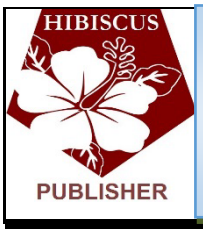
JOBIMB, 2023, Vol 11, No 2, 20-23
https://doi.org/10.54987/jobimb.v11i2.847



JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY
Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

Biological and Physicochemical Evaluation of Palm Oil Mill Effluent Final Discharge from Negeri Sembilan, Malaysia

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HISTORY

Received: 27th Oct 2023
Received in revised form: 21st Dec 2023
Accepted: 25th Dec 2023

KEYWORDS

Atrazine
Bacteria
Biodegradation
Bioremediation
Pollution

ABSTRACT

Palm Oil Mill Effluent (POME) contains a high number of organic materials that cause deleterious effects on the aquatic ecosystem when discharged into water bodies without proper pretreatment. The quality of POME final discharge is usually determined based on chemical monitoring methods such as Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). In this study, biological monitoring methods were used to evaluate the toxicity effects of POME final discharge and to characterize the physical and chemical nature of toxicants present in the effluent through acute Whole Effluent Toxicity (WET) using *Daphnia magna*. The Toxicity Unit (TU) and median lethal concentration (LC_{50}) of POME final discharge from the WET test were 11.09 and 9.02% (v/v), respectively. It is recommended that this method be improved to explore more effects of POME final discharge on the aquatic ecosystem.

INTRODUCTION

A tremendous number of organisms with approximately 6.5 million species on land, while another 2.2 million marine species exist globally [1]. Given these numerous organisms, it is impossible to perform ecotoxicity tests and ecological risk assessments of certain chemical effects towards a wide range of organisms across all environments. The United States Environment Protection Agency (EPA) has summarised the guidelines for Whole Effluent Toxicity (WET) tests using living aquatic organisms by highlighting the recommended keystone species of plants, invertebrates and vertebrates such as *Daphnia magna* (Planktonic crustacean), *Pimephales promelas* (Fathead Minnow), *Selenastrum capricornutum* (Green alga) [2]. Assessment of aquatic toxicity is very important because changes in water parameters such as temperature, pH or dissolved oxygen (DO) result in negative impacts on the diversity of the aquatic ecosystem [3]. Discharge of domestic and industrial waste such as palm oil mill effluents (POME) and other industrial wastes must be treated and restricted to a certain permissible limit before being released into the environment to avoid severe water pollution. POME is the waste generated from crude palm oil (CPO), estimated to be produced in an even greater amount at approximately thrice the quantity of CPO itself [4]. Biological toxicity-based monitoring using whole effluent toxicity (WET) has been acknowledged. In addition, biological monitoring can demonstrate the responses of biological communities towards the presence of contaminants or other factors of environmental stress in the ecosystem [5].

Currently, the common method for monitoring the quality of POME in palm oil mills in Malaysia is physical monitoring such as effluent clarity, colour or smell, and chemical monitoring such as BOD, COD, DO, pH, and temperature. Although chemical and physical monitoring methods can provide reliable results, these methods are unable to detect the whole changes in the composition of biological communities caused by pollutants in the water [6]. Besides, hazardous substances and metals in POME final discharge cannot easily be found using physical or chemical monitoring techniques [7]. In order to give additional

platforms for appropriate evaluation, this research also attempts to assess the sample toxicity using biological testing such as by employing *Daphnia magna* in highly potential toxic media.

MATERIALS AND METHODS

Collection of sample

The Palm Oil Mill in Negeri Sembilan, Malaysia, was a location for the sample of POME final discharge collection (GPS Latitude and Longitude: 3.0506, 102.2864). The lab and the palm oil factory are located 117 kilometres apart. Therefore, in order to make the sampling process easier, the tests and sample analysis were done off-site, and extra precautions were taken to maintain the quality of random sampling and reduce analytical mistakes. Three glass laboratory bottles with screw lids were filled with the POME final discharge sample (3L), and the bottles were covered with aluminium foil. These bottles were kept in a cold box (>6°C) to guarantee there were no air gaps between the contents and lid. POME was tested and analyzed following US EPA, 2002 [2].

Physicochemical characterization of POME final discharge Determination of five days biochemical oxygen demand (BOD_5)

The biochemical oxygen demand (BOD_5) test was performed according to the American Public Health Association 5210-B, 5-Day BOD test [8].

Preparation of BOD_5 test reagents

Phosphate buffer solution

Potassium dihydrogen phosphate, KH_2PO_4 (8.5 g), dipotassium hydrogen phosphate, K_2HPO_4 (21.8 g), disodium hydrogen phosphate heptahydrate, $Na_2HPO_4{\cdot}7H_2O$ (33.4 g) and ammonium chloride, NH_4Cl (1.7 g) was dissolved in 500 mL of distilled water and diluted to 1 L.

Magnesium sulphate solution

Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$ (22.5 g) was dissolved in distilled water and diluted to 1 L.

Calcium chloride solution

Anhydrous calcium chloride, $CaCl_2$ (27.5 g) was dissolved in distilled water and diluted to 1 L.

Ferric chloride solution

Iron (III) chloride hexahydrate, $FeCl_3{\cdot}6H_2O$ (0.3 g) was dissolved in distilled water and diluted to 1L.

Sodium hydroxide solution

Sodium hydroxide, NaOH (40 g) was dissolved in 20 mL distilled water and diluted to 1 L.

Sulphuric acid solution

Concentrated sulphuric acid, H_2SO_4 (56 mL) was diluted to 1 L.

Sodium sulphite solution

Na_2SO_3 (1.6 g) was dissolved in 1 L distilled water.

BOD dilution water

Distilled water free of heavy metals and toxic substances was used to make sample dilutions.

Determination of dissolved oxygen (DO) concentration of POME

The instrument manual's instructions were followed in order to calculate the sample's DO concentration [10]. In order to generate a 100% relative humidity calibration environment, the portable DO meter was calibrated utilizing the air calibration technique, which involved placing the DO meter probe above water within a BOD bottle. Following calibration, the POME sample was submerged in the DO probe, which was then attached to a stirrer, and the dissolved oxygen content in mg/L was measured [10].

BOD_5 testing procedure

DO concentration was measured using a portable dissolved oxygen meter (YSI Incorporated, United States). In distilled water (1.5 L), one mL of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride was added and mixed thoroughly. Water and POME were adjusted to 20 ± 3°C, and the pH of the sample was around 7.0 to 7.2 using sulphuric acid (or sodium hydroxide). Five dilutions (5%-25%) were prepared in triplicates, including blanks in 300 mL BOD bottles (with a stopper). The bottles were filled with dilution water and parafilm tape and aluminium foil were used for complete sealing. Initial DO concentration was determined within 30 min after preparation, and then the BOD bottles were incubated in the dark (20°C). The final DO was measured after five days and readings were gathered based on various concentrations of samples [8].

Calculation of BOD_5

The equation that was used to determine BOD_5 value:

$$\text{BOD (mg/L)} = \frac{([\text{DO initial} - \text{final}] \times [\text{Volume bottle}])}{\text{Sample volume}} \quad \text{(Eq. 1)}$$

where:
DO initial = DO of diluted sample after preparation, mg/L
DO final = DO of diluted sample after incubation (five days, 20°C), mg/L
Volume of BOD bottle = Volume of BOD bottle, mL
Sample volume = Volume of the sample within the dilution, mL

Determination of chemical oxygen demand (COD): Reactor digestion procedure

The chemical oxygen demand (COD) value was determined using HACH Digital Reactor Block 200 (DRB 200) Instrument Manual [9]. The reactor was pre-heated to 150°C. POME sample (2 mL) was inserted into a digestion reagent vial of range 3-150 mg/L COD at an angle of 45 degrees using a clean pipette. For the blank, distilled water (2 mL) was added into the vial, closed tightly and inverted gently. After 2 hours, the vials (120°C) were cooled down and inverted while still warm to room temperature.

Colorimetric procedure

A wavelength of 420 nm was used to measure the COD's sample colourimetrically. The measurement was carried out in mg/L [9].

Daphnia magna cultures feed preparation

For culture feed preparation, 10 mg of dry yeast (*Saccharomyces cerevisiae*) was added to a glass bottle filled with 100 mL of distilled water. A magnetic stirrer was used for uniform mixing and fresh solution was prepared every week.

Set-up and maintenance of *Daphnia magna* culture

D. magna (fifteen neonates), aged 24 hours, were raised in a plastic aquarium filled with 13 litres of water and one milliliter of dechlorinator. The culture was inspected every day at 20 ± 1°C and was photoperiod regulated, with 16 hours of light illumination and 8 hours of darkness (US EPA, 2002). Daphnids were fed on a consistent schedule, once a day, with 0.5 mL of yeast stock solution and every two weeks, fresh cultures were made [12].

Acute Whole Effluent Toxicity (WET) testing of POME

According to the acute toxicity test [2], a multi-concentration or definitive test was carried out using four replicate chambers for each of the five effluent concentrations, plus a control. The POME sample concentrations (0%, 6%, 12%, 25%, 50%, and 100% v/v) were applied with distilled water. For the 48-hour acute toxicity test of POME, *D. magna* was subjected in a static non-renewal condition. After transferring 20 mL of the test solution (including the control) into a 50 mL glass beaker, *D. magna* was added and allowed to remain in each concentration for 48 hours. The photoperiod condition was used for the WET test. The survival of *D. magna* was considered as the endpoint, which served as a toxicity indicator of the POME sample. Probit analysis was used in order to determine the LC50 values and the final result was presented in the Toxic Unit (TU). This analysis was done in 4 replicates [2].

RESULTS AND DISCUSSION

Collection, handling and preservation

Several precautionary measures were carried out in order to maintain the integrity of the POME sample. The sample was immediately placed in a chilled box to maintain its temperature at 0-6 °C until used to reduce microbial degradation, chemical alterations and depletion of highly volatile toxicants [2].

Physicochemical characteristics of POME final discharge sample

There are two standards outlined by DOE, Malaysia: Standard A and Standard B for an acceptable limit of parameters for industrial effluent, which includes pH value, BOD, COD, total suspended solids, and mineral contents [13]. **Table 1** shows the BOD_5 value of POME (23.40 mg/L) that met the minimum allowable effluent discharge of Standard B (40 mg/L) but exceeded the limit for Standard A (20 mg/L) [13]. The DO obtained was 3.05 mg/L. To survive, the DO is necessary for various life forms, including fish, invertebrates, bacteria, and plants. DOE has imposed a more stringent limit for the permitted discharge limit of BOD value in East Malaysia, whereby BOD value must be less than 20 mg/L [14].

The BOD is one of the significant measures used to indicate organic material pollution in water, whereby a higher BOD value indicates poorer water quality. The chemical oxygen demand (COD) value of POME final discharge obtained was 220 mg/L, exceeding the minimum permissible limit for both Standard A (80 mg/L) and Standard B (200 mg/L). The COD analysis measures the oxygen-depletion capacity of a water sample, which correspondently indicates the quantity of organic and inorganic matter that is susceptible to oxidation in polluted water. Thus, COD analysis can be done to measure the degree of pollution in water bodies and also the efficiency of treatment plants.

The pH value of POME was 8.04 and met the minimum limit of acceptable pH value of effluent discharge of both Standard A (pH 6-9) and Standard B (pH 5-9). Acidic conditions caused by low pH and basic conditions caused by high pH can alter the enzyme's structure and inhibit growth. However, most microorganisms can survive within the pH range of 6.5 to 8.5, where this range is parallel to the standard limit implemented by DOE [15].

Table 1. Summary of physicochemical characteristics of POME final discharge.

Parameter	Replicate 1	2	3	Mean	Stdev*	RSD (%)*
BOD_5 (mg/L)	23.49	23.50	23.20	23.40	0.170	0.73
COD (mg/L)	220.00	220.00	220.00	220.00	0.000	0.00
DO (mg/L)	3.03	3.06	3.06	3.05	0.017	0.56
pH (mg/L)	7.99	8.09	8.03	8.04	0.050	0.62

*Stdev, Standard deviation; RSD, Relative standard deviation

Culture of *Daphnia magna*

The culture of *D. magna* was started with individuals that were less than 24 hours old in order to minimize clonal differences in traits like age maturity and brood size. *Daphnia magna* are filter feeders that can separate tiny food particles, therefore, a yeast suspension was provided as a useful feeding alternative [12].

Acute Whole Effluent Toxicity (WET) of POME final discharge

To assess the toxicity of POME final discharge on *Daphnia magna*, an acute whole effluent toxicity (WET) test was performed (**Table 2**). The term "WET" describes an effluent's total harmful effect and the endpoint of a WET test is the organism's death test such as the percentage of mortality [16]. The percentage of *D. magna* individuals that die increases with increasing POME concentration (%, v/v) (**Fig. 1**). For the POME final discharge sample, the median lethal concentration (LC_{50}) was determined to be 9.02% (v/v) (**Table 3**). According to Albanese et al. [17], the higher the toxicity, the lower the LC_{50} value. Therefore, the LC_{50} value found indicates that half of the starting population of *D. magna* could become fatal at a low concentration of a final discharge. The final discharge sample had a TU of 11.09 (**Table 3**), which indicates that the sample was toxic.

Secondary data was taken for comparison, and the LC_{50} value of the POME final discharge sample (this study) was compared with other samples (from a palm oil mill in the East Coast of Malaysia, Terengganu) [7]. LC_{50} was much lower (9.02%, v/v) for the POME sample from this study than it was for the other sample (15.63%, v/v). The other POME samples (from Terengganu) and this study yielded TU values of 6.40 and 11.09 respectively. Because of this, the LC_{50} and toxic unit values found suggested that the sample from this study was more harmful to *D. magna* even at low concentrations. Conversely, the outcomes of the five-day chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) tests contradicted the findings of the biological monitoring. In contrast to the COD and BOD_5 samples from the earlier study (Terengganu), which had values of 236 mg/L and 73 mg/L, respectively, the BOD_5 and COD levels in this investigation were 220 mg/L and 23.40 mg/L, respectively. The Terengganu sample was found to be more harmful, which is counter to the findings of the biological monitoring. These results showed that both approaches are crucial for water quality monitoring, maybe because POME contains low concentrations of pollutants that are fatal to some aquatic life. As a result, biological monitoring may be seen as an additional strategy for interpreting water quality.

Table 2. Corrected percentage mortality for various POME concentrations in a 48 hours acute WET test.

POME (%)	Replicate 1	2	3	Mean	Stdev*	RSD (%)*
0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.0	20.0	20.0	20.0	20.0	20.0	0.0
12.0	40.0	40.0	40.0	40.0	40.0	0.0
25.0	80.0	80.0	80.0	80.0	80.0	0.0
50.0	100.0	100.0	100.0	100.0	100.0	0.0
100.0	100.0	100.0	100.0	100.0	100.0	0.0

*Stdev, Standard deviation; RSD, Relative standard deviation

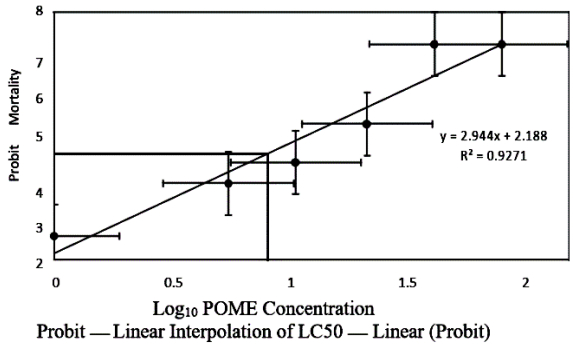

Fig. 1. Acute WET test (48-hour) of probit transformed responses for *Daphnia magna*'s total death when exposed to graded concentrations of POME final discharge.

The information displayed showed the mean (± SEM) percentage of *D. magna* corrected mortality exposed to various POME concentrations. The vertical solid line indicates the genuine median lethal concentration (LC50) for the concentration-response curve, whereas the solid horizontal line reflects the Probit score linked to a 50% death of *D. magna*. N=10 *D. magna* individuals for each treatment of POME concentration.

Table 3. Comparison of properties of POME final discharge samples obtained from different palm oil mills.

Parameter	Unit	POME final discharge (Negeri Sembilan)	POME final discharge (Terengganu)
BOD_5	mg/L	23.40 ± 0.73	73.00 ± 17.00
COD	mg/L	220.00 ± 0.00	236.00 ± 0.00
LC50	(%, v/v)	9.02	15.63
TU	-	11.09	6.40

CONCLUSION

The toxicity of Palm Oil Mill Effluent (POME) final discharge was evaluated by acute Whole Effluent Toxicity (WET) test using *Daphnia magna.* The chemical and physical nature of toxicants in POME final discharge was characterized to identify the causative toxicants in the effluent. The values of five days of Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO) concentration, and pH of POME final discharge were 23.4 mg/L, 220 mg/L, 3.05 mg/L and 8.04, respectively. The conventional chemical parameter, BOD_5 value, met the Standard B effluent standard limit by the Department of Environment, Malaysia. However, by using the biological monitoring method, an acute WET test showed contradictory results as the Toxic Unit (TU) of the POME final discharge was 11.09 and the median lethal concentration (LC50) was 9.02% (v/v). The high TU and the low LC50 obtained for the POME final discharge suggested that the POME sample had a high toxicity level and contained potential toxicants that were lethal to aquatic organisms even at low concentrations. Therefore, further research for the broader applicability of biological monitoring needs to be conducted to detect a broader and more diverse set of chemicals that may be released from the mills.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Higher Education, Malaysia, for providing the research grant under the Fundamental Research Grant Scheme (FRGS/1/2020/STG01/UPM/02/14; Vote 5540357; Project Code 01-01-20-2225FR) along with necessary facilities to carry out the research.

ABBREVIATION

Palm Oil Mill Effluent – POME
Chemical Oxygen Demand – COD
Biochemical Oxygen Demand – BOD
Whole Effluent Toxicity – WET
The Toxicity Unit – TU
Median lethal concentration - LC50
Environment Protection Agency – EPA
Whole Effluent Toxicity – WET
Dissolved oxygen – DO
Crude palm oil – CPO
Whole effluent toxicity – WET

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