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Full Length Research Paper

Impact analysis of palm oil mill effluent on the aerobic bacterial density and ammonium oxidizers in a dumpsite in Anyigba, Kogi State

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The effects of palm oil mill effluent (POME) on the total aerobic bacterial populations and ammonium oxidizers in the soil were assessed. This was done by culturing soil samples from an effluent dumpsite for the total aerobic bacterial counts and ammonium oxidizers. Results showed that the total aerobic bacterial populations in the POME soil samples ($9.6 \times 10^8 \pm 0.3$ at 20°C, $1.64 \times 10^9 \pm 0.2$ at 30°C and $1.07 \times 10^9 \pm 0.4$ at 40°C) were significantly higher ($P \leq 0.05$) than the counts for the non-POME soil samples ($4.5 \times 10^8 \pm 0.3$ at 20°C, $7.6 \times 10^8 \pm 0.3$ at 30°C and $5.9 \times 10^8 \pm 0.3$ at 40°C). In addition, ammonium oxidizers were isolated from the non-POME soil samples but not from the POME soil samples.

Key words: Palm oil mill effluent, total aerobic bacteria, ammonium oxidizers.

INTRODUCTION

Palm oil processing is carried out in mills where oil is extracted from the palm fruits. Large quantities of water are used during the extraction of crude palm oil from the fresh fruits and about 50% of the water results in palm oil mill effluent (POME). It is estimated that for 1 tonne of crude palm oil produced, 5 - 7.5 tonnes of water will end up as POME (Ahmad et al., 2003). It has been observed that most of the POME produced by the small-scale traditional operators in Anyigba, Kogi State undergo little or no treatment and is usually discharged into the surrounding environment.

The roles of total aerobic bacteria and ammonium oxidizers are very crucial to the fertility and agricultural quality of soil. It has been reported that the total aerobic bacteria help in the degradation of macromolecules from plant and animal remains, regulate most elemental cycles in nature and reduce the effects of pesticides in the soil by breaking them down to more soluble products (Dommergues, 1992). The ammonium oxidizers on the other hand participate in nitrogen fixation and other agriculturally beneficial processes (Dommergues, 1992). Nitrification is a microbial process by which reduced nitrogen compounds (primarily ammonia) are oxidized to nitrite and nitrate in the soil. The study of nitrification in soil is important from the standpoint of soil fertility and because of the potential adverse impact that nitrate and its denitrification products can have on the environment.

Untreated POME contains high concentrations of free fatty acids, starches, proteins and plant tissues (Bek-Nielsen et al., 1999) but it is non-toxic (Ma and Ong, 1982; Ngan et al., 1996). It has a high biological oxygen demand (BOD) which averages around 25,000 mg/l making it in this respect a 100 times more polluting than domestic sewage (Maheswaran and Singam, 1977). In terms of its population equivalent, the BOD generated by the palm oil industry in Malaysia in 1998 is equivalent to that generated by 38 million people (Bek-Nielsen et al.,

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1999). Organic matter plays an important role in soil productivity and the solids in raw POME are a good source of organic matter (Chan et al., 1980).
The aim of this paper, therefore, is to study the impact of POME on total aerobic bacterial populations and ammonium oxidizers in the soil.

MATERIALS AND METHODS

Sample collection

Sampling was done from two sites; a palm oil mill effluent dumpsite, and a non-effluent soil about 100 metres away which served as control. Sampling was done from the rhizosphere, which is that portion of the soil under the immediate influence of the plant root and also the region of the soil where microorganisms are found in greater numbers and diversity (Wollum, 1982). The samples were air-dried, crushed to fine particles and sieved using a 2 mm sieve. Stock solutions and subsequently, 10-fold serial dilutions were prepared by adding 1 g of each soil sample to 10 ml of sterile deionised water and serially diluted until the 10^{-7} dilution was obtained for each sample. Fresh samples were obtained for every test.

Total aerobic bacterial population isolation

This was carried out by the method of Wollum (1982) and involved the use of nutrient agar (International Diagnostic Group) modified with 0.05 g/l of *Nystatin* incorporation to prevent the growth of fungi. Plating was done in triplicates for each dilution by the spread plate technique. Inoculated plates were inverted and incubated at 3 different temperatures, 20, 30 and 40°C for a maximum of 72 h.

Ammonium oxidizers isolation

This was carried out using the method and composition described by Schmidt and Belser (1982). The inoculated plates were allowed to remain uninterrupted for about 30 min on the bench before they were inverted and incubated at 2 different temperatures, 25 and 30°C for a minimum period of 14 days (2 weeks). Isolated cultures were purified by repeated sub-culturing and microscopy carried out after Gram staining for morphology.

Test for the presence of nitrite

The test for the presence of nitrite was carried out using a modification of the method described by Schmidt and Belser (1982) which involves Greiss-Ilosvay reagent; the only modification being that suspensions had to be prepared by dissolving some part of the isolated cultures from the selective medium in 1 ml of sterile deionised water.

Statistical analysis

Data generated were analyzed using the parametric test, analysis of variance (ANOVA) at the $P \leq 0.05$ and $P \leq 0.01$ confidence limits.

RESULTS AND DISCUSSION

Total aerobic bacterial populations

After incubation, plates were counted with the aid of a colony counter (Mac-Anderson Instruments, U.K.) (Table 1).

Table 1. Total aerobic bacterial count at different temperatures.

Temperatures (°C)	Non-POME (x 10^8)	POME (x 10^8)
20	4.5 ± 0.3	9.6 ± 0.3
30	7.6 ± 0.3)	16.4 ± 0.3**
40	5.9 ± 0.3	10.7 ± 0.4**

Values are means of triplicate results ± standard error.
*Significant difference at 99% confidence limit ($P \leq 0.01$).
**Significant difference at 95% confidence limit ($P \leq 0.05$).

Ammonium oxidizers and presence of nitrite

The number of ammonium oxidizers on the selective medium was determined and are presented in Table 2. The presence of nitrite was also determined as a confirmatory test for ammonium oxidizers.

Total aerobic bacterial populations

From the result of the analysis of the total aerobic bio-load, there was a significant difference ($P \leq 0.01$) at all

Table 2. Number of ammonium oxidizers in the soil.

Sample description	25°C	30°C	Gram reaction	Morphology	Presence of nitrite
POME (cfu/g)	No growth	No growth	NA	NA	NA
Non-POME (cfu/g)	8.0±0.4 x10^2	5.0±0.4x10^2	Gram negative	Short rods	Positive (red coloration)

Values are means of quadruplet results ± standard error.
NA, Not Applicable.

the temperatures between the POME and non-POME soils. At 20 and 30°C, there was a significant difference ($P \leq 0.01$) in the growth of bacteria for both soil samples. There was however, a significant difference ($P \leq 0.01$) in the growth of bacteria at 40°C when compared to the other temperatures, which implied that in this study, 40°C was the most favourable temperature for the growth of aerobic bacterial populations in both the POME and non-POME soils.

The two main locations for active bacteria are believed to be soil pores (within the surrounding water film), in regions of preferential flow or alternatively entrapped within the soil matrix (Grundmann et al., 2001). Schmidt (1973) considers the plate count method acceptable particularly as media that are specific for fixed physiological groups are developed and as the incubation conditions approach the natural environment. As with all enumeration techniques, it must be realized that organisms are not uniformly distributed throughout the soil environment but rather are found in a point distribution frequently dependent on a localized feature that allows for the maximum expression of that particular organism (Wollum, 1982). However, it must be borne in mind that the plate counts of bacteria results usually represent only 1 - 5% and at the most only 50% of the number determined by direct microscopy (Paul and Clark, 1989). The results discussed are also subject to these limitations.

Numerous organisms invade and grow in POME breaking down complicated molecules into simple ones. The high organic matter in palm oil effluent, which has been shown to be higher than in the control soil samples (Wood, 1977), may have played a major role in the proliferation of aerobic micro organisms.

Ammonium oxidizers

From the results, ammonium oxidizers were only recovered from the non-POME soil sample (8.0×10^2 at 25°C and 5.0×10^2 at 30°C) and not from the POME, which suggests that the POME may have had a deleterious effect, which probably inhibited the growth of ammonium oxidizers. The fact that 25°C seemed to have encouraged the growth of ammonium oxidizers in the non-POME soil more than the 30 - 35°C that is considered to be the optimum for ammonium oxidizers (Paul and Clark, 1989), is also worthy of note. More work may be necessary to determine if the species of ammonium oxidizers is a factor in this difference.

The factors responsible for the apparent inhibition of ammonium oxidizers in POME-treated soil may include decreased aeration, increased moisture and organic matter contents associated with POME soil. Nitrification is said to be inhibited by these conditions; (Paul and Clark, 1989) and may be the reason for the inability of ammonium oxidizers to grow in the POME-treated soil. The ammonium oxidizing isolates were grayish, smooth, colonies with convex surfaces, which were gram-negative rods isolated after 2 weeks incubation on the selective medium and also tested positive to nitrite using the modified Greiss-Ilosvay reagent.

It must be noted that while the total aerobic bacteria required a maximum of 72 h of incubation for enumeration, the ammonium oxidizers on a selective medium required at least 2 weeks of incubation to show the first signs of growth. This suggests that ammonium oxidizers were most likely, not present in the total aerobic bacteria enumerated with the nutrient agar and an indication of the fastidiousness of the ammonium oxidizers (Schmidt and Belser, 1982).

Schmidt and Belser (1982) and Persson and Wiren (1995) reported that the activity of nitrifiers in soil is limited mainly by concentrations of ammonia and oxygen as may be expected in view of their distinctive and specific energy-yielding reactions. Other factors such as soil pH, aeration, moisture, temperature and organic matter also play individual roles and the interplay of these factors may limit nitrification under otherwise non-limiting circumstances. The report of Yeop and Poon (1983) that land application of palm oil mill effluent has no adverse effects on the environment has been contradicted as nitrification has been shown to be obviously affected by the results of this study.

Conclusion

It can be concluded that palm oil mill effluent (POME) has an obvious effect on total aerobic bacterial populations since the counts from the POME soil ($9.6 \times 10^8 \pm 0.3$ at 20°C, $1.64 \times 10^9 \pm 0.3$ at 30°C and $1.07^9 \pm 0.3$ at 40°C) were significantly higher ($P \leq 0.05$) than the counts on the non-POME soil ($4.5 \times 10^8 \pm 0.3$ at 20°C, $7.6 \times 10^8 \pm 0.3$ at 30°C and $5.9 \times 10^8 \pm 0.3$ at 40°C). Secondly, POME may have an obvious effect on ammonium oxidizers as they were not isolated from the POME soil sample. This highlights the need for proper treatment and disposal for POME especially for small-scale and intermediate operators who also need the benefits of the apparent increase in total aerobic bacterial populations in the soil in their environment for agriculture. It may be desirable to recycle the POME to other useful products as is done in other developed countries. This would avoid the apparent negative effects of POME on nitrification in the soil and by extrapolation, agriculture.

REFERENCES

Ahmad A, Ismail S, Bhatia S (2003). Water recycling from palm oil mill effluent (POME) using membrane technology. *Desalination*, 157:87-95.

Bek-Nielsen C, Singh G, Toh TS (1999). Bioremediation of palm oil mill effluent. In: Proceedings Porim International Palm Oil Congress 16th February 1999, Kuala Lumpur, Malaysia.

Chan KW, Watson I, Lim KC (1980). Use of oil palm waste material for

increased production. Paper presented at the Conference on Soil Science and Agricultural development in Malaysia, Kuala Lumpur, Malaysia.

Dommergues YR (1992). Management of soil microbial populations to optimize tree establishment and growth, with particular reference to nitrogen fixing bacteria: In: Biological Nitrogen Fixation and Sustainability of Tropical Agriculture. K Muolongoy, M Gueye, DSC Spencer (eds.), Wiley Sayce Co. : 113-121

Grundmann, G.L., Deschne, A., Bartoli, F., Flandrois JP, Chasse JL, Kizungu R (2001). Spatial modeling of nitrifier microhabitats in soil. Soil Science Society of America Journal, 65 :1709-1716.

Ma AN, Ong ASH (1982). Anaerobic digestion of palm oil mill effluent. *PORIM* Bulletin (4):24.

Maheswaran A, Singam G (1977). Pollution control in the palm oil industry – promulgation of regulations. *Planter* 53: 470– 476.

Ngan MA, Zajima Y, Asahi M, Junit H (1996). A novel treatment process for palm oil mill effluent. PORIM TECHNOLOGY, October 1996.

Paul EA, Clark FE (1989). Soil Microbiology and Biochemistry. Academic Press Incorporated San Diego, California.: 11-234.

Persson T, Wiren A (1995). Nitrogen mineralization and potential nitrificaion at different depths in acid forest soils. Plant and Soil. 169:55- 65.

Schmidt EL (1973). The traditional plate count technique among modern methods: Chairman's summary. In: T. Roswall (ed.) Modern methods in the study of ecology. Bulletin of Ecological Resource Commission, Stockholm 17: 451-454.

Schmidt EL, Belser IW (1982). Nitrifying bacteria. In: A.L. Page, R.H. Miller and D.R. Keeney (eds.) Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. Madison, Wisconsin: 1027 – 1042.

Wollum AG (1982). Cultural methods for soil micro-organisms In : A.L. Page, R.H. Miller and D.R. Keeney (eds.) Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. Madison, Wisconsin: 781- 801.

Wood BJ (1977). A review of current methods for dealing with palm oil mill effluents. *Planter* 53: 477-495.

Yeop KH, Poon KC (1983). Land application of plantation effluent. Proceedings of the Rubber Research Institute of Malaysia o n oil palm by product utilization. Kuala Lumpur.