Increasing the fertilizer value of palm oil mill sludge:
bioaugmentation in nitrification


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Abstract Malaysia is essentially an agricultural country and her major polluting effluents have been from agro-based industries of which palm oil and rubber industries together contribute about 80% of the industrial pollution. Palm oil sludge, commonly referred to, as palm oil mill effluent (POME) is brown slurry composed of 4–5% solids, mainly organic, 0.5–1% residual oil, and about 95% water. The effluent also contains high concentrations of organic nitrogen. The technique for the treatment of POME is basically biological, consisting of pond systems, where the organic nitrogen is converted to ammonia, which is subsequently transformed to nitrate, in a process called nitrification. A 15-month monitoring program of a pond system (combined anaerobic, facultative, and aerobic ponds in series) confirmed studies by other authors and POME operators that nitrification in a pond system demands relatively long hydraulic retention time (HRT), which is not easily achieved, due to high production capacity of most factories. Bioaugmentation of POME with mixed culture of nitrifiers (ammonia and nitrite oxidizers) has been identified as an effective tool not only for enhancing nitrification of POME but also for improving quality of POME as source of liquid nitrogen fertilizer for use in the agricultural sector, especially in oil palm plantations. Nitrate is readily absorbable by most plants, although some plants are able to absorb nitrogen in the form of ammonium. In this study, up to 60% reduction in HRT (or up to 20% reduction in potential land requirement) was achieved when bioaugmentation of POME was carried out with the aim of achieving full nitrification.

Keywords Aerobic; anaerobic; bioaugmentation; facultative; nitrification; palm oil mill effluent

Introduction and background

Malaysia is the world’s leading producer and exporter of palm oil. Crude palm oil (CPO) is obtained through milling of oil palm fresh fruit bunches (FFB). Malaysia’s edible oil production rose from 92,000 metric tonnes in 1960 to 7.4 million metric tonnes in 1995 (Journal of Oil World Annual, 1995). O’Holohan (1997) projected an estimated annual production capacity of 10 million metric tonnes by the year 2000. In 1997, Malaysia produced about 32 million tonnes of POME from 290 palm oil mills. In terms of biochemical oxygen demand (BOD), this is equivalent to 25,000 mg/l. POME is mainly organic in nature with relatively high organic nitrogen (Ma, 1999), as shown in Table 1. Due to this tremendous volume and its polluting potential, POME requires proper management and handling by the industry and government authorities. The government therefore enacted the Environmental Quality (Prescribed Premises, Crude Palm Oil) Regulations in 1977 (Table 1). Over the past 20 years, the technique available for the treatment of POME in Malaysia has been basically biological treatment consisting of anaerobic, facultative, and aerobic pond system. This system has been found to be stable, and easy to operate. Its major problems are that it occupies a vast amount of land and requires relatively long hydraulic retention time (HRT) for effective performance. De-oiling tank, acidification, anaerobic, facultative and aerobic ponds would require 1, 4, 45, and 16 days respectively (Ashhuby et
Long HRT is a big problem for most oil mill operators due to their high production capacity. The consequence is that the discharge standards are difficult to achieve, with respect to various forms of nitrogen. Nitrification process (oxidation of ammonium nitrogen to nitrate via nitrite) is most adversely affected by this practice due to low HRT in the aerobic pond. From Table 1, it could be observed that although the discharge limits for ammoniacal and total nitrogen have been set at 150 mg/l and 200 mg/l respectively, the discharge of effluent containing nitrate is not limited by regulatory restriction in Malaysia.

Biologically treated POME has been widely used in the oil plantations for irrigation purposes and can be used as liquid fertilizer. Its use as a fertilizer has to be with caution because of imbalance in the nutrient composition of POME. Prolonged improper utilization may cause accumulation of magnesium and inhibit the availability of potassium (Kittikun et al., 2000). Table 2 shows the nutrient requirements of the various growth stages of the plant and the amount of POME to be applied in order to avoid soil damage. Von Vexkull and Fairhurst (1991) had obtained similar values.

**Why nitrify POME?**

Only a fraction of all the POME generated can be utilized as fertilizer, the unutilized portion has to either be recycled or discharged. The discharge of effluent containing ammonia is undesirable because it causes excessive oxygen demand in the receiving waters. Ammonia toxicity to fish even at very low concentration has been reported (Sawyer et al., 1994, Bernet et al., 2000). According to the recommendation of EPA (1976), not more than 0.02mg/l free ammonia should be allowed in the receiving waters. Discharge of organic nitrogen is equally dangerous, and will eventually be mineralized into inorganic nitrogen.

Furthermore, nitrification is a step closer to denitrification. Recycling the nitrified POME into the anaerobic pond brings about quick denitrification, and then less pollution. Application of organic nitrogen as fertilizer is not uncommon, though. Some environmentalists favour this practice for the fact that it discourages leaching of nitrate into the

**Table 1** POME characteristics and limits for discharge of POME (Maheswaran, 1982; Environmental Quality Act 1974 (1999))

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>10,250–47,500</td>
<td>5,000</td>
<td>2,000</td>
<td>1,000</td>
<td>500</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>COD</td>
<td>11,500–106,360</td>
<td>10,000</td>
<td>4,000</td>
<td>2,000</td>
<td>1,000</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total solids</td>
<td>11,450–164,950</td>
<td>4,000</td>
<td>2,500</td>
<td>2,000</td>
<td>1,500</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Suspended solids</td>
<td>410–60,360</td>
<td>1,200</td>
<td>800</td>
<td>600</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>130–86,430</td>
<td>150</td>
<td>100</td>
<td>75</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ammoniacal nitrogen</td>
<td>0–110</td>
<td>25</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>150</td>
<td>150**</td>
</tr>
<tr>
<td>Organic nitrogen</td>
<td>180–1820</td>
<td>200</td>
<td>100</td>
<td>75</td>
<td>50</td>
<td>300</td>
<td>200**</td>
</tr>
<tr>
<td>pH</td>
<td>3.8–4.5</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
</tr>
</tbody>
</table>

* All in mg/l except for pH and temperature
** Value of filtered sample

**Table 2** POME application (m³/acre/year): Kittikun et al., 2000

<table>
<thead>
<tr>
<th>Crops</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young palms</td>
<td>25–70</td>
<td>27.5–32</td>
<td>5.1–10</td>
<td>1.2–10</td>
</tr>
<tr>
<td>Adult palms</td>
<td>90–128</td>
<td>52.5</td>
<td>10–18.5</td>
<td>15</td>
</tr>
<tr>
<td>Old palms</td>
<td>162</td>
<td>52</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>
groundwater from where drinking water could easily be contaminated. The risk for nitrate leaching however depends on a number of factors and is limited in the tropics because of continuous uptake of nitrogen by the vegetation. Application of organic nitrogen had however been associated with lower yields. Alaerts et al. (1996) noted that the microbial hydrolysis of more complex organic N and P into NH$_4^+$ and ortho-PO$_4^{3-}$ was the limiting step for enhanced biomass production.

**Nitrification of POME**

Significant nitrification rarely takes place in most pond treatment systems treating POME. Some of the reasons for this have been highlighted above. In the face of strict discharge regulations, the operators of the oil mills would be left with two options: a) to expand the size of the lagoon in order to attain the hydraulic retention time for effective performance, which would mean greater coverage of precious land areas; b) to reduce the retention time by bioaugmenting POME with efficient, nitrifying bacteria, thereby achieving the parameter limit. This study reported in this paper examines the later option since it appears to offer a more cost-effective method of achieving effective nitrification. Bioaugmentation is defined in this paper as the application of indigenous or allochthonous (wild type) or genetically modified organisms to polluted hazardous waste sites or bioreactors in order to accelerate the removal of undesired compounds (van Limbergen et al., 1998). Mixed cultures of nitrifiers used for the bioaugmentation were isolated from soil in a paddy field.

**Materials and methods**

**Performance evaluation of POME treatment ponds**

Samples were initially collected from four different mills with pond treatment systems to determine similarities or otherwise in parameter characteristics. Subsequent samplings were done from only one factory for a period of 15 months. The samples were analysed for heavy metals, phosphorus, chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD$_5$), suspended solids, and various forms of nitrogen.

**Laboratory-scale bioaugmentation studies of POME**

The first phase of the study was the isolation and identification of nitrifiers from soil in a paddy field. Isolation was carried out using the growth medium of Walker and Skinner (1961) as described by Soriano and Walker (1968). More details of the isolation and identification of the paddy field nitrifiers can be found elsewhere (Onyia et al., 2001). The isolates were used as inoculum in the second phase of the study. Three 2-litre capacity flat bottom fermentors (B. BRAUN BIOLAB CP, Germany) were used, which consisted of series of batch cultures. Each culture vessel was equipped with a sensor and heater for temperature control, antifoam sensor for foam detection, pH electrode for pH measurement, and polarographic electrode for pO$_2$-measurement, stirring impeller and sparger, for homogenous mixing of the sample, exhaust cooler, and a sampling device. Batch cultures were performed in 3 different fermentors. One fermentor represented the control and contained only the synthetic medium stated above and without nitrifying inoculum (for sterility check). The second fermentor was fed with POME from an aerobic pond containing resident bacteria only, while the third reactor was fed with POME also from the same aerobic pond as in the second reactor and inoculated with 15 mg/l of the mixed culture of nitrifiers prepared during the first phase. The three fermentors were operated at temperature of 30°C, and pH of 7.5 maintained with 2N sterile sodium hydroxide solution. These optimal conditions had been determined for the isolates in the first phase of the study (Onyia et al., 2000). The stirring impeller was set to rotate at 350 rpm. Filtered air was pumped to each reactor to ensure supply of dissolved oxygen concentrations of...
3 mg/l–5 mg/l. The fermentors and the synthetic medium were pre-autoclaved to avoid contamination.

**Analytical methods**
Heavy metals were analysed using microwave assisted digestion method with an Ethos 900 Milestone laboratory station. Readings were thereafter taken with the flame Atomic Absorption Spectrometer (AAS-Perkin Ermer 3110 model), as described in the *Standard Methods* (APHA, 1992). Total phosphorus was determined as described in *Standard Methods* using ascorbic acid method. Chemical oxygen demand (COD) was determined using open reflux, titrimetric method while biological oxygen demand (5-day BOD) test was conducted at 20°C (APHA 1992).

Total suspended solids determination was carried out with Direct Reading Spectrophotometer (DR/2000) as described by Hach (1992). Total nitrogen, ammonium nitrogen, and total oxidized nitrogen and nitrite and nitrate were determined according to *Standard Methods* (APHA 1985, 1992). To separate the two forms of oxidized nitrogen, nitrite was determined by the method described in *Hach’s Water Analysis Handbook* (Hach, 1992). Nitrate concentration was calculated from the difference between the concentrations of the total oxidized nitrogen and nitrite.

**Results and discussions**

**Performance of a POME treatment pond system**
A serial combination of anaerobic digestion pond and extended aerobic pond system is widely employed for the treatment of POME in Malaysia. This technique has been found to be very stable and economical (where land is not a limiting factor), and effective for achieving discharge limits for most of the parameters. Table 3 shows the average result of a 15-month monitoring program of these pond systems treating POME. Table 3 indicates that nitrification is not a usual phenomenon in the pond system. The presence of small quantities of nitrate in the facultative pond, which increased slightly in the later ponds, suggests that although nitrifiers were present in the ponds, their activities were adversely affected by the prevailing conditions there. Apart from dissolved oxygen deficiency, fluctuating pH values were believed to contribute to the inhibition. Onyia *et al.*, (2000) had observed that pH value above 8.5 slows down the activities of nitrifying bacteria. Table 3 on the other hand reveals that POME contains all ingredients essential for a mixed fertilizer.

**Bioaugmentation studies**
Varying degrees of nitrification activities were observed in the two reactors containing

**Table 3 Material flow through the pond treatment system**

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Influent (de-oiling tank and sterilization tank)</th>
<th>Anaerobic pond</th>
<th>Facultative pond</th>
<th>Aerobic pond</th>
<th>Settling tank (discharge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺-N</td>
<td>42.22</td>
<td>103.47</td>
<td>184.58</td>
<td>163.17</td>
<td>159.50</td>
</tr>
<tr>
<td>NO₃⁻-N</td>
<td>0.00</td>
<td>0.00</td>
<td>2.24</td>
<td>4.48</td>
<td>6.50</td>
</tr>
<tr>
<td>TKN</td>
<td>595</td>
<td>–</td>
<td>287</td>
<td>–</td>
<td>227.4</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.028</td>
<td>0.02</td>
<td>0.21</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Calcium</td>
<td>326.5</td>
<td>325</td>
<td>266</td>
<td>218.5</td>
<td>196</td>
</tr>
<tr>
<td>Magnesium</td>
<td>610</td>
<td>518.4</td>
<td>445</td>
<td>4,369</td>
<td>279</td>
</tr>
<tr>
<td>Potassium</td>
<td>2,267.5</td>
<td>1,787.5</td>
<td>1,807.5</td>
<td>1,730</td>
<td>1,310</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>12.1</td>
<td>7.1</td>
<td>4.1</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>pH</td>
<td>3.7</td>
<td>4.9</td>
<td>7.6</td>
<td>8.5</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* All in mg/l except for pH and temperature
POME with and without nitrifying inoculum (Figures 1 and 2). The reactor containing POME but without nitrifying inoculum achieved less than 50% nitrification during the experimental period of 11 days (Figure 3), while 100% nitrification was achieved in 7 days in the POME inoculated with the nitrifying inoculum (Figure 4). Taking a comparative look at Figures 1 and 2, it could be concluded that the initial low population of nitrifying bacteria in POME without inoculum was the reason for inefficient nitrification since all other conditions were uniformly set in the two reactors. It could also be noticed from Figure 1 that nitrate production was not commensurate with ammonium removal. The possible reason could be attributed to the activities of faster growing heterotrophs (due to the presence of organic matter) that might have out-competed the nitrifiers, and possibly utilized most of the ammonium nitrogen for cell growth. Inoculating POME with efficient nitrifiers greatly reduced the HRT by more than 60% (Figure 4) with respect to complete nitrification.

**Conclusion**

Nitrification of POME is necessary, as not all the POME generated could be used as liquid fertilizer; a great proportion of it would end up in the watercourse. In comparison with organic nitrogen and ammonia, discharge of effluent with nitrate is considered more stable in the aquatic system since it does not exert oxygen demand. As a fertilizer source, nitrified POME should be a better option than POME with high organic content (especially in the tropics where nitrate leaching is not much of a problem) because nitrate is more easily absorbable by most plants. Application of organic nitrogen had been associated with lower yields, because ammonia liberated during mineralization of organic matter is complexed with soil particles and cannot often reach the root zone of many plants. This study has shown that bioaugmentation is a useful tool in achieving high nitrification efficiency at relatively low HRT in the aeration pond treatment system. In this study, up to 60% reduction in HRT (or up to 20% reduction in potential land requirement) was achieved, when bioaugmentation of POME was carried out with the aim of achieving full nitrification.
References


