Phytotoxicity effect of palm oil mill effluent (POME) on lettuce seed (*L. sativa L.*) after vermifiltration treatment

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Abstract- Palm oil industry is one of the leading agricultural industries in Malaysia and been contributing significantly towards the country's economy and increase standard of living among Malaysians. The raw palm oil mill effluent (POME) is acidic, with a pH level 4.6 and it may cause environmental problems, so that treatment of POME is necessary. In Malaysia, high generation of POME from crude palm oil production is currently treated using conventional method known as ponding system, which is space inefficient. As an attempt to resolve the long-standing dilemma, recent and alternative method such as vermifiltration is being studied and introduced to wastewater treatment. Vermifiltration is a filtration process where solids are separated from liquid aided by earthworms. In this research, the performance of a vermifilter (VF) containing the earthworms, African Night Crawler and a control filter (CF), without earthworms were compared using the mixture of sand and garden soil as vermibed. Worm density of 8 g/L with flow rates 100 ml/min was used in the vermifilter for a 150 day period. The research was conducted at laboratory scale. Both filters, vermifilter and control filter were set as triplicates. The POME pH increased from acidic to neutral. It was observed that vermifilter reactor shows the highest reduction rate of BOD_5, COD, TSS and turbidity by 92%, 90%, 95% and 86% respectively compared to control filter by 55%, 45%, 65% and 52% respectively. Moreover, the phytotoxicity in treated POME in vermifiltration system was showed through germination test. The result of germination percentage showed germination percentage higher than 50% only for control, 1% and 3% treatments, so this three treatments indicates the maturity of the compost. For 10% and 30% treatments showed germination percentage lower than 50%. For, 100% treatment showed 0% and its proved that 100% of treated POME not safely used for agricultural purposes. Vermifiltration technology can therefore be applied as an environmentally friendly technique and has potential to treat POME.

Index Terms- African night crawler, palm oil mill effluent, phytotoxicity, vermifiltration.

I. Introduction

Palm oil industry is one of the leading agricultural industries in Malaysia. Palm oil industries have been contributing significantly towards the country's economy and increase standard of living among Malaysians. During the production of one tonne of crude palm oil, more than 2.5 tonnes of POME is produced [1]. The POME is a thick brownish viscous liquid waste and non-toxic because no chemicals are added during oil extraction but has an unpleasant odor. It is highly polluting [2]. Generally, the average chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in the POME are around 50,000 and 25,000 mg/L, respectively [3]. The POME can cause environmental problems if discharged without effective treatment because it contains high concentrations of oil and grease, organic matter, suspended solids (SS) and plant nutrients. Recently, palm oil mills face a huge challenge in meeting increasingly stringent environmental standards.

Vermifilter (VF) was widely used to treat the wastewater, and appeared to have high treatment efficiencies, including synchronous stabilization of wastewater and sludge. Earthworms have also proved to be master bio-processing agents for the management of organic effluents from diverse sources ranging from domestic sewage to industrial refuse [4]. Therefore, vermifiltration may work as a reliable tool for treatment the POME. *Vermifiltration* using waste eater earthworms is a newly conceived novel technology with several advantages over the conventional systems. A typical system will separate the wastewater solids by allowing wastewater to be gravity fed over filtration material such as fine mesh. The wastewater solids are then biodegraded by worms into humus material [5]. Earthworms' body works as a 'biofilter' and have been found can remove the 5-days' BOD (BOD_5) by over 90%, COD by 80–90%, total dissolved solids (TDS) by 90–92%, and the total suspended solids (TSS) by 90–95% from wastewater by the general mechanism of 'ingestion' and biodegradation of organic wastes, heavy metals, solids and pathogens from wastewater and also by their 'absorption' through body walls. Besides, earthworms have the capacity to bio-accumulate high concentrations of toxic chemicals in their tissues and kill any pathogen by discharge of anti-pathogenic 'coelomic fluid' and the resulting treated wastewater becomes almost free of chemicals and pathogens to be reused for non-potable purposes [6]. Earthworms will provide low cost solutions to several social, economic and environmental problems of human society. They are both 'protective' & 'productive' for environment and society [7].

In previous studies by [8], the POME had been neutralized by adding Calcium Hydroxide into the POME before introduced into the system. The pH of the POME was adjusted to pH 5-7. The performance of the vermifilter and control system for palm oil mill effluent (POME) treated using the earthworm, *Lumbricus rubellus* were compared based on the water quality measured, with results pointing to the vermifilter's promising potential in reducing the COD and TSS against the control.

The present study employed African Night Crawler *(Eudrilus Eugeniae)* in treating raw untreated POME and focused on remove BOD, COD, and TSS from untreated raw POME using vermifilter system without change the pH of the POME. Besides, in this study the effect of treated POME after through vermifilter system on the growth of selected plant in soil-less culture through seed germination will be investigate to be platform for further scientific study in palm growing countries and to suggest that the POME vermifiltrate can be used safely for agricultural cultivation.

II. MATERIALS AND METHODS

A. Raw Sewage

Raw palm oil mill effluent (POME) that used in this experiment was obtained from MALPOM Sdn. Bhd. in Nibong Tebal, Pulau Pinang. It was stored in 10L plastic bottles and was placed in laboratory under room temperature (21 – 25 °C) to allow the POME to cool down. Table 1 shows the characteristics of raw POME that obtained during the experiment. There is great fluctuation in these values depending upon catchment area and flow rate.

Table 1: Characteristics of raw POME

Characteristics	pH	BOD_5 (mg/L)	COD (mg/L)	TSS (mg/L)	Turbidity (NTU)
Raw POME	4.9 - 5.3	25 000 – 30 000	50 000 – 61 000	20 000 – 25 000	15 000 – 20 000

B. Vermifilter setup

Figure 1 presents the schematic diagram of the vermifilter reactor system which was constructed in close area at the laboratory of School of Industrial Technology, Universiti Sains Malaysia. The bottom most layer is made of gravels of size 2 cm – 3 cm in diameter and it fills up to the depth of 5 cm. Above this lies 5 cm layer of gravels of 0.5 cm – 1.0 cm in diameter. On the top of this is the 5 cm layer of sand of 5 mm – 10 mm sizes. The topmost layer of about 15 cm consists of the mixture of sand and garden soil act as vermibed in which the earthworms are released. Then, the POME is soaked in the medium for one night. This purpose is to stabilize the medium before earthworms is released into reactor. The next day, the earthworms were released at the topmost layer. Earthworms, African Night Crawler were introduced into the vermibed at density of 8 g/L with the flow rates 100 ml/min. The worms were given around one week settling time in the vermibed to acclimatize in the new environment. The density of the earthworms were chosen based on the studies by [9] concerning the performance and mechanisms of a microbial-earthworm ecofilter for removing organic matter and nitrogen from synthetic domestic wastewater. The POME percolated down through various layers in the vermifilter passing through the vermibed inhabited by earthworms, the sandy layer and the gravels under gravitational influence and at the end, the vermifiltrate (the treated wastewater) was collected from the bottom of the system.of raw POME that obtained during the experiment. There is great fluctuation in these values depending upon catchment area and flow rate.

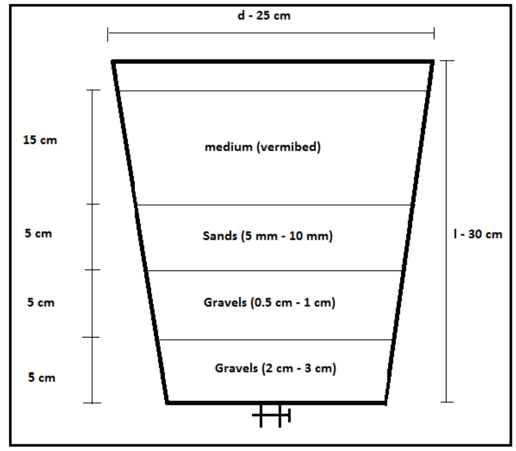

Figure 1: Schematic diagram of filter reactor

C. The control reactor without earthworms : comparison to assess the precise role of earthworms as biofilter

A control reactor (CF) is the exact replica of vermifilter reactor (VF) but devoid of earthworm. The CF was organized for reference and comparison. It is important to note that the medium, sand particles and the gravels in the reactor also contribute in the filtration and cleaning of POME by adsorption of the impurities on their surface. They provide ideal sites for colonization by decomposer microbes which work to reduce BOD_5, COD, turbidity, and TSS from POME. As the POME passes through, a layer of microbial film is produced around them together they constitute the 'geological' and the 'microbial' system of POME filtration. With more POME passing through the gravels there is more formation of 'biofilms' of decomposer microbes. Hence it is important to have control reactor to determine the precise role of earthworms in the removal of $BOD_{5,}$ COD, turbidity and TSS.

D. Parameter studied in POME vermifiltration

The POME that was fed to the vermifilter reactor (VF) and control reactor (CF) which was collected at the bottom of the reactor in a chamber were analyzed to study the biochemical oxygen demand of 5 days (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), turbidity and the pH value based on the guidelines of APHA Standard Methods for examination of water and wastewater and Standard Methods For The Examination of Water & Wastewater 21st Edition.

E. Germination test

Germination tests were conducted to obtain data on germination percentage of lettuce seed (L. sativa L.) with commercial hydroponic solution as control along with five different concentrations (1%, 3%, 10%, 30% and 100%) of treated POME collected after vermifiltration process. A piece of filter paper moistened with sample (10ml) was placed in petri plate. Seeds were treated with 0.2 N mercuric chloride for 2 minutes and washed with distilled water prior to its germination to remove contamination on seed coat. Lettuce seeds were placed on filter paper in a petri plate and incubated for 120 hours in dark condition at constant temperature 22°C ± 2°C. Germination of lettuce seeds were observed after 120 hours.

III. RESULTS AND DISCUSSION

A. The pH value of treated POME

Results indicate that the pH value of raw POME was improved during the treatment process in both reactor, vermifilter reactor (VF) and control reactor (CF) (Figure 2). The pH of the raw POME is within the range of 4.9 to 5.3. Based on studies, after passing through the both treatment process (VF and CF), the pH of effluent increased to the 6.9 - 7.8 range. In the VF, the pH was within the 7.0 - 7.8 range, which could be due to earthworm mediated rapid mineralization of organic fractions of wastewater. Earthworm activity caused an in-built pH buffering ability by increasing the pH, hence neutralizing the sewage wastewater [10]. In the CF, the pH was within the 6.8 – 7.3. The pH value of the VF and CF improved throughout the experimental study.

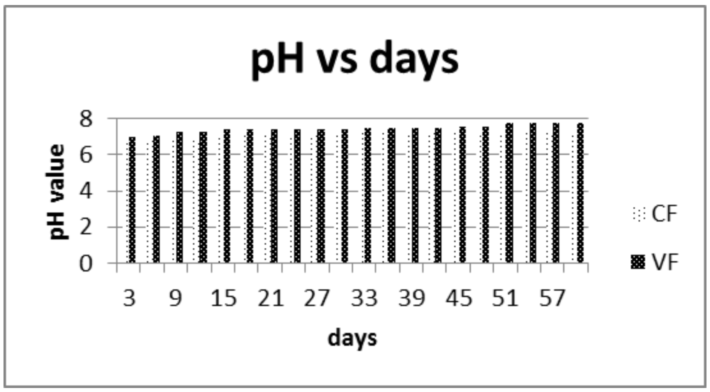

Figure 2: pH value vs day (CF and VF)

B. Removal of BOD_5

The Results in Figure 3 shows that the removal efficiency of BOD_5 of the treated POME in the VF is higher than removal efficiency of BOD_5 in CF, 92% and 55 % respectively. Since the earthworms are primarily accountable to biodegrade waste as compared to inorganic waste through enzyme as a biocatalysts to quicker the rate of biochemical reaction, BOD removal efficiency was found to be much better than that of BOD_5 removal efficiency in CF.

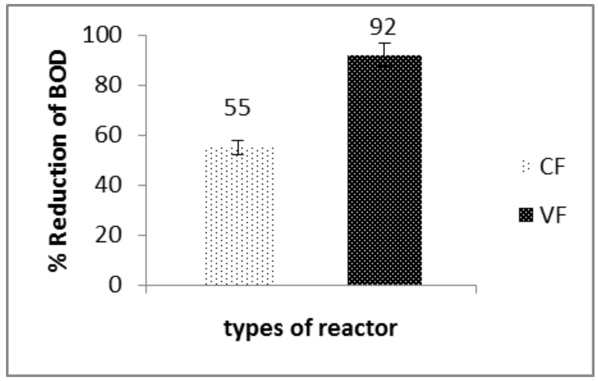

Figure 3: Percentage of reduction in BOD_5 of POME treated by CF and VF.

C. Removal of COD

Figure 4 indicates the COD removal efficiency for both types of reactor, vermifilter reactor, VF (with earthworms) and control reactor, CF (without earthworms) during the experimental time. Results show that the average COD removed from the POME by earthworms is over 90% while that without earthworms is just over 45%. COD removal by earthworms is not significant as the BOD, but at least much higher than the microbial system. The enzymes in the gut of earthworms help in the degradation of several of those chemicals which cannot be decomposed by microbes.

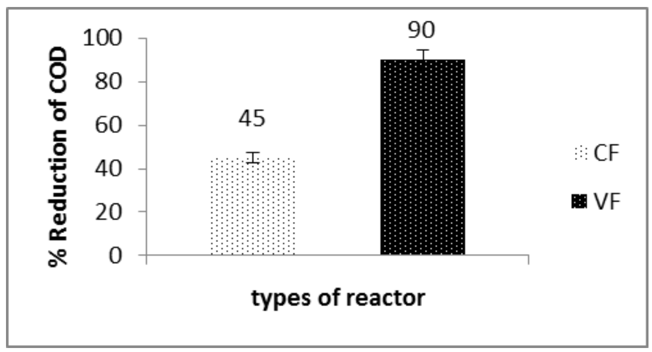

Figure 4: Percentage of reduction in COD of POME treated by CF and VF.

D. Removal of TSS

Results shows that the earthworms can significantly remove the suspended solids from the POME by over 95%, which in the control reactor (where geological and microbial system works together) is over 65% only (Figure 5). These solids accumulate over time as 'sludge' and choke the system which ceases to work. However, in the VF, the results suggest earthworms to possess the capability in removing solid fractions from wastewater during VF processes. Suspended solids are trapped on the surface of vermifilters and processed by earthworms, being subsequently fed to soil microbes immobilized in the vermifilter [5]. This explains why the vermifilter reactor did not choke and work smoothly. Earthworms eat up the solids and also improve the 'adsorption' properties of the geological system by grinding them in their gizzard.

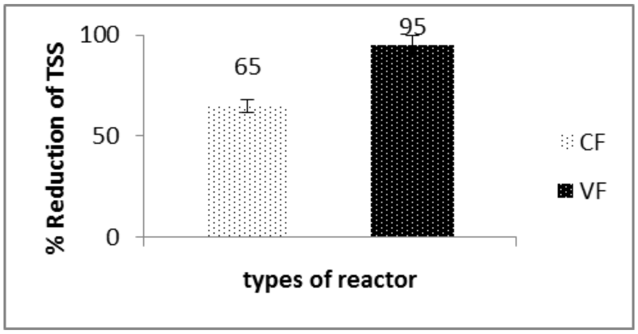

Figure 5: Percentage of reduction in TSS of POME treated by CF and VF.

E. Removal of turbidity

Results shows that the average reduction in turbidity is over 86% for vermifilter reactor (VF) and 52% for control reactor (CF) (Figure 6). The geological system plays very important role in turbidity removal by 'adsorption' of suspended particles on the surface of the vermifilter medium, sand and gravels in addition to the earthworms' activity.

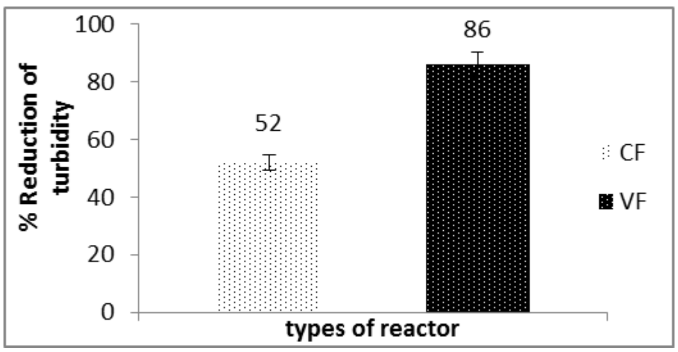

Figure 6: Percentage of reduction in turbidity of POME treated by CF and VF

F. Phytotoxicity

Germination test was conducted by using lettuce seed (L. sativa L.). Germination percentage was calculated and showed in Figure 7. Seed germination has been used for evaluating the maturity of compost in degree of decomposition of phytotoxic organic substances [11]. However, according to [12], seed germination also a well-known and rapid method for phytotoxicity test. Germination percentage of >50% indicates the maturity of the compost [13] and findings (Figure 7) showed germination percentage higher than 50% only for control, 1% and 3% treatments. For 10%, 30% and 100% treatments showed germination percentage lower than 50%. Phytotoxic effects of organic wastes are due to several factors, including heavy metals [14] and salts [15]. POME contains high electrical conductivity that might be one of the factors that may inhibit plant growth and germination [16]. Therefore, it explained the lower germination percentage showed by 10%, 30%, and 100% concentration of treated water because its contains POME with high electrical conductivity. Besides, according to [14] the delay in germination can cause by metal content present which explain lower germination percentage exhibits in treated water because POME contains high heavy metals.

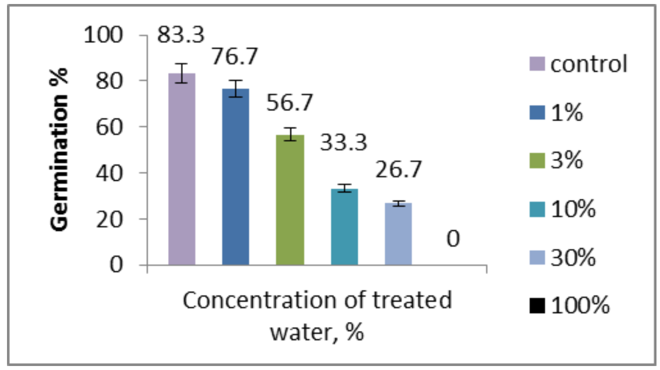

Figure 7: Germination percentage of control (commercial hydroponic solution) and different concentration of treated water (POME 1%, 3%, 10%, 30% and 100%).

IV. CONCLUSION

Vermifiltration system was effective to remove biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solid (TSS) and turbidity in POME. However, in the control reactor (CF) the systems fails to work for longer time as it is frequently choked due to the formation of sludge and colonies of bacteria and fungi in the absence of the earthworms. POME treated by vermifilter (VF) indicates a better reduction in BOD_5, COD, TSS and turbidity compared to CF system. The pH of the treated POME in VF increased from pH 7.0 to pH 8.0 and remained stable throughout the process. The pH comply the Malaysia Sewage and Industrial Effluent Discharge Standard. The result of germination percentage showed germination percentage higher than 50% only for control, 1% and 3% treatments, so its proved the maturity of the compost. For 10% and 30% treatments showed germination percentage lower than 50%. For, 100% treatment showed 0% and its proved that 100% concentrations of treated POME not safely used for agricultural purposes. Therefore, vermifiltration of POME had been found to be generally good and effective way to treat POME but not good enough for agricultural purposes because it contains high toxicity level.

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