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## **Prediction of Optimal C: N Ratio in Different Palm Oil Mill** Waste Mixtures and Its Evaluation of Earthworm Biomass

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Abstract. The oil palm industry has been recognized for its contribution towards economic growth and rapid development, it has also contributed to environmental pollution due to the production of huge quantities of by-products from the oil extraction process. Current research reports prediction of optimal CN ratio of different mixture percentages of palm oil mill effluent (POME) and palm pressed fiber (PPF) namely 100% (only POME), 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% and 0% (only PPF). Different mixture percentages with varying initial characters results in different C:N ratio which could affect the earthworm growth and the vermicomposting stability. Therefore, the present research aims to predict the optimal mixture by establishing a 3D base model. The models were generated having an average unified formula using mathematical software Matlab. The results obtained in this study indicates that the 3D polynomial graph can explain the relationship between different POME-PPF concentration with respect to earthworm growth and time showing ideal  $R^2$  value of 0.99. The regression analysis showed positive correlation between different mixture percentages and earthworm growth in 50% (r = 0.412), 60% (r = 0.509) and 70% (r = 0.441). Therefore, from the model it can conclude that 60% mixture of POME-PPF is an optimal mixture for the vermicomposting process.

#### **1. Introduction**

This experiment therefore, was carried out to test how the different mixtures of POME-PPF namely 100% (only POME), 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% and 0% (only PPF) with having different starting CN ratios can affect the resultant CN ratio and earthworm growth. Carbon and nitrogen are two primary nutrients required for cell growth; therefore an optimal carbon to nitrogen ratio is necessary for vermicomposting process [1]. Technological application is to make the best use of epigeic group of earthworms for their ability to feed on partially degraded organic wastes. Earthworms that can survive on different organic substances, tolerate a wide range of temperature and pH are best suited for culturing [2].



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POME is one of the by-products which produces in abundant from the palm oil mills. Palm oil mill board of Malaysia reported discharge of 44 million ton in 2014 [3]. POME is acidic in nature and releasing it naturally is harmful for the environment and causes depletion in water bodies. Rupani et al [4], studied on the applicability of acidic POME for vermicomposting technology and could successfully achieve matured compost (CN-14) in 40 days of experiment. Yet, no further study conducted on evaluating the optimal mixture for vermicomposting process of palm oil mill wastes. This study aims to evaluate the kinetic study of different mixtures of POME-PPF and generate a mathematical model in order to predict the optimal mixtures.

#### 2. Material and Methods

#### 2.1. Material

The fresh palm oil mil effluent (POME) and palm pressed fiber (PPF) were obtained from MALPOM industry Sdn. Bhd, Nibong Tebal, Malaysia. The earthworms belong to E. eugeniae species were achieved from the vermiculture unit (Penang, Malaysia). The culture of the earthworm was maintained in plastic containers with partially decomposed mixture of bio-waste (as a growth medium) under laboratory conditions  $(26\pm2 \text{ C})$  for further use.

#### 2.2. Experimental Setup

A fixed amount of 50 g of well mixed POME and PPF of different combination (namely: 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% and 0% POME - PPF) on a wet weight basis were placed in each reactor. All the vermireactors were set in triplicates, with a total of thirty three experimental units. As this experiment focuses on the efficiency of the earthworm in different mixtures, hence, each reactor offered as a comparison study for the other. Therefore, after 5 days of pre-composting, 10 g sample from each mixture was placed in a petri dish to conduct the vermicomposting experiment. pH were analysed using pH Meter (Hach Sension 3); total organic carbon (TOC) and total nitrogen (N) were measured by CHN analyser (Shimadzu Model 2400), [5]

#### 2.3. Statistical Analysis and Modelling

The data was subjected to ANOVA, Post-Hoc Tukey's test to determine the significant differences between the parameters at 5% confidence level. Data were analysed using the regression analysis to find the model that describes the relationship between independent variables (pH and C: N ratio) and the dependent variable (earthworm biomass). Polynomial model was used to fit the data for different POME-PPF mixtures to predict the optimal changes in carbon to nitrogen (C: N) ratio in relation to different POME-PPF mixtures by using Matlab software [6].

#### 3. Results and Discussion

#### 3.1. pH Changes in Different POME-PPF Mixtures

The pH of different POME-PPF mixtures during the vermicomposting process through 15 days is shown in Figure 1. The initial pH was in the range of 5.3 - 5.8 in different mixtures which increased to 6.3 to 7 in 15 days of vermicomposting. The highest pH value (7.1- 7.13) on the 15<sup>th</sup> day was observed in 50 and 60% mixtures of POME-PPF. The results of ANOVA for pH during vermicomposting of different POME-PPF mixtures show significant difference (P < 0.05) on 15<sup>th</sup> day, while on 0, 5<sup>th</sup> and 10<sup>th</sup> day no significant difference (P > 0.05) was observed. The pH may be due to the rapid metabolic degradation of organic acids, as well as intense proteolysis of alkaline ammonia due to protein degradation [7], [8]. Earthworms in 100% mixture of POME-PPF could not survived, therefore, data pertaining 100% eliminated from the results.

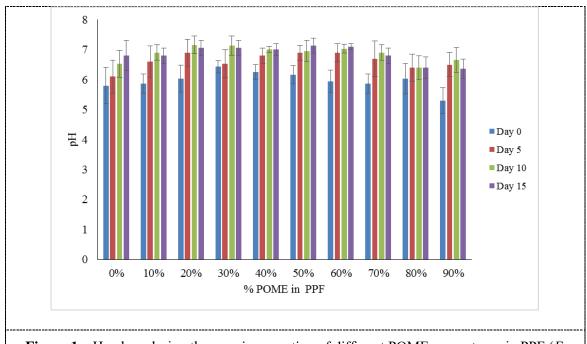
#### 3.2. CN Changes in Different POME-PPF Mixtures

Figure 2 shows the CN ratio of different POME percentages mixed with PPF using *E. eugeniae*. Initially CN was in the range of 50.26 - 25.2 in POME-PPF mixtures. The figure revealed that all the treatments showed a reduction in the CN ratio throughout the vermicomposting process, ranging between 35.27 and 9.6 in different mixtures using *E. eugeniae*. Standard deviation from 40 to 90% was minimal and is not feasible to illustrate graphically in bar charts. The results of ANOVA for CN

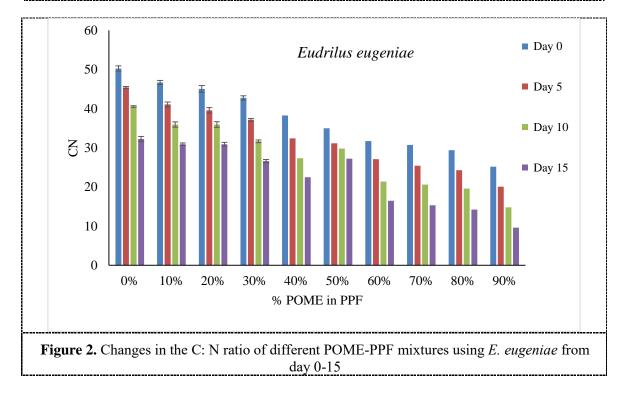
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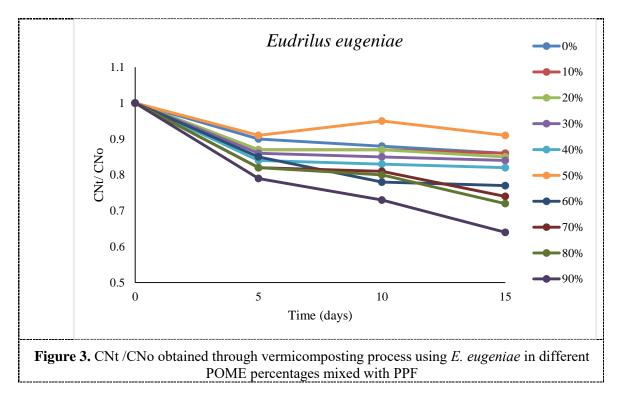
ratio of different POME-PPF mixtures show significant difference (P < 0.05) in all the mixtures during the vermicomposting process.

In order to normalize the CN ratio, the CN ratio at any time of measurement (CNt) was divided to CN at time zero (CNo). Thus, at starting time, t=0 the CNt /CNo is equal to 1.0 to measure the efficiency of CN stabilization (Figure 3). The normalized CN ratio which starts from 1 could clearly demonstrate the significant decreasing pattern in all the mixtures (P < 0.05). The results show that the maximum normalized value was achieved in 50% mixture of POME-PPF due to the equal ratio of POME and PPF.



**Figure 1.** pH values during the vermicomposting of different POME percentages in PPF (*E. eugeniae*)





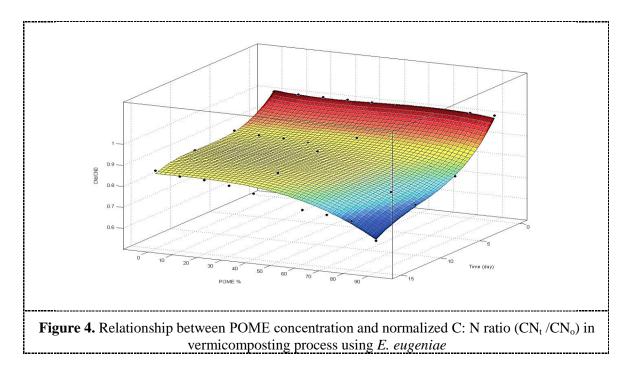
#### 3.3. The Relationship between POME Concentration and CN Ratio with Time

In order to relate dynamic CN ratio of different POME concentrations, a surface fitting method was applied. A 3D polynomial graph of normalized CN ratio obtained from different POME-PPF percentages as shown in Figure 4. The equations represents the fitted polynomial surface (Eq) which can be very useful in order to identify the corresponding values lying in between the collected data. In other words, the equation gives the fraction of the reduction in CNt /CNo with respect to time and make possible predictions and comparison.

 $Y = 1.008 - 0.0426 x - 0.001247 z + 0.004784 x^{2} + 0.0004697 xz + 0.0000378 z^{2} - 0.0001743 x^{3} + 0.000003133 x^{2}z$ (Eq.1)

The goodness of the fitting techniques for CNt /CNo and the square root of coefficient (R2) and the sum of squares of errors (SSE) were calculated. The values demonstrated as the goodness of the surface fitting is very high (0.027) which is very close to the ideal value (zero). It indicates that the model has a smaller random error component, and the fit can be more useful for the prediction of CNt /CNo. The square root of coefficient showed above 0.9 and very close to the ideal value (1). Therefore, it can be concluded that the fittings are very close to the actual data and it can serve as an ideal equation to predict the CN ratio at any time given.

The regression analysis were performed to evaluate the effect of pH and CN ratio on earthworm biomass. Regression models for earthworm biomass of different POME-PPF mixtures are given in Table 1. The results shows that the relationship between earthworm biomass and pH was highly positively related (r = 0.784) at 20% mixtures of POME-PPF and moderately positive related at 10% (r = 0.539), 50% (r = 0.412), 60% (r = 0.509) and 70% (r = 0.441) mixtures of POME-PPF, while in other percentages of POME-PPF, earthworm biomass were negatively correlated with pH. However, C: N ratio at 80% (r = 0.312) and 90% (r = 0.685) mixture of POME-PPF were positively related to earthworm biomass and other percentages were negatively related.



| <b>Table 1.</b> Regression analysis between earthworm biomass on pH and CN in different |  |  |  |  |
|---|--|--|--|--|
| POME % concentrations   |  |  |  |  |
|   |  |  |  |  |

| Earthworms as<br>dependent<br>variable in | Beta<br>Coefficient | Beta<br>Coefficient | Regression models                                       |
|---|---------------------|---------------------|---|
| POME-PPF                                  | (pH)                | (C: N)              |   |
| 0%  | - 0.045             | - 0.642             | $Ew_0 = 0.954 - 0.10 \text{ pH} - 0.16$<br>CN           |
| 10%                                       | 0.539               | - 0.420             | $Ew_{10} = 0.306 + 0.125 \text{ pH} - 0.08 \text{ CN}$  |
| 20%                                       | 0.784               | - 0.081             | Ew <sub>20</sub> = 1.128 + 0.214 pH - 0.002 CN          |
| 30%                                       | - 0.086             | - 0.643             | Ew <sub>30</sub> = 0.940 -0.029 pH - 0.015<br>CN        |
| 40%                                       | 0.429               | - 0.576             | Ew <sub>40</sub> = 0.938 + 0.309 pH - 0.024 CN          |
| 50%                                       | 0.412               | - 0.651             | Ew <sub>50</sub> = 0.784 + 0.238 pH - 0.065 CN          |
| 60%                                       | 0.509               | - 0.474             | Ew <sub>60</sub> = 0.724 + 0.238 pH - 0.020 CN          |
| 70%                                       | 0.441               | - 0.572             | Ew <sub>70</sub> = 0.307 + 0.166 pH – 0.020 CN          |
| 80%                                       | - 0.695             | 0.312               | $Ew_{80} = 0.088 - 0.017 \text{ pH} + 0.108 \text{ CN}$ |
| 90%                                       | - 0.096             | 0.685               | Ew <sub>90</sub> = 0.624 - 0.002 pH + 0.139 CN          |

#### 4. Conclusion

The results of this experiment showed that pH value showed significant differences through vermicomposting process except for 20% and 80%. However, CN value decreased significantly in all mixtures of POME-PPF. The results of regression analysis indicates that 60% mixture of POME-PPF can be select as an optimal mixture for vermicomposting of POME-PPF. This experiment can serve as

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a novel findings with regards to kinetic studies during vermicomposting process of palm oil mill by products. Further studies are recommended to generate a mathematical prediction model for any biowaste products.

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