VERMIFILTRATION OF PALM OIL MILL EFFLUENT

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VERMIFILTRATION OF PALM OIL MILL EFFLUENT (POME)

By

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LIST OF SYMBOLS AND ABBREVIATIONS

Chemicals

$Ca(OH)_2$	Calcium Hydroxide
CO_2	Carbon Dioxide
CH_4	Methane
NH_3	Ammonia
NH_4^-	Ammonium
NH_3-N	Ammoniacal Nitrogen
NO^{3-}	Nitrate
PO_4^{3-}	Phosphate
TN	Total Nitrogen

Other

ANC	African Nightcrawler
APHA	American Public Health Association
BF	Biofilter
CF	Control Filter
Char.	Characteristic
EPA	Environmental Protection Agency
FFB	Fresh Fruit Bunches
h	Height
GHG	Green House Gas
HRT	Hydraulic Retention Time
HLR	Hydraulic Loading Rate

LR	Lumbricusrubellus
l	Length
PVC	Polyvinyl Chloride
POME	Palm Oil Mills Effluent
PT	Pretreatment Tank
RBD Palm Olein	Refined Bleached Deodorised Palm Olein
RBD Palm Stearin	Refined Bleached Deodorised Palm Stearin
RBD Palm Oil	Refined Bleached Deodorised Palm Oil
SP	Starting POME
VSFCW	Vertical Subsurface-Flow Constructed Wetlands
VF	Vermifilter
BVF	Batch vermifilter
BVC	Vermicast filter
WD	Worm Density
w	Width

Parameters

BOD_5	Biological Oxygen Demand
COD	Chemical Oxygen Demand
EC	Electro Conductivity
SS	Suspended Solid
TSS	Total Suspended Solid
TDS	Total Dissolved Solid

Units

^{o}C	Degrees Celcius
^{0}F	Degrees Fahrenheit
cm	Centimeter
d	Day
kg	Kilogram
L	Liter
m^3	meter cube
m	Meter
mg	Milligram
min	Minutes
m^2	meter square
mm	Millimeter
mL	Mililiter

PENURASAN VERMI EFLUEN KILANG MINYAK KELAPA SAWIT

ABSTRAK

Prestasi sistem penuras vermi (VF) menggunakan spesis cacing *Lumbricus rubellus* and *Eudrillus eugeniae* dalam merawat efluen kilang minyak kelapa sawit (POME) telah dikaji. Di dalam kajian ini, POME adalah singkatan kepada 'Palm oil mill effluent' iaitu sisa air organik yang terjana daripada penghasilan minyak kelapa sawit. Sifat-sifat POME yang kompleks seperti nilai kepekatan COD, BOD dan TSS yang tinggi, nilai pH yang rendah, dan bau yang tidak menyenangkan menyebabkan ia sukar untuk dirawat bagi memenuhi piawaian alam sekitar. POME telah dirawat menerusi sistem ini dan perubahan pH, COD dan TSS direkodkan. Kedua-dua spesis cacing iaitu *Eudrilus eugeniae* dan *Lumbricus rubellus* dalam sistem penuras vermi boleh digunakan untuk merawat POME. Sistem ini dapat menyingkir kepekatan TSS dan COD dalam julat 80%-97%. Penambahan pernuras vermikas juga dapat meningkatkan kecekapan penyingkiran TSS daripada 88% kepada 97% dan kecekapan penyingkiran COD daripada 84% kepada 95%. Kehadiran tumbuhan di dalam penurasan vermi juga diperhatikan dan daripada keputusannya, didapati tiada sebarang kesan terhadap kualiti POME yang telah dituras jika tumbuhan digunakan atau tidak digunakan. Kajian ini juga menunjukkan kehadiran cacing dapat meningkatkan kecekapan penyingkiran TSS dan COD. Kecekapan penyingkiran TSS penuras vermi adalah 95% di mana ianya lebih tinggi daripada pernuras tanpa cacing, 87%. Manakala, kecekapan penyingkiran COD penuras vermi adalah 91% dan kecekapan penyingkiran penuras tanpa cacing hanya 82%. Melalui kajian ini juga, didapati kadar beban limpah dan ketumpatan cacing yang sesuai untuk sistem

penurasan vermi yang selanjar untuk kecekapan penyingkiran yang melebihi 90% adalah masing-masingnya 940 ml/min m^2 dan 8 g/L. Idea awal ini boleh digunakan untuk membina sistem VF dalam skala yang lebih besar untuk merawat POME yang dihasilkan daripada kilang kelapa sawit.

VERMIFILTRATION OF PALM OIL MILL EFFLUENT

ABSTRACT

The performance of vermifiltration (VF) systems using the *Lumbricus rubellus* and *Eudrillus eugeniae* earthworm in treating palm oil mill effluents (POME) was studied. In this work, POME is an organic wastewater generated from the production of crude palm oil that is high in COD, BOD, TSS concentration, low in pH, oily and has unpleasant odor and is difficult to treat and to comply with the environmental standard. POME was treated through this system, after which the changes in pH, COD and TSS were recorded. In vermifiltration system, both earthworms' species, *Eudrilus eugeniae* and *Lumbricus rubellus* can be used to treat wastewater. They could remove TSS and COD concentrations in the range of 80%-97%. The addition of vermicast filter could also increase the percentage of TSS reduction from 88% to 97% and percentage of COD reduction from 84% to 95%. The presence of plant showed no effect on water quality of treated POME in vermifilter with plant and without plant. This study has showed that the presence of earthworms could aid in increasing the removal efficiency of TSS and COD. TSS reduction in vermifilter was 95% which is higher than control filter, 87%. Meanwhile, for COD reduction, vermifilterremoved 91% of COD concentration and control filter removed only 82%. Based on this study also, it was found that for vermifiltration system, the most suitable hydraulic loading rate and worm density to be used in vermifiltration of POME where the efficiency of treatment was more than 90% were at 940 mL/min m^2 and 8 g/L respectively. This preliminary idea can be

extended for further studies to scale up a vermifiltration system to treat POME produced at palm oil mills.

CHAPTER 1

Introduction

1.1 Research Background

The main concern of this study is to treat organic wastewater which comes from various sectors such as agricultural, industrial and domestic. Examples of organic wastewater are households' wastewater, palm oil mill effluent (POME), swine wastewater and dairy wastewater. Organic wastewater has been treated using a few systems such as biofilter (Rushton *et al.,* 2000), membrane filtration (Wu *et al.,* 2007) and ponding system (Wong, 1980). Biofilter system uses living materials to separate solids from liquid while in membrane filtration, it provides physical barriers that remove solids, bacteria and other small microorganisms that exist in wastewater (Rushton *et al.,* 2000). Ponding system is a series of pond comprised of anaerobic, aerobic and facultative ponds and has been used to treat POME (Wong, 1980).

POME is a large volume of dark brown water with pH between 4-4.5 and also very oily and greasy (Sivapalan and Ripin, 1997). It has unpleasant odor and high in chemical oxygen demand (COD) and biological oxygen demand (BOD). Releasing POME into the river without being well treated can cause serious water pollution. Stringent regulations have been enforced under the Environmental Quality Act of Malaysia, 1974 to control the quality of effluent discharged from the crude palm oil process. The effluent discharge from the mills must comply with the effluent discharge standard stated in the Environmental Quality (Prescribed Premises) (Crude Palm Oil) (Amendment) Regulations 1982.

There are many treatments that have been applied to treat POME such as anaerobic treatment process (Ling, 2007), treatment of POME using ultrafiltration membrane (Wu *et al.,* 2007) and treatment of POME using membrane bioreactor (Hazlan, 2006). The widely favoured treatment system by the palm oil industry in Malaysia is the ponding system. (Hassan *et al.,* 2006).

The operation processes of palm oil produce a large amount of wastes. The problems faced by palm oil industry are the requirement of large land area, cost of operation and maintenance in order to treat the waste. Long hydraulic retention times (HRT), high sludge production, low treatment efficiency, wide land area requirement, emission of green house gases (CO_2 and CH_4) in large amount are some of the drawbacks of this conventional POME treatment method (Ali Akbar, 2006).

1.2 Problem Statement

Vermifilter is said to be one of the effective and economical wastewater treatment system to treat organic polluted water such as wastewater from industrial, agricultural and household sectors. It does not require high operational cost and vast land area in comparison to the conventional wastewater treatment system (Zhao *et. al.,* 2010). The idea of including earthworms in filtration system was first advocated by Jose Toha (Zhao *et. al.,* 2010) which resulted in several studies to evaluate the performance of vermifiltration system in treating domestic wastewater treatment

(Sinha *et. al*., 2008), municipal wastewater treatment (Yang *et al*., 2008), domestic greywater (Kharwade and Khedikar, 2011) wastewater from gelatin industry (Ghatnekar *et al*., 2010), swine wastewater treatment (Li *et al.,* 2007) and pilot scale studies of sewage wastewater (Manyuchi *et al.,* 2013).

Most researchers have classified that the earthworms from epigeic group such as *Eudrilus eugeniae, Lumbricus rubellus* and *Eisenia fetida* are best suited for vermi treatment under all climatic conditions (Sinha and Bharambe, 2008). For example, Liu *et al*. (2009) used *Eisenia fetida* to treat domestic wastewater, Asha *et al.,* (2008) used *Eudrilus eugeniae* in vermicomposting and Ghatnekar *et al.,* 2010 used *Lumbricus rubellus* in treating liquid effluents from gelatin industry. Since there is no known work in relation to vermifiltration of POME, usage of two or more types of epigeic worms should be tried.

In vermifiltration system, it is important to take into account the composition of filter materials. Numerous filter medias have been used by earlier studies such as gravel, sand and soil (Sinha *et al*., 2008), wood chips, bark, peat and straw (Li *et al*., 2007) and ceramic pellets (Zhao *et al*., 2010). However, less attention has been given in the potential of vermicast as a filter material. Vermicast has high porosity, good aeration and drainage system and is a suitable substrate for living microbes (Parthasarathi, 1999). Therefore, the interaction between earthworm and microorganisms is important to increase the treatment efficiency of POME (Zhao *et al*., 2010). Thus, vermicast has been used in this study to determine the feasibility of the vermicast in improving the water quality of POME.

In previous studies, some researchers (Ghatnekar *et al*., 2010 and Morand *et al*., 2011), attempted on combining vermifilter and wetlands to treat wastewater and from their results, it showed a significant removal in the water quality of the wastewater. This system is also known as root-zone technology or reed bed system which used aquatic plant such as *Canna indica, Hymenocallis caribae and Phragmites australis* in vermifiltration system. Most studies had used these plants in their studies to treat wastewater (Ghatnekar *et al*., 2010, Wang *et al*., 2011 and Morand *et al*., 2011) however, application of wetlands in treating POME has yet to be done. Root-zone technology using *Hymenocallis caribae* (Spider Lily) has been studied to determine the potential of this plant to treat POME. Spider Lily was used because it is one of the ornamental garden plant which is commonly used in wetlands due to its aesthetically appealing (Varma, 2002).

Worm density and hydraulic loading rate may also play an important role in determining the treatment efficiency (Sinha *et al*., 2007). Thus, a study to determine suitable worm density and hydraulic loading rate with respect to vermifiltration of POME was proposed to be conducted.

Finally, although vermifiltration is quite well practiced, vermifiltration of acidic POME is not known yet and this work is probably the first of success attempt.

1.3 Objectives

The specific objectives in this research are:

1. To compare the performance of two epigeic earthworm species in ingesting and digesting organic matter using vermifiltration system.
2. To evaluate the performance of vermifiltration system with and without vermicast filter.
3. To determine the performance of vermifiltration system with and without *Hymenocallis caribaea* (Spider Lily).
4. To assess the performance of continuous vermifiltration system using a selected epigeic earthworm.
5. To measure the performance of vermifiltration system at various hydraulic loading rate (HLR) and worm density (WD).

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CHAPTER 2

Literature reviews

2.1 Filtration

Filtration is a process where solids or foreign particles are separated from liquid by filter materials. According to Rushton *et al.* (2000), during filtration, the mixture of solid-liquid is directed towards a medium where the filtrate flows through the medium and the solids sustained on the surface or within it. There are practically two types of filtration used in industries namely surface filtration and depth filtration.

Surface filters are normally used for cake filtration where particles build up in the form of cake on thin filter medium. Meanwhile, depth filters are usually used for deep bed filtration in which the building up of particles take place inside the medium and cake formation on the surface is undesirable (Svarovsky, 1981). There are a few kinds of filter materials that could be used such as cloth, asbestos, sand and gravels.

The major drawback of cake filtration is that the filtration rate decreases with time when cake resistance increase on the top surface of the filter medium during filtration (Iritani *et al.,* 2012). This cake resistance can also cause clogging in filtration. According to environmental protection agency (EPA, 1995), clogging may be defined as a buildup of head loss across the filter media until it reaches some predetermined design limit. In rapid gravity filtration, the filter media will remove the impurities and particles, thus causing the filter to clog after some period of time (EPA, 1995). If filtration alone was to apply in treating POME, it would definitely cause this system to chock very fast because POME itself is very sludgy and oily (Hassan *et al*., 2006).

2.2 Vermifiltration (VF)

Vermi is a latin word which means earthworm and filtration is the process of separating solids and liquid as mentioned previously. Vermifiltration is thus a filtration process where solids are separated from liquid in the presence of earthworms (Xing *et al*., 2010). This process is efficient and energy-saving because it could treat both sewage and sludge (Jian *et al.,* 2010). According to Kwon *et al.,* (2009), vermifiltration could process organically polluted water using earthworms. The presence of earthworms in filtration system is important as it can digest various kinds of organic materials including organic waste from earth (Jayakumar and Natarajan, 2012).

Earthworms are great decomposer as it hosts million of microbes in their gut and excrete them in soil as vermicast which contain nutrients such as phosphorus and nitrogen (Singleton *et al*., 2003). The microbes will use the nutrients content in soil for multiplication and enrichment. The number of bacteria and actinomycetes contained in the ingested material increased up to 1000 fold while passing through earthworm's gut, (Edward and Fletcher, 1988). About 15, 000 of earthworm's population will nurture and develop a microbial population to billions (Morgan and Burrows, 1982).

Vermifilters have high specific area up to 800 m^2/g of soil and the porosity is up to 60%. Earthworms process the suspended solids that are trapped on top of vermifilter and fed to the soil microbes immobilized in the vermifilter (Sinha *et al*., 2006). Complex biodegradation processes that take place in soil which inhabited by earthworms and aerobic microbes are stabilized and dissolved and suspended solids are trapped by adsorption (Sinha *et al*., 2006). The aeration by earthworms allows the soils to stabilize and filtration system to become effective (Sinha *et al*., 2007).

By granulating clay particles, earthworms increase the hydraulic conductivity and natural aeration in the soil (Sinha *et al*., 2007). They also transform the insoluble organic into soluble form which increase the total surface area and enhance the ability to adsorb the organic and inorganic matter from wastewater. Earthworms also ingest excessive harmful and ineffective microbes in wastewater selectively which prevent choking of the medium and sustain the function of a culture of effective biodegrader microbes (Sinha *et al*., 2006).

2.2.1 Vermifiltration treatment for domestic greywater

A finding by Kharwade and Khedika (2011) on domestic grey water passing through vermifilter, indicated that the performance of vermifilter based on water quality parameters was more efficient than the non-vermifilter. They carried out their study using vermifilter kit containing gravels, sand and black cotton soil on top as vermibed. *Eudrilus eugeniae* was inoculated in the vermibed. The non-vermifilter kit was a replica of vermifilter kit but devoid of earthworm. From their studies, it was found that vermifilter could remove the concentration of BOD by 85% to 93% while in non-vermifilter it was 72% to 80%. As for the COD concentration, vermifilter removed up to 74-80% whereas in non-vermifilter, COD percentage reduction was only 52-60%.

2.2.2 Vermifiltration treatment for wastewater from dairy industry

According Sinha *et al.* (2006), waste products from dairy industry consisting of large amount of white liquid with high turbidity and cannot be processed further and has to be discarded. It contained high organic matter thus high in BOD, COD and TSS. By introducing vermifiltration system, they successfully removed BOD by 98%, COD by 80-90% and TSS by 90-95%. They concluded that any non-toxic wastewater from industries can be effectively treated by earthworms and the vermifiltration technology can be designed to suit a particular wastewater (Sinha *et al.,* 2006).

2.2.3 Vermifiltration treatment for sewage wastewater

Manyuchi *et al.,* (2013) recently did a pilot scale study for vermifiltration of sewage wastewater at hydraulic loading rate, of 1000 m^3/day using *Eisenia Fetida*. The vermifilter comprised of garden soil particles, mixed sand and gravel particles. The vermifiltration system has effectively resulted in 90% of BOD, COD, TDSS and turbidity reductions as well as neutralization in pH of the sewage wastewater. The pH ranges from pH 5.6-6.3.

Another study was done by Sinha *et al.* (2008) with respect to sewage treatment by vermifiltration with synchronous treatment of sludge by earthworms, *Eisenia fetida*, *Perionyx excavatus*, *Eudrilus eugeniae*. Based on the results obtained, it showed that earthworms were able to remove 90% of BOD, 80-90% of COD and 90-95% of TSS concentration and the pH range between pH 6.5-7.5. They also concluded that vermifiltration is a logical extension of soil filtration and can be a most ecological, cost-effective and odor-free sewage treatment.

2.2.4 Vermifiltration system incorporating terrestrial plant

Researchers like Ghatnekar *et al.,* (2010) broaden up the function of vermifilter by including terrestrial plant, *Canna indica* into the system using *Lumbricus rubellus*. The working principle of the system is filtration through sand, gravel and biological degradation of wastes by aquatic plants. This system is also known as root-zone system (Headley *et al.,* 2003). *Canna indica* was planted in the upper layer of the bedding materials. After few months, the bedding material gradually was converted into humified vermicompost in which seedlings of *Canna indica* were planted.

The combination of vermiculture biotechnology or root-zone system and vermifitration system showed the potential to treat wastewater and at the same time produced vermicompost that can be used as probiotics and soils conditioners (Ghatnekar *et al*., 2010). The physical conditions of the soils can also be improved when earthworms ingest and excrete plants and soils residues and at the same time contribute to the stabilization of soil to nurture plant growth (Karaca, 2011).

Wang *et al.,* (2011) in another study used three-stages vermifiltration system integrated with *Penstemon campanulatus* plant to treat rural domestic wastewater using *Eisenia fetida*. Results showed extensive improvement in reducing COD (83.1%), ammonium (98%) and total phosphorus (98.4%). They also concluded that the presence of earthworms intensified the bacterial activity in soils.

Tomar and Suthar (2011) constructed a small-scale vermifiltration system using vertical subsurface-flow constructed wetlands (VSFCW) aided with local earthworms *Perionyx sansibaricus*. The coco grass; *Cyprus rotundus* was used in VFSCW. They claimed that vermifiltration had caused significant decrease in level of TSS (88.6%), TDS (99.8%), COD (90%), NO^{3-} (92.7%) and PO_4^{3-} (98.3%). Their results suggested that vermifiltration system is more efficient than VSFCW in terms of contamination removal efficiency.

The presence of plants in vermifiltration system or root-zone system not only enhances the efficiency of wastewater filtration but at the same time provide shelters to bacterial communities (e.g. N-fixers, ammonifying and denitrification bacteria) that are responsible for nutrient removal from wastewater (Tomar and Suthar, 2011).

In vermifiltration system, microbes are also crucial in treating wastewater and provide some extracellular enzymes to facilitate the earthworms and increase the surface area for multiplication of bacteria for rapid degradation of organic substances (Suthar, 2010). Therefore, it is best to introduce wastewater in the environment where colonies of bacteria are presence before passing through the vermibed. This can be done by including trickling system before the vermifiltration system.

2.3 Pretreatment using Trickling System

Trickling system is often used as pretreatment of wastewater before entering the vermibed. Trickling system consists of a fixed bed of sand, gravels, ceramic materials, hard coal and plastic media where sewage flows downward and forms a layer of microbiofilm or slime (Evangelho *et al.,* 2001). According to Henze *et al.,* (1995), trickling filter is very suitable with respect to adhesion to bacteria, interaction between water and biofilm and reaeration of water.

The cell forming agelatinous matrix called a biofilm develops when adsorbed microorganisms grow, reproduce and produce extra-cellular polymeric substances (Characklis and Marshall*,* 1990). These exopolymers consist mainly of a variety of heterogeneous polysaccharides depending on the type of microorganisms involved (Lazarova and Manem, 1995). Complex biofilm comprised of bacteria, fungi, protozoa, metazoa, macroinvertebrates (larvae, worms) and algae (Bruce and Hawkes, 1983).

Bacteria are the most abundant microorganisms in fixed film processes and the first to colonize the biofilm (Mack, 1975). The dominant bacteria that are usually found in fixed film treatment processes are *Zooglea, Pseudomonas, chromobacter, Achromobacter, Alcaligenes and Flavobacterium* (Lessard and Bihan, 2003). These bacteria species need idle environment to develop and multiply.

Operational parameters such as substrate, nutrients, dissolved oxygen, temperature, pH and hydraulic loading rate are crucial in bacteria growth. For example, temperature could influence the performance of fixed film processes by slowing down the microbial metabolism of microbial and affects the microbial organisms' populations (Lessard and Bihan, 2003). Under standard condition, the microbial activity doubles when temperature increases by 10°C between 5°C to 30°C (Bruce and Hawkes, 1983). As for pH, bacteria growth is favored under neutral condition (Gray, 1989).

Biofilm development is based on the capacity of different microorganisms to grow on and attach to solid surfaces. This fixed film processes happens due to various reasons such as substrate availability, protection from high velocity currents and interaction of physical forces like adsorption, attraction and adhesion (Senthilnathan and Ganczarczyk, 1990). Removal of organic matter allows bacteria to grow and makes thicker biofilm and this accumulation of bacteria can be balanced by biofilm sloughing off (Lessard and Bihan, 2003). A schematic view of biofilm is shown in Figure 2.1 (Metcalf and Eddy, 1991).

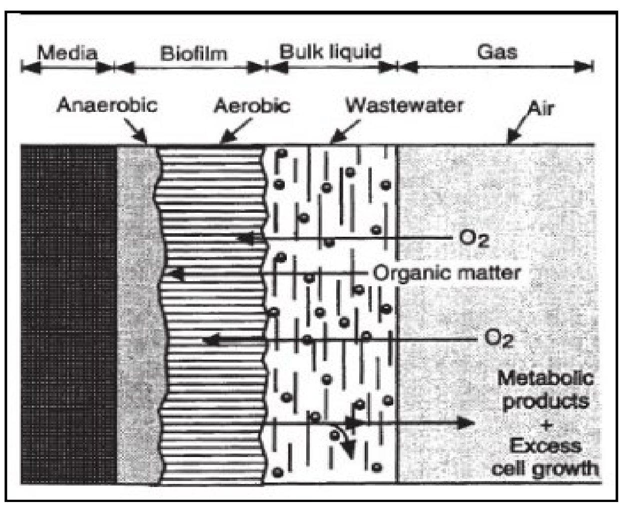

Figure 2.1 A schematic view of biofilm (Metcalf and Eddy, 1991)

The ideal solid substratum is a material that has a high surface area per unit volume, relatively cheap, high durability, and does not clog easily (Metcalf and Eddy, 1991). Based on earlier study by Sanford *et al.,* (1995), their results indicated that the finest substrate (a sand and-gravel mixture) became almost completely clogged.

The advantages of trickling filters include: low maintenance, cheap installation, and great tolerance of differences in hydraulic and organic loads (Lekang and Kleppe, 1999). Trickling system also could nurture oxygenation and remove carbon dioxide from water (Lekang and Kleppe, 1999). A study by Matthews *et al.,* (2006) on aerobic biological treatment of landfill leachate has suggested that effective microbial film had developed in trickling system using plastic media. In another study, Kornaros and Lyberatos (2006) claimed that the microorganisms developed in the trickling filter were able to remove COD levels efficiently up to 36 000 mg/L, under aerobic conditions at pH values between 5.5 and 8.0.

2.4 Hydraulic Loading Rate in Wastewater Treatment System

In wastewater treatment system, hydraulic loading rate (HLR) is an important factor influencing performance of the system (Li *et al.,* 2011). HLR is the volume of sewage wastewater applied per unit area of the soil bed per unit time (Sinha *et al*., 2007). The formula to calculate HLR is shown in Equation 2.1.

$$\text{Hydraulic loading rate (HLR)} \; (\text{mL/m}^2\ \text{min}) = \frac{\text{Volume (mL)}}{\text{Area (m}^2\text{) time (min)}} \qquad (2.1)$$

Low hydraulic loading rate will prolong the hydraulic retention time (HRT) in vermifilter and this will increase the treatment efficiency of vermifilter (Sinha *et* *al.,* 2008). HRT is the time taken by wastewater to percolate through the vermibed in which the earthworms are inhabiting (Sinha *et al.,* 2008). For optimum removal of organic pollutants, the organic wastewater must remain in the vermibed for a certain period of time to allow interaction between wastewater and earthworms (Azuar and Ibrahim*,* 2012). During this period, nutrients are removed from the activity of earthworms which involve physical and biochemical processes, thereby reducing the BOD, COD, TSS and turbidity of wastewater (Manyuchi *et al.,* 2013).

A finding by Xing *et al.,* (2010) showed that hydraulic loadings rate could affect the removal rates of COD, $BOD_{5,}$ SS, TN and NH_{4-} and higher hydraulic loading indicated more processing capacity of waste water. According to Li *et al.,* (2011), relatively high loading rates could not maintain stable running for the long term and relatively low loading rates would require much soil. Based from their results, it shows that when the average hydraulic loading of 3.3 mL/min m^2 was increased to 9.3 mL/min m^2, the soil became clogged due to overfeeding and penetrability decreased.

Most wastewater treatment systems are constructed to treat domestic sewage, and suitable hydraulic loading rates are recommended to ensure long-term performance with no clogging (Zhou, 2007). If loading is too high, these types of wastewater treatment systems tend to clog (Zhou, 2007).

2.5 Earthworms

2.5.1 Earthworms species and their characteristics

Earthworms can be classified into three types of groups which are epigeic, endogeic and anecic by color and size. Epigeics (epi=top, geic=earth) are the species being reddish brown in co or and live on the surface of the soil with body length about 1-7 cm (as adults) (WSAC, 2013). This species lives in trash layer and consumes primarily on fungi and bacteria present in the trash. The examples of epigeic earthworms are *Eisenia fetida*, *Lumbricus rubellus, Eudrillus eugeniae and Dendrobaena octaedra*.

Soil dwelling species is called endogeics (endo= internal, geic= earth) inhibit mineral soil horizons feeding on soil can range from 2-12 cm (Gajalakshmi and Abbasi, 2004). This species lacks skin pigmentation therefore they may look pale and yellowish or whitish in color (Gajalakshmi and Abbasi, 2004). They rarely appear at the surface instead live in the burrow system and feed on decayed organic matter (Willie and Martina, 2009).

Several endogeic earthworms have been identified which are *Aporrectodea caliginosa* and *Octolasion tyrtaeum* (Garg and Gupta*,* 2011). Anecic earthworm is known as deep burrowing species living in burrows (about 2 m) but come to the surface to feed on dead leaves and fresh surface litter (GLWW, 2013). This earthworm species has a very large body length (8-15 cm) and reddish brown in color.

Anecic earthworm has the ability to consume large amount of wastes and build permanent burrows from the soil surface down through mineral soil layer. An example of anecic species is *Lumbricus terrestris*.

The earthworm used in the vermifiltration process is mostly from the epigeic group such as *Eisenia fetida, Lumbricus rubellus*, *Dendrobaena octaedra* and *Eudrillus eugeniae* which are from the phylum of Annelida and subclass of oligochaeta (Garg and Gupta, 2011). The examples of epigeic earthworms are shown in Figure 2.2. Earthworms are long, narrow, cylindrical, bilaterally symmetrical, segmented animals without bones measuring few centimeters (Am-Euras, 2009). The length of *Lumbricus rubellus*, or the "Red Earthworm", ranges from 1 to 4 inches (25-105 mm) and has smooth, semi-transparent, reddish, flexible skin segmented into circular sections. The total number of segments per matured organism ranges from 95-105 and each segment contains four pairs of setae (Edwards and Lofty, 1972).

The segmentation of *Lumbricus rubellus* identifies the organism as a member of Phylum Annelida, while the enlarged segments towards the anterior of the organism called the clitellum classified it as membership to Class Clitellata (Edwards and Lofty, 1972). Members of this class also have permanent gonads (Nancarrow and Taylor, 1998).


Eisenia fetida *Eudrillus eugeniae*


Lumbricus rubellus *Dendrobaena octaedra*

Figure 2.2 Examples of epigeic Earthworms species

(Storey, 2010)

2.5.2 Earthworms Habitat

Earthworm naturally lives in soils which have high in organic matter, preferably dung and feces (Edwards and Lofty, 1972). The worms require loose and moist soil to burrow in and for gas exchange (Wallwork, 1983). Further requirements include abiotic factors such as pH and temperature. Abiotic factors such as light and temperature are significant to *Lumbricus rubellus* and *Eudrillus eugeniae*. Reynolds (1998) noted the importance of pH and the range of 5.5 to 8.7 is acceptable with a preference for neutral solids.

Edwards and Lofty, (1972) also noted that temperature is also an important factor, with implications for growth, reproduction, metabolism and respiration amongst other things. They noted an ideal temperature of 25-30°C. Another abiotic factor is moisture which is important for their respiration.

A similar species, *Millsonia anomala*, is most active at 10-17% moisture content. The substratum for *Lumbricus rubellus* is related to the species food sources, pH and moisture requirements. With regards to light intensity, Edwards and Lofty, (1972) noted that most earthworm species are photonegative to strong sources of light and photopositive to weak sources of light. This is attributable to the effect of intense light, such as drying and insufficient of food sources found above ground for earthworms.

2.5.3 The Behavior of Earthworms

Earthworms' sense organs (chemoreceptors) associated with feeding are on the prostomium, located at the anterior end of the organism (Edwards and Lofty, 1972). The chemoreceptors are sensitive to alkaloids, polyphenols and acids. Negative responses are caused by acid and alkaloids (at certain levels), while polyphenol sensitivity identifies different food sources.

Chemoreceptors, as noted by Edwards and Lofty, (1972), can also be found on other parts of the organisms' body. The function is to direct the organism away from dangers such as pH variations or temperature and direct the organism towards available food sources. Earthworms ingest wastes and digest them into a fine material called vermicompost and produce more soluble and available nutrients to plants such as nitrogen, calcium, potassium and phosphorus (Coulibaly and Zoro, 2010).

2.5.4 Reproduction of Earthworms

According to the Appelhof (1982), earthworms can be considered as an enviable creature on earth. They meet, eat, procreate, sleep, and excrete. Red worms are sexually mature at eight to 10 weeks. They mate throughout the year at any time, in their bedding at different levels and even on the lid of their worm bin, if that is where they live. A red worm is sexually mature and able to reproduce when it has formed a thick band, or clitellum around its body about one-third of the way between its mouth and anus at around three months of age.

Worms are hermaphrodites and contain both male and female sexual organs. Two worms will nestle against one another in opposite directions so that their clitella are touching (Gajalakshmi and Abbasi, 2004). Each worm will secrete mucus forming a band around both worms at the clitella. Sperm from each worm move down a groove into receiving pouches of the other worm. The sperm enters a storage sac. The worms slide apart, withdrawing backwards through the band and continue on their separate ways (Appelhof, 1982). After the worms have separated, their clitella secrete another substance called albumin. This material forms a cocoon in which the eggs are fertilized and baby worms hatch.

Redworm cocoons, which are round and small, change color during their development (Edwards and Lofty, 1972). First they are white, become yellow, later brown and gradually enlarge. When the new worms are ready to emerge, the cocoons are turning red and are about the size of a grape seed (Edwards and Lofty, 1972). Worm development within the cocoon takes at least three weeks. Temperature and other conditions are factors in the development of the hatchlings.

A cocoon might hold as many as 10 eggs, but usually only three or four worms will emerge. The young hatchlings are whitish with a pink tinge showing their blood vessels. Redworms can lay 2 cocoons a week for six months to a year (Dominguez *et al.,* 2001).

According to Dominguez *et al.* (2001), the greatest number of cocoons per week and the number of hatchlings per cocoons were obtained at 25°C and cocoons of *Eudrillus eugeniae* hatched in only 12 days at 25°C. This species, popularly known as Night Crawler, is the second most widely used earthworm for vermicomposting (Garg and Gupta, 2011). It grows faster than other species accumulating mass at the rate of 12 mg/day.

2.5.5 Vermicasts from earthworms

Earthworm's feces or their "poop" is known as castings. In vermicomposting process, their castings are called vermicast. Vermicast contain nutrients that help improve the soil's condition. Vermicast also absorb water making it available for the plant when needed. Some species cast within their burrows and others on the surface of the soil (Gajalakshmi and Abbasi, 2004). Vermicast have the potential to enhance the fertility of soil and could be suitable substrate for living microbes (Parthasarathi and Ranganathan, 1999). It is formed from the ingesting and egesting process of organic materials by earthworms and transforms them as a peat-like material called vemicompost (Coulibaly and Zoro, 2010).

Vermicompost has been established as a good fertilizer to plants as it is rich in micro/macronutrients, inorganic minerals, organic matter, enzymes and microbes (Parthasurathi and Ranganathan, 1999). Vermicast is much more fragmented, porous and microbially active (Edwards and Bohlen, 1996). The stabilization of the wormcasts depends on their age, organic matter and microbial activity (Parthasarathi and Ranganathan, 1999). Worm casts are enriched with available form of carbon, phosphorus and nitrogen (Aira *et al.,* 2007).

Previous studies have found that the vermicasts was dominated by members of the phylum *Proteobacteria* and *Pseudomonas* sp. (Zhao *et al.,* 2010). It could also accelerate the growth of bacteria, fungi, algae and protozoans (Suthar and Singh, 2008). According to Gajalakshmi and Abbasi (2004), casts contain enzymes such as protease, amylase, lipase, cellulose and chitinase which could degrade organic matter even after casts have been excreted. This is the reason of higher bacteria diversity in filters containing earthworms in comparison to the one without earthworms. Microbial activity also has been reported to be greatly stimulated by vermicast (Aira *et al.,* 2002).

2.5.6 Role of Earthworms in Vermifiltration

The most important thing in vermifiltration system is the presence of earthworms. Charles Darwin has described earthworms as great benefactors of soils and agriculture and Aristotle called worms the “intestines of the earth while other scientists have defined it as unheralded soldiers of mankind (Am-Euras, 2009). Recently, the potential of earthworms in recycling nutrients, degrading solid wastes and treating organic wastewater have been discovered and more efforts have been done to apply these systems at commercial scale (Garg and Gupta, 2011).

2.5.6.1 Improve the physical structure of soil

Earthworms have the ability to break down organic matter and act on soil structure by creating burrows and producing casts (Yasemin and Remzi, 2011). Earthworms move in the soil and create tunnels by pushing soils and and granulate clay particles to create natural aeration (Yasemin and Remzi, 2011). This aeration helps the soil drain water instead of running off and avoid blockage of wastewater. When the soil is very compact and dense, they literally eat their way through (Gajalakshmi and Abbasi, 2004).

2.5.6.2 Break down organic matter and act on soil structure

Vermifiltration technology employs earthworms and effectively process organically polluted water. According to Dominguez (2004), earthworm played a critical role in degrading wastes and earthworm could improve activity of microorganism and stabilization of organic matter. In vermifiltration systems, earthworms help stimulate and accelerate microbial activity by increasing the population of microorganisms in the soil besides improving aeration by their burrowing actions (Binet *et al.,* 1998). Therefore, microbial processes and vermiprocesses are two distinct yet relevant features to vermifiltration. Earthworms are the idlest creature to treat wastes because they consume all organic wastes as their food and at the same time treat the wastes (Gajalakshmi and Abbasi, 2004).

2.5.6.3 Castings enrich with nutrients which contribute to soil fertility

Earthworms act as soil conditioner by improving its physical, chemical, biological properties and also allow plants to grow healthily by providing nutrients through soil fragmentation and aeration, degradation of organic matter and secretion of plant growth hormones (Am-Euras, 2009). Earthworms ingest decayed organic matter that passes through earthworm's gut and deposit them as casts which are rich in nutrients.

2.6 Palm Oil

The oil palm tree (*Elaeis Guineensis Jacq*.) originated from West Africa in a belt from Angola to Senegal (Sumathi *et al*., 2008). It was introduced to Malaysia at the start of the 20th century and commercially produced in 1917 (Hazlan, 2006). The oil palm has a lifespan of over 200 years, while the economic life is about 20-25 years (Parveen *et al.,* 2010). The nursery period is 11-15 months for plants and first harvest is done after 32-38 months after planting (Hazlan, 2006). It takes 5-10 years for palm oil plant to reach maximum yield (Sumathi *et al*., 2008).

Today, palm oil is one of the 17 major oils traded in the global edible oils & fats market and can be found in one out of every ten food products worldwide (Sime Darby, 2009). Palm oil has a bright future as it is not only used in food products (vegetable oil, margarine, shortening, and milk fat replacer and cocoa butter substitute) but also for non-food applications (detergent, soap, cosmetics, biodiesel). Table 2.1 shows the palm oil value chain and applications.