

BIODEGRADATION OF OIL PALM FIBRE WASTE

This invention relates to a method for degradation of biomass, the product of that method, and use of that product.

In particular, this invention relates to a method for biodegradation of oil palm fibre waste by vermicomposting.

DESCRIPTION OF THE PRIOR ART

Oil palm is the main agricultural crop in Malaysia with the total planted area recorded in 2009 at 4.69 million hectares. Based on the distribution rate of biomass per hectare, total oil palm biomass (empty fruit bunch (EFB); oil palm trunk (OPT) and oil palm fronds (OPF)) availability was estimated at about 65.46 million tonnes (dry weight).

Agro-waste from the oil palm industry (EFB, OPT and OPF) are a potential source for new value-added materials. Oil palm biomass is an agricultural by-product of the re-planting, pruning and milling processes of the palm oil industry. This by-product is largely unutilized after the 25-year economic life of the oil palm trees and is often a cause of environmental pollution. Oil palm waste is a suitable raw material for recycling because it is produced in large quantities in localized areas. Due to environmental air pollution control, incinerator burning of oil palm waste occurs less frequently in recent times. Palm oil mill effluents (POME) and other mill waste by-products such as decanter cake slurries require treatment, prior to use as a biomass source for the production of new materials.

Composting of EFB, OPT, OPF, POME or decanter cake slurry could substantially reduce the emission of methane, carbon dioxide and foul smell from anaerobic waste ponds of palm oil mills. However, due to the presence of toxic compounds such as heavy metals in the compost product, application of such products on agricultural land is presently limited.

Environmental legislation limits the heavy metal (Fe, Mn, Zn, Cu, Pb) content allowable in agricultural produce. After composting, the concentration of heavy metal in the compost substrate is relatively high. This prevents compliance with the provisions of the law, and hence, the resultant compost cannot be applied to soil.

The 2009 publication by Djunita, Tengku Sabrina and Musa, Mohamed Hanafi and Tengku Muda Mohamed, Mahmud and Abd Aziz, Nor Azwady in the Compost Science and Utilization Journal, 17(1) pp 61-67 entitled "*Vermicomposting of oil palm empty fruit bunch and its potential in supplying nutrients for crop growth*" describes the use of earthworms in vermicomposting of oil palm by-products.

In this prior publication, the authors noted that only one species of earthworm was viable for vermicomposting of oil palm waste, namely, *Eisenia foetida*. They mentioned that only *E. foetida* was able to survive in a compost substrate comprising oil palm by-products. This prior method necessitates addition of cow dung as a food supplement in order to reduce mortality rate and maintain growth of the worms.

The authors concluded that the optimum pre-composting duration was one month. This publication disclosed that a shorter pre-compost period resulted in the substrate having a low pH reading (acidic, undesirable).

This invention thus aims to alleviate the problems of the prior art and to provide an eco-friendly, affordable vermicomposting method and a high quality vermicompost product.

SUMMARY OF INVENTION

In accordance with an aspect of this invention, there is provided a method for biodegradation of oil palm waste by vermicomposting.

The method comprises the steps of:

- (i) pre-treating oil palm waste to form fibrous waste material;
- (ii) adding effective microbes to the fibrous waste and subjecting the waste to a pre-composting phase; and
- (iii) adding earthworms to the pre-composted waste of step (ii) and subjecting the mixture to a vermicomposting phase.

A bioadsorbance material is added to the vermicomposting mixture and the mixture is maintained in a dark, moist and warm condition.

In an embodiment, the pre-treatment of step (i) may comprise shredding the oil palm waste so as to reduce the waste to fibrous material. The pre-treatment may further comprise refining the shredded fibrous waste by using a thermo-mechanical refiner. The refined fibrous waste material may comprise fine fibers having a length of approximately 3.68 mm and a width of approximately 165.45 μm .

In another embodiment, step (ii) may comprise adding three different cultures of effective microbes to the fibrous waste material. The effective microbes of step (ii) may comprise microorganisms cultured in a mixture of vermileachate, sugar or molasses and animal-derived additives.

The effective microbe culture may comprise lactic acid bacteria, photosynthetic bacteria, yeast, organic acids and goat's milk.

The effective microbe culture may comprise a microbial component, waste material such as chicken manure, soil and water.

In yet another embodiment, the pre-composting mixture of step (ii) may be maintained in an environment having a moisture content of between 80% to 90%.

The pre-composting mixture of step (ii) may be maintained at a temperature of between 30°C to 50°C.

The pre-composting mixture of step (ii) may be maintained at a pH of between 6 to 8.

According to an embodiment, step (iii) may further comprise adding enhancement material comprising agricultural and/or animal waste. The enhancement material may comprise agricultural waste such as mushroom waste, jatropha waste or palm oil mill waste and/or animal waste such as cow dung or goat manure.

According to another embodiment, step (iii) may further comprise draining of leachates produced during vermicomposting.

In a further embodiment, step (iii) may further comprise adding bioactive additives such as egg shells or egg casts to increase the lifespan of the earthworms.

In an embodiment, the earthworms of step (iii) may comprise clitellated worms.

In another embodiment, the earthworms of step (iii) may comprise epigeic worms.

The earthworms of step (iii) may comprise *Eisenia spp.* worms. The *Eisenia spp.* worms may be *Eisenia foetida*.

The earthworms of step (iii) may comprise *Eudrilus eugeniae*.

In yet another embodiment, the bioadsorbance material of step (iv) may comprise zeolites. The zeolite may be clinoptilolite. The zeolite used may have a particle size of between 0.16 to 2.5 mm.

According to an embodiment, the vermicomposting mixture may be maintained in an environment having a moisture content of between 70% to 90%.

According to another embodiment, the vermicomposting mixture may be maintained at a temperature of between 25°C to 27°C.

According to a further embodiment, the vermicomposting mixture may be maintained at a pH of between 6 to 8.

In an embodiment, the oil palm waste may comprise any one or more of empty fruit bunches (EFB), oil palm fronds (OPF), oil palm trunk (OPT), and palm oil mill effluent (POME).

Additionally, there is also provided a vermifertilizer produced by the method of this invention. The vermifertilizer may have an N : P : K content of at least 1 : 1 : 1, between 1 : 1 : 1 to 2 : 5 : 5 or more than 2 : 5 : 5.

Further, the leachate produced in step (iii) of the method of claim 1 may be used as a plant growth medium.

The vermicast produced in step (iii) of the method of this invention may also be used as a plant growth medium.

The object of this invention is to provide a sustainable approach to handling the conversion of oil palm wastes into a useful recyclable product on site by way of an eco-friendly and economically sustainable method.

Vermicomposting is a decomposition process involving interactions between earthworms and microorganisms. The vermicomposting method of this invention provides an economical, viable and sustainable option for managing oil palm waste.

It is easily implemented and operable and only involves a small footprint for the production of high quality fertilizer.

The market for vermicompost in Malaysia is presently still small due to high production costs and concerns regarding the quality of the compost product. Advantageously, the method of this invention allows for production of high quality compost at affordable cost by utilizing readily available biomass waste.

Addition of cultures of effective microorganisms (EM) during the pre-composting phase (step ii) in the method of this invention enables a shortened overall composting duration (pre-compost and vermicompost).

Additionally, physico-chemical changes within the compost substrate caused by metabolic activities of these EM cultures aids in the production of a higher quality vermicompost product (vermifertilizer) i.e. increased N : P : K value.

Further, addition of bioadsorbance material such as zeolites during the vermicompost phase (step ii) in the method of this invention aids in substantially lowering heavy metal content in the vermicompost product, and therefore enabling production of a vermifertilizer that is applicable on agricultural land with substantially minimized (or no) harmful effect. Addition of bioadsorbance materials also aids in the reduction of ammonia emissions during the composting process.

It was observed that the bioadsorbance material added also serves as a bulking agent to increase vermisiludge porosity, which will in turn influence temperature and moisture of the sludge i.e. improved bio-environment during vermicomposting. The bioadsorbance material added also acts as an enhancing material for the vermicompost by aiding in further increasing the N : P : K value of the vermicompost product.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated, although not limited, by the following description of embodiments made with reference to the accompanying drawings in which:

Figure 1 is a graph showing the trend of EFB cellulose degradation (with and without earthworms) as per the study detailed in the Example 1.

Figure 2 is a graph showing the trend of OPF cellulose degradation (with and without earthworms) as per the study detailed in the Example 1.

Figure 3 is a graph showing the trend of OPT cellulose degradation (with and without earthworms) as per the study detailed in the Example 1.

Figure 4 is a graph showing the trend of EFB lignin degradation (with and without earthworms) as per the study detailed in the Example 1.

Figure 5 is a graph showing the trend of OPF lignin degradation (with and without earthworms) as per the study detailed in the Example 1.

Figure 6 is a graph showing the trend of OPT lignin degradation (with and without earthworms) as per the study detailed in the Example 1.

Figure 7 is a graph showing the trend of EFB cellulose degradation (with and without earthworms) as per the study detailed in the Example 2.

Figure 8 is a graph showing the trend of OPF cellulose degradation (with and without earthworms) as per the study detailed in the Example 2.

Figure 9 is a graph showing the trend of OPT cellulose degradation (with and without earthworms) as per the study detailed in the Example 2.

Figure 10 is a graph showing the trend of EFB lignin degradation (with and without earthworms) as per the study detailed in the Example 2.

Figure 11 is a graph showing the trend of OPF lignin degradation (with and without earthworms) as per the study detailed in the Example 2.

Figure 12 is a graph showing the trend of OPT lignin degradation (with and without earthworms) as per the study detailed in the Example 2.

Figure 13A is a perspective view of a set-up comprising four vermireactors that may be used in the method of this invention.

Figures 13B and 13C are front and top views, respectively, of the set-up of Figure 13A.

DETAILED DESCRIPTION OF THE EMBODIMENTS

A method for biodegradation of oil palm waste by vermicomposting and the resultant vermifertilizer product are provided as follows.

Method for biodegradation of oil palm waste by vermicomposting

A method for biodegradation of oil palm waste by vermicomposting according to the present invention generally involves three main steps, namely, pre-treating oil palm waste to form fibrous waste material; subjecting the waste to a pre-composting phase; and subjecting the pre-composted waste to a vermicomposting phase.

Pre-treatment of the oil palm waste

The biomass substrate (oil palm waste) used in the vermicomposting method of this invention may be in the form of a "mix substrate" or a "pure substrate".

A mixed substrate comprises a mixture of oil palm waste e.g. EFB, OPF, OPT and/or POME. These different types of oil palm biomass may be combined in differing ratios depending on the desired end use of the compost product. It was observed that a mixed substrate of EFB and POME having a ratio of 1 : 1 resulted in a vermifertilizer of preferable quality.

On the other hand, a pure substrate comprises wholly EFB, OPF, OPT or POME. It was observed that a pure substrate comprising EFB resulted in a vermifertilizer of preferable quality.

Prior to formation of either a mixed or pure substrate, the oil palm biomass is pre-treated to form a fibrous waste material suitable for vermicomposting.

EFBs, OPFs and OPTs may be subjected to any form of size reduction processing such as chipping, milling and/or shredding to form a fibrous material. Screw pressed biomass followed by shredding in a hammer mill produces shredded fibers with ranging from 5 to 10 cm in size having a moisture content of about 40 to 45%. The biomass may also be cut using cutting standard knife mechanisms into chips of a 5 to 10 cm size.

Preferably, the fibrous material may be refined to form loose fine fibrous material using a suitable device such as a thermo-mechanical refiner (by Andritz – 30.5 cm (12 inch) refiner segment) which utilizes refining plates that rotate at up to 3,000 rpm. Fiber chips are digested at a temperature of between 100°C to 160°C in a digester tank prior to refining. Most preferably, the fine fibers are ≈ 3.68 mm in length and ≈ 165.45 μm in width.

The above reduction in size and refining pre-treatment of the oil palm waste increases the surface area of the fibres, hence, enhancing the decomposition process (pre-composting and vermicomposting). This aids in enabling a reduction in the duration of the process (pre-composting and vermicomposting) from 3 months (conventional method) to less than 2 months.

Pre-composting phase

The fibrous waste material is then subjected to a pre-composting phase. Self-cultured effective microbes (EM) are added to the material. Up to three different cultures of EM can be used in the pre-composting phase. The EM may comprise microorganisms cultured in a mixture of vermiculite, sugar or molasses and animal-derived additives. Preferably, the EM cultures comprise mesophilic bacteria. The effective microbe culture may comprise a laboratory derived mixture of lactic acid bacteria, photosynthetic bacteria, yeast, organic acids and goat's milk or a commercially available mixture, for example, comprising microbial component, waste material such as chicken manure, soil and water.

Application of the EM cultures aid in expediting the pre-composting process i.e. shortened pre-composting phase. This will further aid in enabling a reduction in the total duration of the process (pre-composting and vermicomposting).

In the method of this invention, pre-composting is preferably carried out for about three weeks prior to vermicomposting. During that time, the pre-compost mixture is maintained in a dark, moist and warm environment. Preferably, the pre-compost mixture is maintained in an environment having a moisture content of between 80% to 90%, at a temperature of between 30°C to 50°C and at a pH of between 6 to 8.

Vermicomposting phase

After completion of the pre-composting phase, earthworms are added to the pre-compost mixture and the mixture is then subjected to the vermicomposting phase for one week.

Earthworms (inoculants) are added to the mixture. Earthworms are generally known to have a capacity to degrade various types of biomass. During vermicomposting, earthworms ingest, grind and digest organic waste with the help of aerobic and anaerobic microflora within their gut, converting the waste into a finer, humified, microbially active material. Cellulolysis occurs as a result of the combined action of

fungi and bacteria on the lignocellulosic components of the biomass. During decomposition, earthworms act either directly by cellulolytic activity of the cellulose enzymes (species dependent) or indirectly by affecting the structure and dynamics of the microbial population within the vermicomposting mixture. Important plant nutrients such as N, P, K, Ca and other micronutrients are converted into more soluble forms and made more readily available to plants.

Any species of earthworm suitable for use as inoculants in vermicomposting can be used in the method of this invention. Preferably, epigeic earthworms (surface dwellers) are used. It has been observed that use of epigeic worms in the method of this invention aids in significantly hastening the decomposition process and also results in production of better quality compost. More preferably, epigeic worms of the *Eisenia* genus are used, in particular, *Eisenia foetida*. It was surprisingly found that *Eisenia spp.* have remarkable lignocellulosic degradation capability when used in the method of this invention. Particularly, *Eisenia foetida* surprisingly showed remarkable capability to degrade major lignocellulotic components (cellulose and lignin) of EFB. A significant degradation rate of cellulose and lignin of EFB was observed. *Eudrilus eugeniae* were also observed to have good degradation capabilities.

In a preferred embodiment, clitellated worms are used as inoculants. This is because of their natural ability to colonize organic wastes, high rate of consumption, digestion and assimilation of organic matter; tolerance to wide range of environmental factors; high reproductive rates; and endurance and tolerance of handling.

The ratio of worm to substrate used in the method of this invention is very much dependent on the volume and composition of substrate as well as the desired duration of decomposition. For example for an 80 g mix substrate about 5 g of worms may be added ($\approx$ 3 months to decompose) and for a 1,000 kg (1 metric tonne) of mix substrate about 50 kg of worms may be added ($\approx$ 2 months to decompose).

Also, bio-active additive such as egg shells and egg casts may preferably be added to the vermicompost mixture to increase the lifespan of the worms. This will result in increased "productivity" (degradation capacity) of the earthworms.

Preferably, enhancement material such as agricultural and/or animal waste may also be added to the vermicompost mixture to aid in increasing the N : P : K content of the resultant vermicompost from less than 1 : 1 : 1 to 2 : 5 : 5 or more. Any type of suitable agricultural waste such as mushroom waste, jatropha waste or oil palm mill waste (e.g. decanter cake slurry) may be used. Examples of animal waste that may be used as enhancement material is cow dung or goat manure.

Any suitable vermireactor may be used in the vermicomposting phase. An exemplary set-up comprising four reactors is shown in Figures 13A to 13C. It is preferred that the reactor 10 used in the method of this invention is provided with a drainage and collection system comprising inner and outer collection pipes 11, 12 as well as an external collection vessel 20 that enables draining of leachates produced during this phase. Leaches are produced due to the metabolic activity of the microorganisms and worms during the degradation of biomass. Draining of leachates is important to prevent saturation of the vermicomposting substrate (biomass).

Bioadsorbance material such as zeolites is added into the biomass substrate during the vermicomposting phase. Preferably, zeolites are added, in particular zeolites having a particle size of between 0.16 mm to 2.5 mm. The porous micro-structure of zeolites enables adsorption of a variety of elements, and the diameter of the input channels allows zeolites to act as a molecular sieve. More preferably, a natural zeolite such as clinoptilolite is added.

It is generally known that vermicompost produced from oil palm waste has high heavy metal content. The vermicompost produced with the method of this invention has increased heavy metal content (in comparison to heavy metal content in biomass prior to vermicompost), although still within the allowable range i.e. range i.e about 58% for copper, 20% for ferum, 49% for zinc and 50% for manganese. It

was observed that addition of about 25% of zeolite during the vermicomposting phase of the method of this invention results in an extraction efficiency of about 10 to 15% for chromium, 28 to 45% for copper and 40 to 46% for nickel.

In addition to reducing heavy metal content, ammonia emissions were also observed to be reduced by addition of bioadsorbance materials.

The inventors found that the bioadsorbance material (e.g. zeolite) added can also serve as a bulking agent to increase vermiculite porosity, which will in turn influence temperature and moisture of the sludge.

Further, addition of bioadsorbance materials such as zeolite also aid in increasing the Na and K concentrations in the vermicompost through ion exchange.

The zeolites may optionally be removed from the vermicompost before its use i.e. application to soil. It has been observed that the zeolite leachates contain very low concentration of metals. Hence, this is an option rather than preferable step in the method of this invention.

The worms and other vermicast may be removed from the vermicompost prior to its use i.e. application to soil. The manner in which the worms and vermicast are removed is dependent on the volume of the substrate. For example for a 1,000 kg (1 metric tonne) substrate, the worms and vermicast may be removed by using a harvester, whereas for a small amount of substrate, the worms may be simply removed by sieving.

Vermicompost product

The vermicompost product produced with the method of this invention is stable, homogeneous, has desirable aesthetics (colour and odour) and reduced levels of contaminants e.g. heavy metal content.

The product was also observed to have significantly decreased C/N ratio and increased total Kjeldahl nitrogen, available phosphorous and potassium levels in comparison with the untreated (prior to compost) biomass making it particularly suitable for use as plant growth medium e.g. for vegetables and in oil palm nurseries or plantations.

The vermicompost product may be palletized to form vermifertilizers using any known conventional process. The efficacy of the vermicompost product of the method of this invention as a plant growth medium is comparable if not better than normal compost fertilizers, chemical fertilizers as well as conventionally produced vermicompost fertilizers. It was observed that a pure substrate of EFB fibre resulted in the best quality vermicompost in comparison with that produced from either a pure or mixed substrate comprising OPF, OPT and/or POME.

Advantageously, it has also been observed that foliar application of vermicast (top layer of vermicompost mixture) and leachates from the vermicomposting phase of the method of this invention resulted in improved growth, yield and quality of plants.

The chemical composition of leachate produced will mainly depend on the chemical composition of the biomass substrate used for vermicomposting. Apart from high nutrient content, the vermicompost leachate produced with the method of this invention also contributes to plant development due to the presence of humic acid which regulate many plant developmental processes including macro and micronutrient absorption.

EXAMPLE

The following Examples illustrate the various aspects and steps of the method for biodegradation of oil palm waste by vermicomposting of this invention. These Examples do not limit the invention, the scope of which is set out in the appended claims.

Example 1: Comparison of product obtained using the method of this invention versus a conventional composting method

A study to compare the product obtained using the vermicomposting method of this invention versus that obtained with a conventional composting method was conducted by the Agro Product Unit, Engineering and Processing Division of the Malaysian Palm Oil Board in Bangi, Selangor.

The study was conducted in five phases as described below.

Phase I: Physical and chemical analysis of oil palm biomass (EFB, OPF and OPT), with and without addition of effective microorganisms (EM) bioadsorbance material.

EFB, OPF and OPT fibres (length $\approx$ 3.68 mm, width $\approx$ 165.45 μ m) were selected.

For physical analysis, the length and width of each fibre was measured using Digital Imaging Microscopy technique. The morphological structure of the EFB, OPF and OPT before vermicomposting was observed with a Scanning Electron Microscope (SEM) machine (Model Leica Cambridge S-360)

For chemical analysis, the fibre samples were submitted to Soxhlet extraction with ethanol/benzene [1 : 2 (v/v)] for 6 hours to determine α -cellulose, holocellulose and lignin.

The above procedures were performed according to the TAPPI Method T 264om-88.

Phase II: Three-week pre-composting phase (oil palm biomass + EM).

Pure and mixed substrates of the EFB, OPT and OPF fibres were prepared in triplicate, with worms and without worms. Samples without worms were prepared as a control.

5 to 10 ml (diluted) of effective microorganisms (EM) was added to 150 g samples of each substrate mixture. Both a laboratory derived culture comprising lactic acid bacteria, photosynthetic bacteria, yeast, organic acids and goat's milk as well as a commercially available culture (from Dazzling Destiny Sdn Bhd) were used. The mixtures were then placed in 1.2 L rectangular plastic containers (length 14 cm, width 12 cm, height 7 cm). These containers were maintained with a moisture content of $80\% \pm 10\%$ (MC portable meter) throughout this phase.

Phase III: Vermicompost analysis (addition of bioadsorbance material in differing ratios).

In the vermicomposting phase, 5 g of clitellated adult *E. foetida* worms (live weight of each individual adult worm between 0.35 g to 1.90 g), decanter cake slurry and zeolite were added to each container in the ratio as stated in Table 1 below. Identical feed compositions were prepared for OPT and OPF fibres.

Container	Composition of feed	Amount of zeolite
V ₁	EFB (100%)	0%
V ₂	EFB (90%) + decanter cake (10%)	5%
V ₃	EFB (80%) + decanter cake (20%)	10%
V ₄	EFB (70%) + decanter cake (30%)	15%
V ₅	EFB (60%) + decanter cake (40%)	20%
V ₆	EFB (50%) + decanter cake (50%)	25%

Table 1: EFB feed composition of vermicomposters (with and without worms)

The containers were kept in the dark and the moisture content, temperature and pH of the substrate in each container was maintained at 70 to 80% (MC portable meter), 26.3 ± 0.39 °C (average temperature recorded $\pm$ SE) and 6 to 8, respectively, throughout this phase. The controls (without earthworms) were maintained under field conditions and moistened twice a week.

Vermicast (top layer of container) was collected every week for earthworm condition analysis.

Biomass of the worms was determined by removing all worms from each container, washing them in distilled water, and drying them on paper towels. The worms were then weighed in a water-filled weighing boat using a Sartorius balance.

Variation in the C/N ratio of the biomass was observed and the results are provided in Table 2 below.

Container	C/N ratio					
	0 Days	15 Days	30 Days	45 Days	60 Days	84 Days
V ₁	178.1 $\pm$ 0.47	165.1 $\pm$ 0.30	125.2 $\pm$ 2.8	100.6 $\pm$ 4.80	75.9 $\pm$ 0.30	54.0 $\pm$ 0.38
V ₂	114.5 $\pm$ 1.50	88.5 $\pm$ 1.30	79.6 $\pm$ 0.48	66.5 $\pm$ 0.71	44.5 $\pm$ 1.20	20.1 $\pm$ 0.19
V ₃	153.3 $\pm$ 0.16	94.8 $\pm$ 0.90	65.0 $\pm$ 0.36	54.0 $\pm$ 0.19	32.6 $\pm$ 0.16	19.5 $\pm$ 0.81
V ₄	73.1 $\pm$ 0.31	56.8 $\pm$ 1.20	46.1 $\pm$ 0.95	36.9 $\pm$ 0.30	18.4 $\pm$ 4.50	12.1 $\pm$ 0.52
V ₅	123.1 $\pm$ 1.50	79.3 $\pm$ 0.09	61.6 $\pm$ 0.10	51.5 $\pm$ 0.23	20.9 $\pm$ 3.70	15.5 $\pm$ 0.66
V ₆	38.6 $\pm$ 0.30	28.2 $\pm$ 0.38	22.4 $\pm$ 1.10	20.6 $\pm$ 1.60	17.0 $\pm$ 2.80	10.5 $\pm$ 0.05

Table 2: Variation in C/N ratio during vermicomposting (mean $\pm$ SE, n= 3)

The rate of cellulose and lignin degradation was observed and the results are shown in Figures 1 to 6.

In order to compare the advantage of having a pure versus a mix substrate, six vermicomposters having the above worm and zeolite compositions (as in Table 1) were established having different ratios of EFB and POME, followed by OPF and OPT, respectively, and POME, as stated in Table 3 below.

Vermicomposter	Composition of feed
V ₁	EFB (100%)
V ₂	EFB (90%) + POME (10%)
V ₃	EFB (80%) + POME (20%)
V ₄	EFB (70%) + POME (30%)
V ₅	EFB (60%) + POME (40%)
V ₆	EFB (50%) + POME (50%)

Table 3: EFB pure and mix substrate composition

The physico-chemical characteristics of the initial biomass feed versus the vermicompost obtained were observed and the results are shown in Table 4 on the following page.

Table 4: Physico-chemical characteristics of initial feed versus vermicompost obtained from different ratios of feed mixtures.

Feed mixtures	pH	TKN	TP	TK
Initial physico-chemical characteristics of feed mixtures ^a				
V ₁	5.85 ± 0.09	0.30 ± 0.19	0.10 ± 0.01	0.03 ± 0.01
V ₂	6.64 ± 0.2	0.45 ± 0.07	0.16 ± 0.08	0.03 ± 0.01
V ₃	5.45 ± 0.26	0.47 ± 0.04	0.21 ± 0.06	0.05 ± 0.01
V ₄	6.25 ± 0.22	0.53 ± 0.41	0.22 ± 0.13	0.06 ± 0.01
V ₅	6.26 ± 0.12	0.61 ± 0.32	0.25 ± 0.05	0.07 ± 0.01
V ₆	6.32 ± 0.19	1.20 ± 0.60	0.28 ± 0.03	0.09 ± 0.01
Physico-chemical characteristics of final vermicompost ^b obtained (mean ± SE, n=3)				
V ₁	7.92 ± 0.24	0.35 ± 0.64	0.22 ± 0.18	0.06 ± 0.08
V ₂	8.05 ± 0.06	0.63 ± 0.20	0.43 ± 0.30	0.07 ± 0.01
V ₃	8.10 ± 0.1	0.70 ± 0.09	0.70 ± 0.39	0.13 ± 0.01
V ₄	8.50 ± 0.16	0.79 ± 0.48	0.76 ± 0.75	0.17 ± 0.16
V ₅	8.25 ± 0.10	1.02 ± 0.96	0.90 ± 0.08	0.23 ± 0.18
V ₆	8.22 ± 0.23	1.67 ± 0.50	1.40 ± 0.45	0.45 ± 0.39

^aInitial physico-chemical characteristic of feed calculated based on percentage of EFB and POME.

^b Mean value followed by different letters is statistically different (ANOVA; Tukey's test, P< 0.05).

Unit of all parameters measured is mg/kg except for pH value.

TP = total phosphorus

TK = total potassium

TKN = total Kjeldahl nitrogen

The heavy metal content of the initial biomass feed versus the vermicompost obtained was observed and the results are shown in Table 5 on the following page.

The standard range of nutrients contained in vermicompost is provided on Table 6 on the following page.

Table 5: Heavy metal content (mg/kg) in initial feed substrate versus vermicompost obtained from EFB + POME

Feed mixtures	Cu	Fe	Zn	Mn
Heavy metal content in initial feed mixtures				
V ₁	0.03 ± 0.03	15.60 ± 1.01	0.56 ± 0.02	1.26 ± 0.10
V ₂	0.18 ± 0.15	18.23 ± 2.29	0.59 ± 0.13	1.49 ± 0.18
V ₃	0.18 ± 0.61	23.98 ± 0.94	0.79 ± 0.01	2.13 ± 0.32
V ₄	0.19 ± 0.14	23.64 ± 0.61	0.78 ± 0.21	2.34 ± 0.30
V ₅	0.25 ± 0.75	25.63 ± 1.18	0.83 ± 0.02	2.50 ± 0.12
V ₆	0.42 ± 0.15	23.88 ± 0.98	1.08 ± 0.08	2.65 ± 0.27
Heavy metal content in final vermicompost ^b obtained (mean ± SE, n=3)				
V ₁	0.07 ± 0.39	22.79 ± 1.38	0.69 ± 0.19	2.01 ± 0.54
V ₂	0.46 ± 0.33	27.17 ± 1.02	1.39 ± 0.26	2.82 ± 0.90
V ₃	0.77 ± 0.45	27.90 ± 1.85	1.74 ± 0.38	3.55 ± 0.64
V ₄	0.80 ± 0.18	28.68 ± 0.97	1.83 ± 0.57	4.13 ± 0.49
V ₅	0.82 ± 0.19	29.46 ± 0.82	2.01 ± 0.35	4.77 ± 0.37
V ₆	1.01 ± 0.28	29.79 ± 0.95	2.13 ± 0.38	5.37 ± 1.64

^b Mean value followed by different letters is statistically different (ANOVA; Tukey's test, P < 0.05).

Table 6: Standard range of nutrients in vermicompost (Kale, 1994)

Calcium and magnesium (meq/100g)	22.67-47.60
Copper (ppm)	2.00-9.50
Iron (ppm)	2.00-9.30
Zinc (ppm)	5.70-11.50
Available sulphur (ppm)	128.00-548.00

Phase IV: Application of vermifertilizer to vegetable plants and observation on the growth of those plants.

Three types of vegetables were used in phase IV of this example, namely, eggplant, chilly and tomato.

Tomato, eggplant and chilly seedlings were removed at random from seedling trays and transplanted into small pots measuring 10 cm (4 inches) in diameter. Each pot was filled with soilless plant growth medium (five replicate pots per treatment) and vermicompost. All pots were watered once every day for forty days. The seedlings were then transplanted into larger pots measuring 40 cm (15.7 inches) in diameter and watered with deionized water or the appropriate dose of a range of vermicompost tea (mixture of vermicompost and water at varying ratios, e.g. between 5% (1:20) and 20% (1:5) solid vermicompost to water). Thirty days after the final transplant, the tomatoes, eggplants and chillies were harvested. The number and weight of fruits for each plant was recorded. The fruits were harvested for 20 days after the first fruits were taken, after which the shoots of all plants were removed from the potting mixtures, oven-dried at 55°C (131°F) for 8 days, and the mean shoot dry-weight and leaf areas were recorded. Leaf tissues were analyzed for nutrient content. Roots were washed, dried at 55°C (131°F) for 5 days, and weighed.

About 15 to 25g of vermifertilizer were added to each type of vegetable once a month.

After three months, all the vegetables were observed to be growing well with improved foliar growth, fruit colour, vibrancy, size and height.

Phase V: Statistical data analysis

One-way ANOVA test was used to analyze the significant difference between different vermireactors for the observed parameters.

Tukey's *t*-test was also performed to identify the homogeneous type of the reactors for the different chemical properties and earthworm growth parameters i.e earthworm weight gain and cocoon production.

The probability levels used for statistical significance were $p < 0.05$ for both tests.

Example 2: Vermicomposting with the method of this invention using *Eudrilus euginae*

A vermicomposting experiment using the method of this invention was conducted by the Agro Product Unit, Engineering and Processing Division of the Malaysian Palm Oil Board in Bangi, Selangor.

The experiment was conducted in five phases as described in Example 1.

Phase I and Phase II were conducted in an identical manner as Example 1.

For Phase III (vermicomposting phase), 5 g of clitellated adult *Eudrilus euginae* worms commonly known as the African Night Crawler (live weight of each individual adult worm between 0.35 g to 1.90 g) and decanter cake slurry were added to each container in the ratio as stated in Table 1 above. As opposed to Example 1, zeolite was not added to the containers.

Similarly to Phase III in Example 1, the containers were kept in the dark and the moisture content, temperature and pH of the substrate in each container was maintained at 70 to 80% (MC portable meter), 26.3 ± 0.39 °C (average temperature recorded $\pm$ SE) and 6 to 8, respectively, throughout this phase. The controls (without earthworms) were maintained under field conditions and moistened twice a week.

The rate of cellulose and lignin degradation was observed and the results are shown in Figures 7 to 12.

The physico-chemical characteristics of the vermicompost obtained from EFB over an 84-day period were observed and the results are shown in Table 7 below.

The heavy metal content of the vermicompost obtained from EFB after an 84-day period is shown in Table 8 below.

Table 6: Chemical analysis of vermicompost prepared from EFB at different time period.

Days	pH	TP	TK
0	6.5 ± 0.1	0.2 ± 1 × 10 ⁻²	2 × 10 ⁻² ± 1.2 × 10 ⁻³
14	6.7 ± 0.2	0.5 ± 1 × 10 ⁻²	4 × 10 ⁻² ± 1.1 × 10 ⁻³
28	7.2 ± 0.3	0.6 ± 1 × 10 ⁻³	5 × 10 ⁻² ± 4.0 × 10 ⁻³
42	7.3 ± 0.1	0.9 ± 2 × 10 ⁻²	8 × 10 ⁻² ± 2.0 × 10 ⁻³
56	7.4 ± 0.1	0.9 ± 1 × 10 ⁻²	0.2 ± 1.0 × 10 ⁻²
70	7.5 ± 0.3	1.0 ± 1 × 10 ⁻²	0.2 ± 3.0 × 10 ⁻²
84	7.5 ± 0.1	1.1 ± 1 × 10 ⁻²	0.3 ± 1.0 × 10 ⁻²

All values are the mean and standard deviation of three replicates; all values are given in percentage except for pH.

TP = total phosphorus

TK = total potassium

Table 7: Heavy metal content (mg/kg) in vermicompost obtained from EFB

Cu	Fe	Zn	Mn
5.58 ± 0.29	3.29 ± 1.64	3.56 ± 0.08	18.75 ± 0.75

All values are the mean and standard deviation of three replicates

Phase IV and Phase V were conducted in an identical manner as Example 1.

Conclusion

Microbial decomposition of plant material resulted in an overall loss of cellulose and carbon and an increase in nitrogen content. These changes, which resulted from microbial activity on the cellulosic substrate and nitrogen from the decanter slurry, increased microbial protein and humic substances.

For Example 1, as the amount of added natural zeolite increased in the compost pile, the concentration of heavy metals in the final compost decreased. By using 15 to 25% of clinoptilolite during the composting process 100% of Cd, 28 to 45% of Cu, 10 to 15% of Cr, 41 to 47% of Fe, 9 to 24% of Mn, 50 to 55% of Ni and Pb, and 40 to 46% of Zn was taken up by the zeolite. Clinoptilolite readily took up the metal content bound in exchangeable and carbonate fractions.

For Example 2, even without addition of zeolite to the vermicompost, the heavy metal content of the final compost were still within the allowable limits (Table 6). Hence, it is postulated that addition of zeolite to a vermicompost utilizing *Eudrilus euginae* would provide similarly positive results vis á vis heavy metal content, as that utilizing *E. foetida*.

As will be readily apparent to those skilled in the art, the present invention may easily be produced in other specific forms without departing from its scope or essential characteristics. The present embodiments are, therefore, to be considered as merely illustrative and not restrictive, the scope of the invention being indicated by the claims rather than the foregoing description, and all changes which come within therefore intended to be embraced therein.

CLAIMS

1. A method for biodegradation of oil palm waste by vermicomposting, said method comprising the steps of:
 - (i) pre-treating oil palm waste to form fibrous waste material;
 - (ii) adding effective microbes to said fibrous waste and subjecting said waste to a pre-composting phase; and
 - (iii) adding earthworms to said pre-composted waste of step (ii) and subjecting the mixture to a vermicomposting phase;wherein bioadsorbance material is added to said vermicomposting mixture and said mixture is maintained in a dark, moist and warm condition.
2. The method as claimed in claim 1 wherein said pre-treatment of step (i) comprises shredding said oil palm waste so as to reduce said waste to fibrous material.
3. The method as claimed in claim 2 wherein said pre-treatment of step (i) further comprises refining said shredded fibrous waste by using a thermo-mechanical refiner.
4. The method as claimed in claim 3 wherein the refined fibrous waste material comprises fine fibers having a length of approximately 3.68 mm and a width of approximately 165.45 μm .
5. The method as claimed in any one of the preceding claims wherein step (ii) comprises adding three different cultures of effective microbes to said fibrous waste material.
6. The method as claimed in any one of the preceding claims wherein the effective microbes of step (ii) comprises microorganisms cultured in a mixture of vermileachate, sugar or molasses and animal-derived additives.

7. The method as claimed in claim 6 wherein the effective microbe culture comprises lactic acid bacteria, photosynthetic bacteria, yeast, organic acids and goat's milk.
8. The method as claimed in claim 6 wherein the effective microbe culture comprises a microbial component, waste material such as chicken manure, soil and water.
9. The method as claimed in any preceding claims wherein said pre-composting mixture of step (ii) is maintained in an environment having a moisture content of between 80% to 90%.
10. The method as claimed in any one of the preceding claims wherein said pre-composting mixture of step (ii) is maintained at a temperature of between 30°C to 50°C.
11. The method as claimed in any one of the preceding claims wherein said pre-composting mixture of step (ii) is maintained at a pH of between 6 to 8.
12. The method as claimed in any one of the preceding claims wherein step (iii) further comprises adding enhancement material comprising agricultural and/or animal waste.
13. The method as claimed in claim 12 wherein said enhancement material comprises agricultural waste such as mushroom waste, jatropha waste or palm oil mill waste.
14. The method as claimed in claim 12 wherein said enhancement material comprises animal waste such as cow dung or goat manure.
15. The method as claimed in any one of the preceding claims wherein step (iii) further comprises draining of leachates produced during vermicomposting.

16. The method as claimed in any one of the preceding claims wherein step (iii) further comprises adding bioactive additives such as egg shells or egg casts to increase the lifespan of said earthworms.
17. The method as claimed in any one of the preceding claims wherein the earthworms of step (iii) comprise clitellated worms.
18. The method as claimed in any one of the preceding claims wherein the earthworms of step (iii) comprise epigeic worms.
19. The method as claimed in any one of the preceding claims wherein the earthworms of step (iii) comprise *Eisenia spp.* worms.
20. The method as claimed in claim 19 wherein the *Eisenia spp.* worms comprise *Eisenia foetida*.
21. The method as claimed in any one of claims 1 to 18 wherein the earthworms of step (iii) comprise *Eudrilus eugeniae*.
22. The method as claimed in any one of the preceding claims wherein the bioadsorbance material comprises zeolite.
23. The method as claimed in claim 22 wherein the zeolite added is clinoptilolite.
24. The method as claimed in any one of claim 22 or claim 23 wherein the zeolite used has a particle size of between 0.16 to 2.5 mm.
25. The method as claimed in any one of the preceding claims wherein said vermicomposting mixture is maintained in an environment having a moisture content of between 70% to 90%.

26. The method as claimed in any one of the preceding claims wherein said vermicomposting mixture is maintained at a temperature of between 25°C to 27°C.
27. The method as claimed in any one of the preceding claims wherein said vermicomposting mixture is maintained at a pH of between 6 to 8.
28. The method as claimed in any one of the preceding claims wherein said oil palm waste comprises any one or more of empty fruit bunches (EFB), oil palm fibres (OPF), oil palm trunk (OPT), and palm oil mill effluent (POME).
29. A vermifertilizer produced by the method of claim 1.
30. The vermifertilizer as claimed in claim 29 having an N : P : K content of at least 1 : 1 : 1.
31. The vermifertilizer as claimed in claim 29 having an N : P : K content of between 1 : 1 : 1 to 2 : 5 : 5.
32. The vermifertilizer as claimed in claim 29 having an N : P : K content of more than 2 : 5 : 5.
33. Use of the leachate produced during step (iii) of the method of claim 1 as a plant grow medium.
34. Use of the vermicast produced during step (iii) of the method of claim 1 as a plant grow medium.