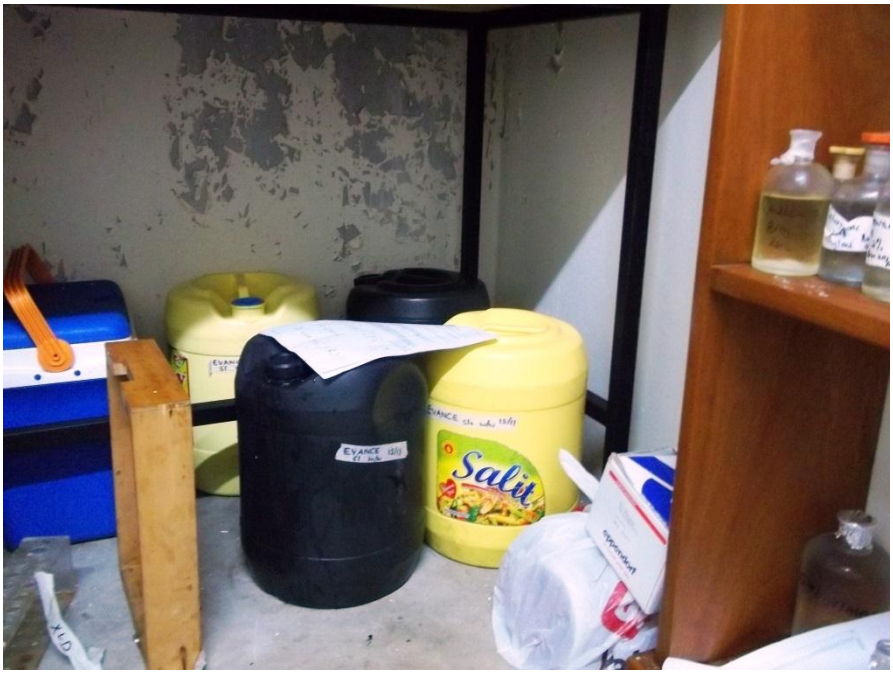

Plate 4: Raw Slaughterhouse Wastewater stored in the Cold Room, Chiromo Campus

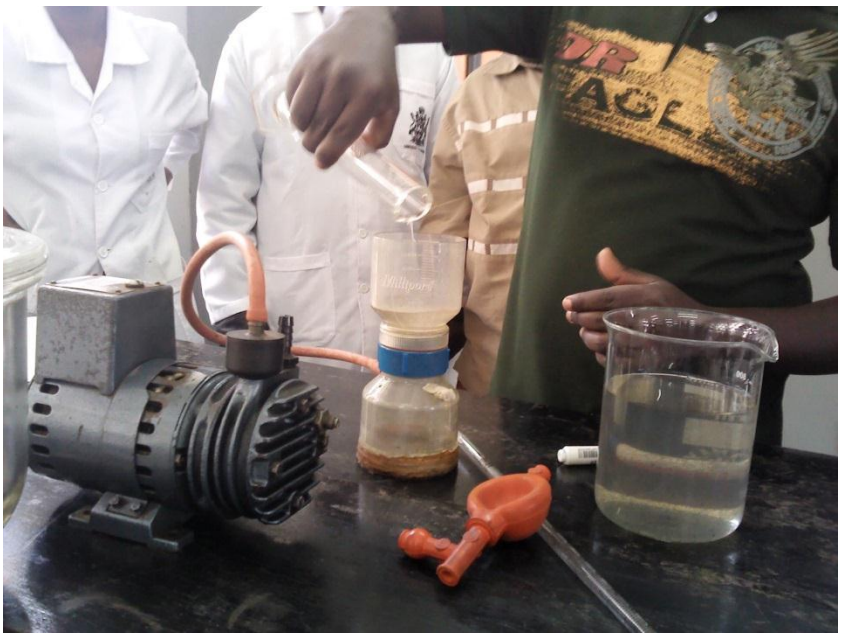

Plate 5: Suspended Solids Testing at Public Health Engineering Laboratory, University of Nairobi

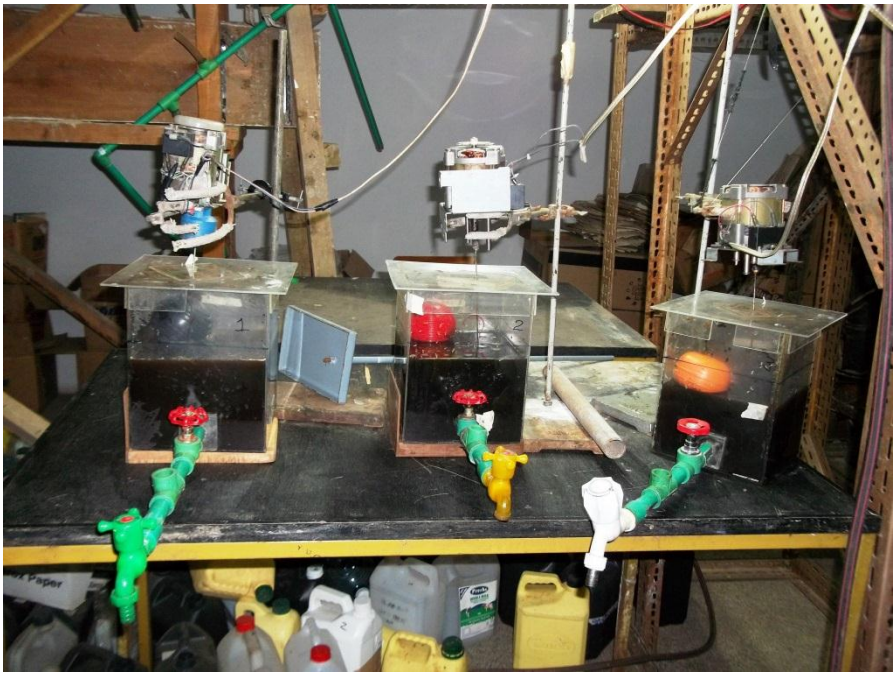

Plate 6: View of ASBR Experimental Set-up, Public Engineering Laboratory, University of Nairobi

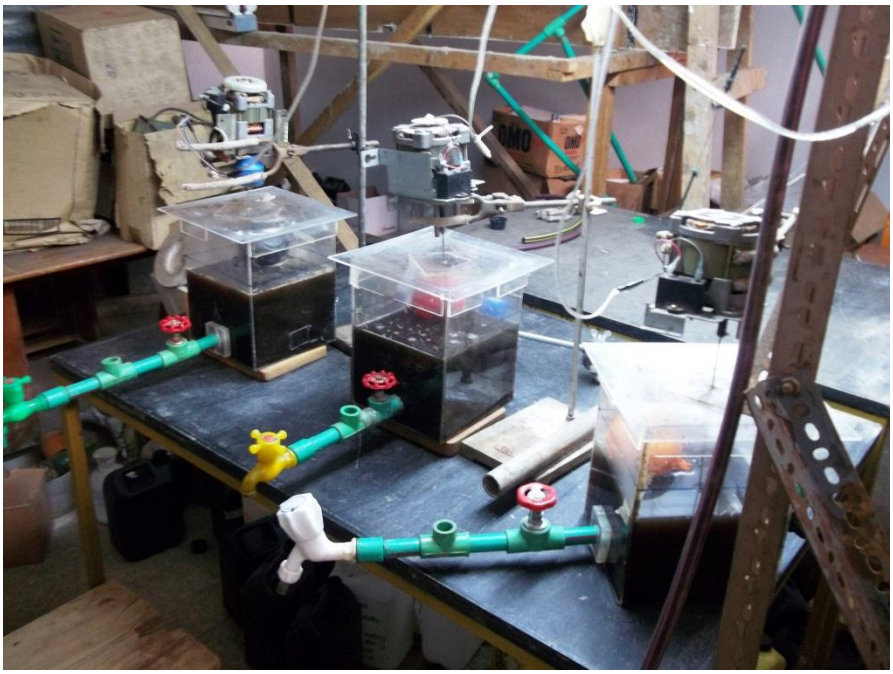

Plate 7: View of ASBR Experimental Set-up, Public Engineering Laboratory, University of Nairobi

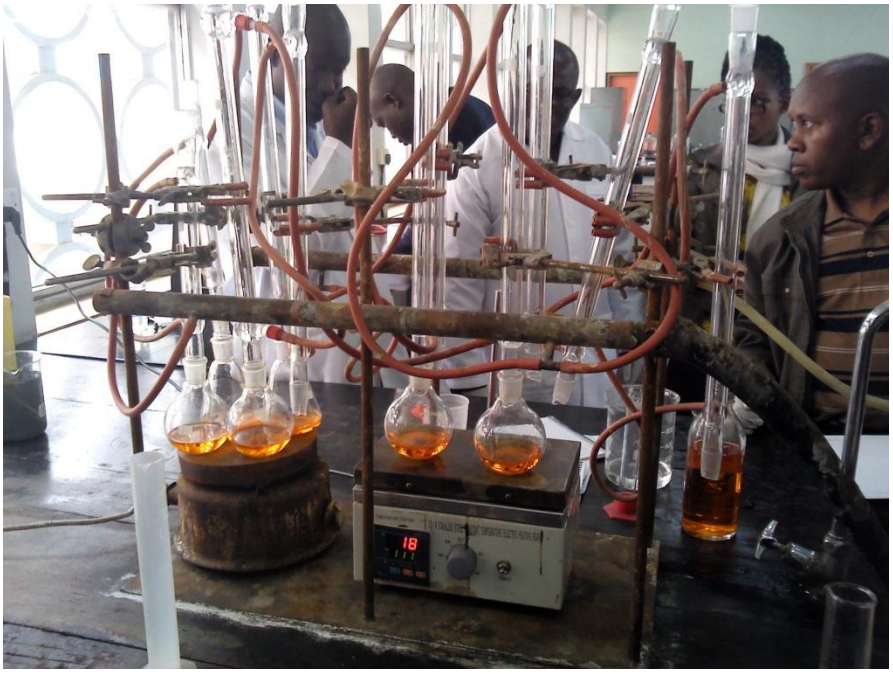

Plate 8: Treated Effluent COD Testing at Public Engineering Laboratory, University of Nairobi