UTILIZATION OF VERMICOMPOSTING DERIVED LIQUIDS AS NUTRIENT SOLUTION IN SOIL-LESS CULTURE

by

QUAIK SHLRENE

**Thesis submitted in fulfillment of the requirements
For the degree of
Master of Science**

JULY 2014

ACKNOWLEDGEMENTS

I would like to express my appreciation to my supervisor Associate Professor Dr. Mahamad Hakimi Ibrahim for the support, patience and understanding in the course of completion of this project. My sincere gratitude goes to my beloved mother, Madam Lau Siew Hong whose affection and undivided support encouraged me in completing this project.

A special appreciation goes to Prof. Sultan Ahmed Ismail (Ecoscience Research Foundation) for the encouragement, guidance and support.

In addition, I would like to thank all academic staff and non-academic staff from Environmental Technology Department who have assisted me throughout the project. My gratitude goes to USM Graduate Assistant Scheme (GA) for the financial support provided.

Special thanks to all my friends especially Asha Embrandiri, Suguna Migeemanathan, and Chuah Li Oon for their moral support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	Certification of earthworm species	109
B	Experimental set up of utilizing VW and VL	110

PENGGUNAAN CECAIR DIPEROLEHI DARIPADA PENGKOMPOSAN VERMI SEBAGAI LARUTAN NUTRIEN DALAM KULTUR TANPA TANAH

ABSTRAK

Tinja lembu terkompos digunakan sebagai substrat dalam penyediaan cecair-cecair pengkomposan vermi. Komposisi cecair vermi sering bergantung kepada substrak yang digunakan dalam penyediaan vermikompos. Cecair yang diperolehi daripada pengkomposan vermi ("vermiwash" (VW) dan "vermicomposting leachate" (VL)) mengandungi jumlah nutrien tumbuhan yang tinggi. Cecair ini bukan sahaja sesuai sebagai suplimen nutrien untuk menggalakkan pertumbuhan tumbuh-tumbuhan, malah berpotensi sebagai larutan nutrien dalam kultur tanpa tanah. Objektif utama kajian ini ialah untuk menentukan kesan VW dan VL terhadap pertumbuhan dalam kultur tanpa tanah. Analisis yang telah dijalankan mengesahkan kewujudan nutrien (P, Ca, Mg, Na, Fe dan K) dalam tinja lembu pada jumlah yang tinggi (13.4 ± 0.08 mg g^{-1} Total P, 42.92 ± 0.02 mg g^{-1} Ca, 4.24 ± 0.03 mg g^{-1} Mg, 18.9 ± 0.12 mg g^{-1} Na, 6.034 ± 0.08 mg g^{-1} Fe dan 99.96 ± 1.76 mg g^{-1} K). Kehadiran unsur logam surih (0.10 ± 0.02 mg g^{-1}) Pb juga dikesan. Analisis kimia mengesahkan kewujudan 0.032 ± 0.01 % N, 4.36 ± 0.00 mg L^{-1} jumlah P, 215.33 ± 5.37 mg L^{-1} K, 107.93 ± 2.27 mg L^{-1} Na, 172.87 ± 6.43 mg L^{-1} Ca, 0.188 ± 0.03 mg L^{-1} Mg, 0.025 ± 0.00 mg L^{-1} Cu and 0.114 ± 0.01 mg L^{-1} Zn dalam VW, dan 0.013 ± 0.01 % N, 3.53 ± 0.00 mg L^{-1} jumlah P, 0.20 ± 7.01 mg L^{-1} K, 111.73 ± 3.02 mg L^{-1} Na, 167.63 ± 1.95 mg L^{-1} Ca, 0.217 ± 0.00 mg L^{-1} Mg, 0.030 ± 0.00 mg L^{-1} Cu and 0.248 ± 0.01 mg L^{-1} Zn dalam VL. Penggunaan VW dan VL dengan pencairan (10, 20 dan 30%) telah menunjukkan kesan terhadap ukuran panjang pucuk, berat akar, berat pucuk dan jumlah biomas labu bawang (*Allium cepa*). Walau bagaimanapun, VW10 dan VL10 (pencairan 10%) mempamerkan keunggulan, terutama dalam kepanjangan akar (12.49 ± 1.28 cm untuk VW10, 12.14 ± 0.64 cm untuk VL10) dan pucuk (43.10 ± 2.31 cm untuk VW10 dan 41.30 ± 1.73 cm untuk VL10) labu bawang berbanding dengan rawatan lain (20% dan 30%). Keputusan bagi *Coleus aromaticus* yang tumbuh dalam VW10 dan VL10 menunjukkan pertumbuhan yang ketara, peningkatan biomass, keluasan daun dan kandungan klorofil. Kandungan Mg dan Ca pada daun *Coleus aromaticus* adalah lebih tinggi dalam rawatan VW dan VL.

UTILIZATION OF VERMICOMPOSTING DERIVED LIQUIDS AS NUTRIENT SOLUTION IN SOIL-LESS CULTURE

ABSTRACT

Pre-composted cow dung was used as the substrates in preparing vermicomposting derived liquids. Composition of vermicomposting derived liquids are often depends on the substrate used during the preparation. Vermicomposting derived liquids (vermiwash (VW) and vermicomposting leachate (VL)) contain high amount of plant nutrients. Not only these liquids are suitable to be used as nutrient supplements for plants for growth promoting, they also have the potential to be used as nutrient solution in soil-less culture. The main objective in this study is to determine the effects of VW and VL towards plant growth in soil-less culture. Analyses had confirmed the presence of high amount of nutrients (P, Ca, Mg, Na, Fe and K) in cow dung (13.4 ± 0.08 mg g^{-1} Total P, 42.92 ± 0.02 mg g^{-1} Ca, 4.24 ± 0.03 mg g^{-1} Mg, 18.9 ± 0.12 mg g^{-1} Na, 6.034 ± 0.08 mg g^{-1} Fe and 99.96 ± 1.76 mg g^{-1} K). The presence of trace elements (0.10 ± 0.02 mg g^{-1} Pb) was also detected. Chemical analyses confirmed the presence of 0.032 ± 0.01 % N, 4.36 ± 0.00 mg L^{-1} total P, 215.33 ± 5.37 mg L^{-1} K, 107.93 ± 2.27 mg L^{-1} Na, 172.87 ± 6.43 mg L^{-1} Ca, 0.188 ± 0.03 mg L^{-1} Mg, 0.025 ± 0.00 mg L^{-1} Cu and 0.114 ± 0.01 mg L^{-1} Zn in VW and 0.013 ± 0.01 % N, 3.53 ± 0.00 mg L^{-1} total P, 0.20 ± 7.01 mg L^{-1} K, 111.73 ± 3.02 mg L^{-1} Na, 167.63 ± 1.95 mg L^{-1} Ca, 0.217 ± 0.00 mg L^{-1} Mg, 0.030 ± 0.00 mg L^{-1} Cu and 0.248 ± 0.01 mg L^{-1} Zn in VL. Utilization of VW and VL with dilution (10, 20 and 30%) showed effect towards shoot length, root weight, shoot weight and total biomass of onion bulb (*Allium cepa*). However, VW10 and VL10 (10% dilution) had exhibited superiority especially in root length (12.49 ± 1.28 cm for VW10, 12.14 ±

0.64 cm for VL10) and shoot length (43.10 ± 2.31 cm in VW10 and 41.30 ± 1.73 cm in VL10) in onion bulbs compared to other treatments (20% and 30%). Results of *Coleus aromaticus* grown in VW10 and VL10 had showed distinctive improvement in terms of growth, biomass, leaf area and chlorophyll content. Concentrations of Mg and Ca in leaf of *Coleus aromaticus* were higher in VW and VL treatments.

CHAPTER 1

INTRODUCTION

1.1 Research background

With the rise of population that leads to increasing crop demand, crop production comes under the spotlight. Fertilizer has been used to increase productivity of crop in order to fulfill the rising demand. Asia region has turned into the largest consumer of fertilizer in the world, making up to be 61% of total world fertilizer consumption (FAO, 2010). Hydroponics culture is a type of soil-less plant cultivation methods. Nutrients needed for plant growth are present in the dissolved form in the hydroponic nutrient solution. Despite the demand in quantity, quality of the crop produced is also one of the major concerns. Production cost of fertilizer used in order to increase crop yield and production will directly affect the price of the crop in the market.

Long term usage of inorganic fertilizer on crop raises concern on health as well as the environmental safety. As an alternative for procuring environmental friendly fertilizer, vermicomposting technique is introduced. Earthworms' feeding behavior allows the wastes to be stabilized through degradation and decomposition. With the help of earthworms activities, microbial population as well as activities are increased. Hence, the time needed for degrading the organic matter in waste used in vermicomposting technology is reduced. Vermicomposting has been used as an environmental friendly technology in waste management for various wastes.

Spain is a major limiting factor for crop production and water resources are limited due to rapid development (Magán *et al.*, 2008). Separate studies on soil-less culture and vermicomposting derived liquids have been carried out. Considering these factors, soil-less culture may offer solution to these problems. Comparison works on vermicomposting derived liquids with commercial hydroponics solution in soil-less culture are limited.

1.3 Objectives of the study

The main objective of this study is to investigate the potential of vermicomposting derived liquids in soil-less culture.

The specific objectives of this study are

a) To determine and compare the physico-chemical properties of vermicomposting derived liquids (vermiwash and vermicomposting leachate)

b) To evaluate the effects of vermiwash, vermicomposting leachate and a commercial hydroponics solution on the growth of selected plant in soil-less culture.

1.4 Scope of study

Focus of this study lies on the utilization of vermicomposting derived liquids (vermiwash and vermicomposting leachate) in soil-less culture on selected test plants especially towards growth promotion and their potential in other agricultural use.

CHAPTER 2

LITERATURE REVIEW

2.0 Animal waste and agricultural waste management

Animal waste and other agricultural waste generated in Malaysia each year is drastically increasing. Improper disposal of animal wastes has been reported to contribute towards the pollution of groundwater due to its bacteria and nitrates content (Gay *et al.* 2003). Singh and Suthar (2012) reported that recycling, reuse and resource recovery as great options for sustainable solid waste management. Most of the animal wastes cannot be used directly without any treatment. Direct disposal may cause environmental contamination problems especially in large amount. Therefore is has become an environmental issue. Meanwhile, Cambardella *et al.* (2003) has stated animal wastes can be useful as reliable organic fertilizers.

During vermicomposting, microbes degrade the organic matter while earthworm conditioned the substrates and therefore enhance the microbiological activity (Dominguez and Edwards, 2004). Studies were carried out on vermicomposting derived liquids such as vermiwash (Ismail, 2005; Tharmaraj *et al.*, 2011), vermicomposting leachate (Garcia-Gomez *et al.*, 2008; Gutierrez-Miceli *et al.*, 2008; Leon-Anzueto *et al.*, 2011) and vermicompost aqueous extract (Pant *et al.*, 2009). Findings from these studies indicated the presence of valuable plant nutrients and their positive impact towards plant growth.

For overcoming soil-borne disease and other soil problems for intensive cultivation in greenhouses and outdoors, soil-less culture was developed (Lal, 2006). The first proposal of commercial water culture was made by Gericke (1929). Gericke (1937) coined the term "hydroponics" to describe the growing of crops with their roots in a liquid medium.

2.1 Vermicomposting

Waste composting process that is helped or stabilized with presence of earthworms is known as vermicomposting. Application of vermicomposting in recycling organic waste has gained acceptance over the past two decades. (Reynolds, 2004) Earthworms help to increase the decomposition rate of surface litter (Tian *et al.* 1997). Important plant nutrients from animal wastes became more soluble and available to plants after undergoing vermicomposting (Ndegwa and Thompson, 2001). Vervicomposting has been recommended as appropriate alternative to aerobic composting as it posses advantages in relation to process duration, recovery of nutrients, microbial richness and phytotoxicity (Singh and Suthar, 2012). Wastes that have used in vermicomposting technology for stabilization as well as production of valuable by product are numerous. Table 2.1 summarized the studies carried out using various wastes in vermicomposting.

Table 2.1 Examples of studies carried out utilizing various wastes in vermicomposting.

Waste	Earthworm species	Reference
Solid textile sludge and cow dung	*Eisenia fetida*	Kaushik and Garg 2003
Mango (*Mangifera indica*) leaf litter	*Eudrilus eugeniae*	Gajalakshmi *et al.*, 2004
Agricultural waste, farm yard manure and urban solid waste	*Perionys sansibaricus*	Suthar, 2007
Coffee grounds and kitchen waste	*Lumbricus rubellus*	Adi and Noor, 2009
Buffalo manure	*Eisenia andrei*	Ngo *et al.*, 2010
Yard leaf manure	-	Kalantari *et al.*, 2011
Tannery sludge with cattle dung	*Eisenia fetida*	Vig *et al.*, 2011
Cow dung, poultry droppings and food industry sludge	*Eisenia fetida*	Yadav and Garg, 2011
Distillation waste of java citronella (*Cymbopogon winteri- anus Jowitt.*)	*Eudrilus eugeniae*	Deka *et al.*, 2012
Pharmaceutical industry waste	*Eisenia fetida*	Singh and Suthar, 2012
Milk processing industry sludge (MPIS)	*Eisenia fetida*	Suthar *et al.*, 2012
Sewage sludge and vinasse bio-wastes with rabbit manure	*Eisenia fetida*	Molina *et al.*, 2013

2.2 Earthworm

Earthworms are under kingdom of Animalia, phylum of Annelida. They have segmented, bilaterally symmetrical body. Earthworms are hermaphrodite and produce cocoon through its clitellum. Newly hatched earthworms are unpigmented and only few millimeters in length (Domínguez and Edwards, 2011). Most of the common earthworms belong to family of Lumbricidae, especially in Europe, North America, western Asia and other parts of the world. Whereas, for earthworms of family Eudrilidae, they are commonly found in West Africa (Reynolds and Wetzel, 2004).

Earthworms are categorized into three categories ecologically by Bouché (1977). The three categories are epigeic, anecic and endogeic. Epigeic species are phytofagous, they feed on litter on soil surface and are non-burrowing in nature. Epigeic are usually heavily pigmented. Intermediate species may burrow on top few centimeters of soil. Anecic species are geophytophagous. They feed on litter on soil surface and some is pulled into burrows. They construct vertical burrows and anecic species such as *L. terrestris* was observed to deposit their coccons in burrows. They are medium to heavy pigmented, and usually only dorsally. For endogeic species, they are geophagous. Their food is mainly mineral soil which is rich in organic matter. They construct sub-horizontal burrows and are unpigmented or lightly pigmented.

2.2.1 Earthworm in vermicomposting

Research works that utilize epigeic earthworms in waste management are well documented. They present high rate of consumption, digestion and assimilation of organic matter. They are able to tolerate wide range of environmental factors and have high reproduction rate. Species that have been extensively used in vermicomposting are *Eisenia andrei* (Savigny), *Eisenia fetida* (Bouché), *Dendrobaena veneta* (Savigny), *Perionyx excavatus* (Perrier), and *Eudrilus eugeniae* (Kinberg) (Domínguez and Edwards, 2011).

2.2.1.1 *Eudrilus eugeniae*

Eudrilus eugeniae, or commonly known as African night crawler is one of the main earthworm species indentified as potentially useful in breaking down organic wastes (Edwards and Arancon, 2004). This species is native to Africa but can be commonly found in many other countries. It has rapid growth and quite prolific. Eudrilus eugeniae is capable of processing large quantity of organic wastes rapidly (Edwards 1988; Neuhauser *et al.* 1988; Kale and Bano, 1991). *Eudrilus eugeniae* has preference to higher temperatures and cannot tolerate long exposure of temperature below 16°C. Therefore, it is suitable to be used in tropical and subtropical countries for outdoor vermicomposting. It is extensively used in tropical countries especially India. Kale and Bano (1991) reported that under laboratory conditions, the carrying capacity of earthworm *E. eugeniae* on organic wastes is 0.015g/cc. *Eudrilus eugeniae* was reported to have more rapidly total biomass increment than *E. fetida*, a species that grows relatively well in most organic wastes and is also widely used in vermicomposting. (Dominguez *et al.*, 2001).

Eudrilus eugeniae is epigeic worm as classified by Bouche (1977) based on their feeding and burrowing strategies. They live in organic horizon in or near surface of litter. They ingest large amount of undercomposed litter. Ephemeral burrows are produced into the mineral soil. Most of their activities are at the upper few centimeters of the soil litter surface.

2.2.1.2 *Eisenia fetida*

Eisenia fetida is the most commonly used species in vermicomposting and vermiculture. It is also known as red worm or red wriggler. It has short life cycles and can tolerate wide range of temperature and moisture (Dominguez and Edwards, 2011). Certain aspects of *Eisenia fetida* and *Eudrilus eugeniae*, the common species used in vermicomposting are shown in Table 2.2.

Table 2.2 Comparison of certain aspects of *Eisenia fetida* and *Eudrilus eugeniae*.

	Eisenia fetida	*Eudrilus eugeniae*
Colour	Brown and buff bands	Reddish brown
Mean weight of adults (g)	0.55	2.7-3.5
Time to maturity (days)	28-30	40-49
Life cycle (days)	45-51	50-70
Optimal temperature	25°C	25°C
Optimal moisture level	80%-85%	80%

Modified from (Dominguez and Edwards, 2011)

2.3 Cow dung

Coulibaly and Zoro Bi (2010) reported pH of cow waste is alkaline. Physico-chemical characteristics of cow dung are stated in Table 2.3 below.

Table 2.3 Physico-chemical characteristics of cow dung

Parameters	Cow dung
Moisture (%)	51.3 ± 4.13
pH	8.37 ± 0.06
Total Organic Carbon (TOC) (%)	47.19 ± 0.01
Total Nitrogen (TN) (%)	0.51 ± 0.03
Carbon: Nitrogen (%)	92.64 ± 4.63
Total Available Phosphorus (TAP) (%)	0.28 ± 0.03
Total Potassium (TP) (%)	0.71 ± 0.01

Source: Coulibaly and Zoro Bi (2010)

Nutrients such as nitrogen, potassium phosphorus and calcium are important for plant growth. Ndegwa and Thompson (2001) reported that these nutrients may be more available to plants after vermicomposting.

Pre-composting of animal manures is vital for avoiding mortality of earthworms. Gunadi and Edwards (2003) reported 100% death of *E. fetida* in fresh cattle wastes after 2 weeks. Hence, pre-composting period of 2 weeks is suggested in order to eliminate volatile toxic gases (Garg *et al.*, 2005).

Coulibaly and Zoro Bi (2010) claimed that cow manure is one of the most favourable wastes for growth and reproduction of *E. eugeniae*. Hence, it is recommended to be used as feed materials in vermicomposting process.

2.4 Vermicomposting derived liquids

Vermiwash (Ismail, 1997), vermicomposting leachate (worm-bed leachate) (Gutiérrez-Miceli *et al.*, 2008), vermicompost aqueous extracts (Pant *et al*, 2009), vermicompost extracts (vermicompost tea) (Edwards *et al.*, 2011) are some of the liquid byproducts commonly found in the literature. Each may be prepared in different ways.

2.4.1 Vermiwash

In vermiwash production, as introduced by Ismail (1997) cow dung was used as the substrate for vermicomposting.

Water percolated through the column of worm action will collect excretory products and mucus secretions of earthworms, as well as the micronutrients from the soil organic molecules (Ismail, 1997). This produces the liquid (vermiwash) that washed away the valuable plant nutrients and microorganism present in drilosphere. Lavelle (1988) termed the affected soil volume due to earthworm activities as drilosphere. The actual benefit of drilosphere towards plants are strongly depends on the ability of extracting nutrients present in drilosphere and soil solution (Brown *et al.*, 2004).

Physico-chemical characteristics of vermiwash reported by Ismail (2005) indicated the presense of valueble nutrients. Table 2.4 shows the physico-chemical characteristics of vermiwash by Ismail (2005). Table 2.5 shows examples of studies carried out utilizing vermiwash.

Table 2.4 Analysis report of chemical characteristics of vermiwash.

Parameters	Vermiwash
pH	7.48 ± 0.03
Electroconductivity (dS m^{-1})	0.25 ± 0.03
Organic Carbon (%)	0.008 ± 0.001
Total Kjeldhal Nitrogen (%)	0.01 ± 0.005
Available phosphate (%)	1.69 ± 0.05
Potassium (ppm)	25 ± 2
Sodium (ppm)	8 ± 1
Calcium (ppm)	3 ± 1
Copper (ppm)	0.01 ± 0.001
Ferrous (ppm)	0.06 ± 0.001
Magnesium (ppm)	158.44 ± 23.42
Managanese (ppm)	0.58 ± 0.040
Zinc (ppm)	0.02 ± 0.001

Modified from Ismail (2005).

Table 2.5 Example of studies carried out on vermiwash.

Waste	Earthworm species	Plant tested	Reference
Cow dung and leaf litter	*Lampito mauritii* *Eisenia fetida* *Eudrilus eugeniae*	Rice	Tharmaraj *et al*, 2011
Coconut leaf litter and cow dung	*Eudrilus sp.*	Cowpea, Maize and Okra	Gopal *et al* , 2010
Animal and kitchen waste	*Eisenia fetida*	Okra (*Abelmoschus esculantus*), lobia (*Vigna unguiculata*) and radish (*Raphnus sativus*)	Nath and Singh, 2009
-	*Eisenia fetida*	*Cyamopsis tertagonoloba* and *Trigonella foenum-graecum*	Suthar, 2010

2.4.1.1 Drilosphere

Lavelle (1998) termed drilosphere as the earthworm activity affected soil volume and Brown *et al.* (2000) defined it as the environment made up of microenvironment in earthworm gut, surface that earthworm in contact with soil, surface and below ground of the vermicasts, middens, burrows and diapause chambers. Earthworms prefer to consume a mixture of soil and organic matter over pure organic matter. They feed selectively and Judas (1992) confirmed it by observing its gut content. Earthworms' ability in digesting organic matter and assimilating nutrients from ingested organic matter varies in species and ecological categories (Lattaud *et al.*, 1998, 1999).

Drilosphere is a dynamic zone of action. It changes constantly in both space and time as earthworm ingest and reingest soil, burrow and cast at different rates and in different locations in the soil (Lavelle, 1997). Microorganisms increases nutrients mineralization rates and releases more plant available N and P in the drilosphere (Edwards and Bohlen 1996; Barois *et al.*, 1999). The cast and burrow are lined with earthworm mucus which is protein and carbon rich (Scheu, 1991). Urine and mucus secreted by earthworm contain nitrogenous compounds and may be source of labile N for soil microorganisms. Earthworm urine is composed of ammonium and urea and mucus is composed of mucoprotein (Scheu, 1991). Although nitrogen is secreted in form of urine and mucus into drilosphere, high amount of N is also returned into soil in form of dead earthworm tissues which are highly labile. Over 70% of the N in dead earthworm tissue was mineralized and returned to the soil (Satchell,1967)

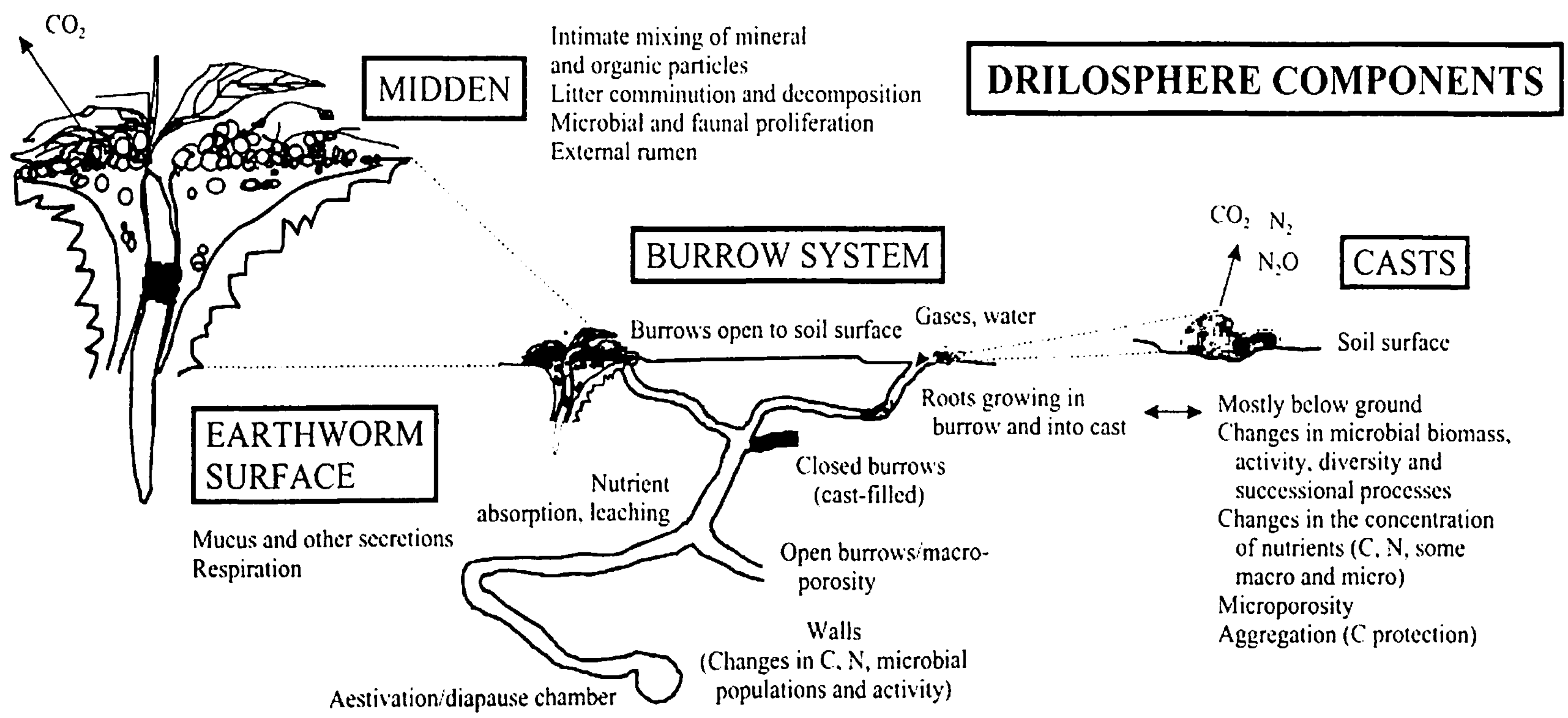

Fig 2.1 Illustration of drilosphere components (Brown and Doube, 2004)

2.4.2 Vermicomposting leachate

Leachate is generated along with vermicomposting process commonly referred to as vermicomposting leachate or worm-bed leachate (Gutiérrez-Miceli *et al.*, 2008). Vermicomposting leachate when collected, can be used as liquid fertilizer as it contains high concentration of plant nutrients as well as humic and fulvic acids. Draining the leachate can prevent saturation of the vermicomposting unit as well as to avoid leaching problem that may cause pollution especially when the site is located near to groundwater source. Studies have found that this leachate that is obtained from vermicomposting contains high amount of plant nutrients that may act as liquid fertilizer to improve plant growth. Chemical composition of vermicomposting leachate derived from cow dung and vegetable wastes were analysed by Singh *et al* (2010) and is reported in Table 2.6.

Table 2.6 Chemical composition of vermicomposting leachate of cow dung and vegetable waste.

	Cow dung	Vegetable waste
pH (1:10)	6.7	7.5
Total carbon(g L^{-1})	8.2	9.4
Total nitrogen(g L^{-1})	0.8	0.7
Total phosphorus (g L^{-1})	0.6	0.4
Total potassium (g L^{-1})	0.6	0.5
Calcium (mg L^{-1})	71	94

Modified from (Singh *et al.*, 2010)

Table 2.7 shows summary of studies carried out on vermicomposting leachate utilizing various wastes, earthworms and plants.

Table 2.7 Summary of studies carried out on vermicomposting leachate.

Waste	Earthworm species	Plant tested	Reference
Cow manure	*Eisenia fetida*	Maize (*Zea mays* L.)	García-Gómez *et al*., 2008
Cow manure	*Eisenia fetida*	Sorghum (*Sorghum bicolor* (L.) Moench)	Gutiérrez-Miceli *et al*., 2008
Cow manure	*Eisenia fetida*	Tomato (*Lycopersicon esculentum* Mill)	Oliva-Llaven *et al*., 2010
Cow dung, vegetable waste and mixture of cow dung and vegetable waste (1:2)	*Eisenia fetida*	Strawberry (*Fragaria x ananassa* Duch.)	Singh *et al*. 2010
Cow manure	*Eisenia fetida*	Lemongrass (*Cymbopogon citrates* (DC) Stapf.)	León-Anzueto *et al*., 2011

2.5 Soil-less culture

Globalization and climate change have severely reduced the agricultural land in all around the world. However, demand of food crop is increasing drastically. Hydroponics was initially introduced by Gericke (1937) to describe growing plants with their roots in liquid media. Soil-less culture however, is defined as the plant cultivation in systems other than soil in situ, which include hydroponics culture and other growing media or substrates (Gruda *et al.*, 2006). Soil-less culture was developed for overcoming soil-borne disease and other soil problems for cultivation in greenhouse as well as outdoors (Gericke, 1937).

Soil-less culture are independent from soil as well as all the soil related problems. The soil-less culture systems may be open or closed. For open system, excess irrigation is allowed to go waste and will not be recycled. However in closed systems, drainage is captured and recycled. Most of the hydroponics systems are closed systems but many aggregate systems, especially until recently were open (Gruda *et al.*, 2006).

Hydroponics becomes important culturing method especially in area with saline or sadic soils which encompass large portion of the cultivated land in the world. In soil-less culture, all essential plant nutrients are supplied through nutrient solution with the exception of carbon which can be taken up from the air as carbon dioxide. Nutrients solution are prepared by mixing water with concentrated stock solutions (Savvas, 2003)

2.5.1 Advantages and challenges of soil-less culture

Soil-less culture provides better control of the nutrients compared to soil cultivations. Hydroponics culture allows reduction of fertilizer application especially in closed systems. In many environmentally protected areas, closed-system hydroponics cultures are demanded through legislation (Savvas, 2003). However, composted material such as green waste and biowaste are not encouraged to be used as a growing medium on its own. The limiting factor of its utilization is the high EC and K content (Maher and Prasad, 2001).

Soil-less culture has the advantage of providing a practically sterile root environment at the beginning of crop cycle. When the nutrients supply is near to optimum, increment in yield and improvement in product quality are often observed. Soil-less culture is mainly found in application of high value crops of fresh vegetables and ornamentals that under protection. Further research and investigation should be focus on enabling this method to remain competitive against open field produce (Gruda *et al.*, 2006).

2.6 Fertilizer

With the rising demand of agricultural production due to increasing of population, marketable yield has become farmers' major concern. In order to increase the production and crop yield, the importance of fertilizers cannot be sufficiently emphasized. High yielding crops requires intense supply of nutrients.

Import and export of food in Malaysia in year 2002 was RM13 billion and RM7 billion respectively (FAO, 2004). Most of the fertilizers in Malaysia are imported. More than 90% of fertilizers used in Malaysia are mineral fertilizers.

However, for reducing the dependence of mineral fertilizers, the government of Malaysia is strongly promoting the use of organic fertilizer (Sabri, 2009).

Table 2.8 Number organic producer and area in Malaysia, by state in year 2001.

State	Number	Area (ha)
Selangor	4	10.8
Negeri Sembilan	10	90
Melaka	2	1.1
Johor	2	3.5
Pahang	6	11.6
Sabah	2	12
Sarawak	1	2
Total	27	131

Source: Wai (2001)

2.6.1 Organic fertilizer

Utilization of organic fertilizer is one of the major components in organic farming (Berner *et al.*, 2008). Organic manures provide essential nutrients to plant which increase crop productivity. However, it also leaves beneficial residual effect on the succeeding crops (Ghosh *et al.*, 2004). Organic fertilizers have been mainly derived from crop residues such as rice bran, oilseed cakes and other animal byproducts. Examples of animal byproducts are meat bone meal, blood meal, fish meal and crab meal. Animal by products are often distinguished from animal manure or compost derived from animal waste (Lee, 2010). Organic materials such as animal manures, sewage sludge and crop residual are used in organic farming in order to improve soil organic matter content, physical, chemical and biological properties of the soil (Debosz *et al.*, 2002).

Usage of chemical fertilizer has increased as the demand of high intensive agriculture. This has lead to soil degeneration and environmental deterioration.

Combining the usage of chemical and organic fertilizers may give proper yield and at the same time maintain the soil fertility

Application of organic manure could improve soil quality and poses least harm to the environment compared with utilization of chemical fertilizer alone (Reganold 1995; Conacher and Conacher, 1998). Soil treated with organic manual continuously are reported to have lower bulk density and higher porosity (Edmeades 2003).

Selvam and Sivakumar (2014) had come up with utilization of seaweed extract as organic liquid fertilizer. It has gain popularity as seaweeds are one of the important marine living resources with high commercial application. Seaweed extract liquid has found to be effective as it promotes faster seed germination and increment in yield in many crops. The upside of organic fertilizer is it biodegradable, and is non hazardous to human health.

Meanwhile, organic fertilizers also raise the concern of the presence of heavy metals due to the raw material or substrates used for deriving the organic fertilizers. Substrates that used in deriving organic fertilizer such as animal manures and sewage waste may contain components that are hazardous to human health, as well as to other animals and plants (Goss *et al.*, 2013). So far, majority of manure are used in land application. Beusen *et al.* (2008) reported 84% of manures were spread on crop land and 16% on grassland.

However, accumulation of NO_3, increased EC in soil in organic farming that utilized animal manure and organic fertilizers have been reported (Lee *et al.*, 2004; Sohn *et al.*, 1996). Hence utilization of organic fertilizer for maximum yield still require further study and the content of heavy metals in crops treated with these organic fertilizers should be closely monitored.

Table 2.9 shows the guidelines of heavy metal concentration for food safety set by few selected countries.

Table 2.9: Guidelines on heavy metal concentrations (mg kg^{-1}) for food safety set by selected countries (Modified from Agrawal *et al.*, 2007)

	Cd	Cu	Pb	Zn
	mg kg^{-1}			
Indian Standard	1.5	30	2.5	50
Food and Drug Administration of the United States (USFDA, 1990)	25	-	11.5	-
FAO/ WHO, 2001	0.2	30	0.3	60
Minister of Public Health, Thailand (MPHT, 1986)	-	133	6.67	667
Malaysia Food Act 1983 (Act 281) & Regulations	1	-	2	-

CHAPTER 3

MATERIALS AND METHOD

3.1 Source of Materials

Earthworm *Eudrilus eugeniae*, or commonly known as African Night Crawler, fresh cow dung and straw were obtained from BP Gemilang Agro Bio Sdn. Bhd.

3.2 Pre-composting

Cow dung was stored in an open plastic container and pre-composted for 2 weeks. Cow dung was turned manually for eliminating toxic gases and to reduce the risk of mortality of earthworms caused by anaerobic condition that may appear in fresh cow dung (Garg *et al.*, 2005). Temperature of composting cow dung was taken every 3 days.

3.3 Set up of vermiwash unit.

The set up of vermiwash unit was a modified version of vermiwash unit as suggested by Ismail (2005). A plastic round-shape container (39 cm x 47cm) of 50 liter volume with a draining tap at the bottom of the container was prepared. Gravels were filled at the bottom of the container till approximately 3cm higher than the draining point of the tap. A layer of pebbles (approximately 5 cm) were placed on top of the gravel layer as the second layer and followed by a layer of coarse river sand (approximately 5 cm, 8 kg). Gravels, pebbles and river sands were all washed with running water until the overflowing water was clear. Loamy soil were loosely filled up to 30 cm as the forth layer. 50 *Eudrilus eugeniae* were then introduced to the vermiwash unit. A layer of pre-composted cow dung (5kg) was introduced followed by a layer of straw. The unit was then covered with jute cloth that functions as moisture trap. Water was added daily to keep the reactor moist. Tap was kept open for the next 15 days as suggested in Ismail (2005).

On the 16th day, the tap was closed. A water dripping system was installed on the top of the reactor. One liter of water (1/50 of the container volume) was slowly dripped (0.04 L min $^{-1}$) into the vermiwash reactor overnight. The tap was opened the next day for vermiwash collection. Set up is shows in Fig.3.1 below.

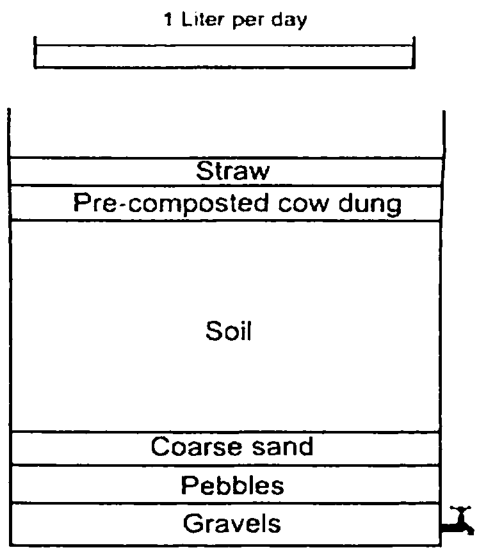

Fig. 3.1 Illustration of the vermiwash set up (Modified from Ismail, 2005).

3.3.1 Collection of vermiwash

Water droplets were allowed to percolate through the reactor. Vermiwash collection was started on the 16th day as suggested in Ismail (2005) to allow the