Microbiology of Palm Oil Mill Effluents

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ABSTRACT

This study was designed to investigate the microorganisms associated with palm oil mill effluent (POME) produced by smallholder processors in Elele, Rivers State, Nigeria. A total of twenty seven samples were aseptically collected from nine oil mills. The POME was subjected to standard microbiological analysis and biodegradation studies using crude palm oil as sole carbon source. The population of total heterotrophic bacteria (THB) ranged from $7.4 \times 10^5 – 2.0 \times 10^6$ cfu/ml, the total heterotrophic fungi (THF) ranged from $3.1 – 5.7 \times 10^4$ cfu/ml. The hydrocarbon degrading bacteria (HDB) ranged from $6.5 \times 10^5 – 2.0 \times 10^6$ cfu/ml and the hydrocarbon degrading fungi (HDF) ranged from $3.1 – 5.6 \times 10^4$ cfu/ml. Among the various mills, analysis of variance showed that there were significant differences $(P < 0.05)$ in THB, THF and HDB but no significant difference $(P > 0.05)$ in HDF. HDB and HDF accounted for 83.34 – 98.62% and 92.17 – 99.28% respectively of the heterotrophic population, indicating that majority of the microbes in the POME are hydrocarbon degraders. Microbial species identified in the POME include Aspergillus niger, Aspergillus flavus, Fusarium, Mucor sp., Penicillin sp., Pseudomonas sp., Serratia sp., Staphylococcus sp., Corynebacterium sp. We conclude that the POME contained microbial species capable of degrading hydrocarbons in the POME to prevent environmental impacts.

Keywords: Biodegradation, lipase, oil mill effluents

INTRODUCTION

Oil palm (Elaeis guineensis) is a monococious plant that bears both male and female flowers on the same tree. It belongs to the palmae family and is the most productive oil producing plant in the world [1-6]. Palm trees may grow up to 60 feet or more in height. The trunks of young and adult plants are wrapped in fronds which give them a rough appearance. The older trees have smoother trunks apart from the marks left by the fronds, which have withered and fallen off [7]. Each tree produces compact bunches of fruitlets weighing about 10 to 25 kg with 1000 to 3000 fruitlets per bunch. Each fruitlet is spherical or elongated in shape. Generally, the fruitlet is dark purple (almost black) and the color turns to orange red when ripe [7].

The process of extracting palm oil requires significant large quantities of water to steam sterilize the palm fruit bunches, clarify the extracted oil and for washing and cleaning processes in the mill [8, 9]. The separated wastewater sludge commonly referred to as palm oil milling effluents (POME) is a brown slurry, which is composed of 4-5 % solids, (mainly organic), 0.5-1.0% residual oil and about 95% water and high concentration of organic nitrogen [10]. POME is a thick brownish liquid that contains solids, oil and grease, chemical oxygen demand (COD) and biological oxygen demand (BOD) [6, 11]. Palm oil mills release POME in tremendous volumes with its attendant
polluting potential. POME has adverse environmental impacts including land and aquatic ecosystem contamination and loss of biodiversity [8].

Palm oil as a lipid derives its distinctive properties from the hydrocarbon nature of a major portion of the structure. The molecules are composed mainly of long chains of carbon atoms. The hydrocarbon group is modified by the presence of small number of more reactive polar groups. The chains can exist in several forms but have long saturated and unsaturated monocarboxylic aliphatic free acids. Since the major portion of a lipid is the hydrocarbon, some microorganisms, which utilizes hydrocarbon have the ability to grow and proliferate in it. A variety of microorganisms have been reported to degrade oil generally causing rancidity [12]. These organisms are widely distributed in both aquatic and terrestrial ecosystem. Palm fruit is the major source of lipase producing microorganisms [13]. Studies have shown that for organisms to degrade palm oil, it must be able to produce lipase. Besides the presence of lipids and other volatile compounds, the inhibitory effects of POME on living tissues, could also be due to presence of water-soluble phenolic compounds [14]. The lipolytic activity of fungi on the triglyceride of the POME which contain oil and fats may cause rancidity, acidity, bitterness soapiness and other off flavors [3]. Microbial isolates with lipase activities include bacteria, fungi and yeast, and they are often associated with specific substrates [15-17]. Heterotrophic microorganisms possessing lipases are generally able to degrade and mineralize POME because such organisms are able to use oil as the sole carbon source. Biodegradation of oily wastes is the cheapest and surest means of managing POME to prevent the attendant environmental impacts. Microbiology of POME is scare in literature. Therefore, this study investigates microorganisms present in POME produced by smallholder oil palm processors in Elele, Rivers State Nigeria.

MATERIALS AND METHODS

2.1. Field Sampling
Smallholder oil palm processing sites were visited for sample collection at Elele, River State Nigeria from 13th – 22nd April 2012. Triplicate POME generated was collected ascetically with sterile microbiological bottles from different mills. The process of POME generation from oil palm processing at these sites is basically the same and is presented in Fig. 1.

2.2 Enumeration of total heterotrophic bacteria and fungi
The populations of microorganisms in the samples were enumerated using serial dilution pour plate method of Akinrele [18]. About 0.1ml of POME sample was serially diluted in sterile distilled/deionized water and aliquots of the dilutions were ascetically plated into the media (Nutrient Agar and Sabouraud Dextrose Agar for bacteria and fungi respectively). The agar plates were incubated at 37°C for 24-48 hours to enumerate the aerobe and facultative bacteria and the fungi culture plates were incubated inverted at 30°C for 3-5 days. After incubation, the colonies that grew on the medium were counted and expressed as colony forming units (cfu)/ml of the samples. Microbial colonies were isolated into pure cultures and preserved in slants for further analysis.

2.3 Enumeration of hydrocarbon degrading bacteria and fungi
Pour plate method was used for the enumeration of hydrocarbon degrading bacteria and fungi. The culture medium used was Bushnell Haas with 0.01% of palm oil (as carbon source) and Agar agar (15g/l). For the hydrocarbon degrading bacteria (HDB), the medium were incorporated with Amphoterim B (to inhibit fungal growth) and for the hydrocarbon degrading fungi (HDF); the medium were incorporated with Tetracycline (to inhibit bacteria growth).

2.4 Identification of microbial isolates
The characterization of POME microorganisms subcultured were identified by comparing their characteristics with those of known taxa using the scheme of Domsch and Gams [19], and Bergey’s Manual of Determinative Bacteriology [20]. Identification of fungi was based on microscopic morphology and cultural characteristics and the bacteria isolates that were characterized based on biochemical test as described by Oyeleke and Manga [21] and Cheesbrough [22, 23].

2.5 Statistical Analysis
SPSS software version 17 (SPSS Inc, Chicago) was used to carry out the statistical analysis. A one-way analysis of variance was carried out at α = 0.05, and Duncan’s multiple range test was used to discern the source of the observed differences.
RESULTS AND DISCUSSION

The microbial population of POME is presented in Table 1. The total heterotrophic bacteria ranged from $7.4 \times 10^5$ – $2.0 \times 10^6$ cfu/ml being significantly different among the nine mills ($P < 0.05$), apart from C and G that is not significantly different ($P > 0.05$). The total heterotrophic fungi population ranged from $3.1 – 5.7 \times 10^4$ cfu/ml, though significantly different ($P < 0.05$) among the mills. The microbial population of hydrocarbon degrading fungi ranged from $3.1 – 5.6 \times 10^4$ cfu/ml not being significantly different ($P > 0.05$) in most of the mills. The microbial population of hydrocarbon degrading bacteria ranged from $6.5 \times 10^5$ - $2.0 \times 10^6$ cfu/ml being significantly different ($P < 0.05$) among the mills. The proportion of HDB and HDF ranged from 83.34 – 98.62% and 92.17 – 99.28% of
the total heterotrophic population respectively. This shows that HDB and HDF thrive well in oily waste water [24]. Awotoye et al. [24] reported THB, THF, HDF and HDB population of 1.8 x 10^6 cfu/g, 9.5 x 10^5 cfu/g, 1.2 x 10^5 cfu/g and 4.0 x 10^4 cfu/g respectively at the point of POME discharge from oil palm milling machine. Ugoji [25] reported that THB and THF are 1.3 x 10^6 cfu/ml and 1.3 x 10^5 cfu/ml respectively in POME. The differences in the various microbial populations are a reflection of many factors such as nutrient, minerals, temperature and oxygen level [26], acidity, volume of wastewater [27] and concentration of oil and grease in the POME. High population of bacteria in the POME may be associated with contaminations from poor sanitation in the mills [3], and irregular disinfection. Also, it may also be due to the handling process and the prevailing environmental conditions in the mills. The microbial species found in POME has the potential of degrading hydrocarbon in the POME. Biodegradation is associated with the saprophytic ability of fungi to grow on and degrade carbon sources in industrial effluents [28].

<table>
<thead>
<tr>
<th>Mills #</th>
<th>Total Heterotrophic Bacteria, x 10^6 cfu/ml</th>
<th>Total Heterotrophic Fungi, x 10^6 cfu/ml</th>
<th>Hydrocarbon Degrading Bacteria, x 10^4 cfu/ml</th>
<th>Hydrocarbon Degrading Fungi, x 10^4 cfu/ml</th>
<th>HDB/THB (%)</th>
<th>HDF/THF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.96 ± 0.02 bc</td>
<td>3.8 ± 0.40 abc</td>
<td>0.95 ± 0.02 bcd</td>
<td>3.5 ± 0.31a</td>
<td>98.62</td>
<td>92.17</td>
</tr>
<tr>
<td>B</td>
<td>1.2 ± 0.06 d</td>
<td>3.5 ± 0.31 ab</td>
<td>1.1 ± 0.05 d</td>
<td>3.4 ± 0.37a</td>
<td>96.75</td>
<td>97.20</td>
</tr>
<tr>
<td>C</td>
<td>0.79 ± 0.1ab</td>
<td>4.2 ± 0.17 bcd</td>
<td>0.71 ± 0.10 ab</td>
<td>4.1 ± 0.49ab</td>
<td>90.34</td>
<td>98.41</td>
</tr>
<tr>
<td>D</td>
<td>1.1 ± 0.05 cd</td>
<td>5.0 ± 0.15 de</td>
<td>1.0 ± 0.06 cd</td>
<td>4.8 ± 0.29bc</td>
<td>95.26</td>
<td>97.33</td>
</tr>
<tr>
<td>E</td>
<td>1.7 ± 0.09 e</td>
<td>5.7 ± 0.27 e</td>
<td>1.6 ± 0.01e</td>
<td>5.6 ± 0.34c</td>
<td>98.04</td>
<td>98.26</td>
</tr>
<tr>
<td>F</td>
<td>1.0 ± 0.06 c</td>
<td>4.4± 0.36 bcd</td>
<td>0.87 ± 0.07 abc</td>
<td>4.1 ± 0.40abc</td>
<td>85.29</td>
<td>93.94</td>
</tr>
<tr>
<td>G</td>
<td>0.75 ± 0.04 ab</td>
<td>3.1 ± 0.32 a</td>
<td>0.70 ± 0.09ab</td>
<td>3.1 ± 0.57a</td>
<td>96.48</td>
<td>97.89</td>
</tr>
<tr>
<td>H</td>
<td>0.74 ± 0.07a</td>
<td>3.2 ± 0.20 a</td>
<td>0.65 ± 0.06a</td>
<td>3.1 ± 0.13a</td>
<td>83.34</td>
<td>96.91</td>
</tr>
<tr>
<td>I</td>
<td>2.0 ± 0.1f</td>
<td>4.6 ± 0.29 cd</td>
<td>2.0 ± 0.08f</td>
<td>4.6 ± 0.23bc</td>
<td>98.25</td>
<td>99.28</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± standard error (n = 3). Different letters in each column indicate significant differences at P < 0.05 according to the Duncan Statistics.

Tables 3 and 4 presents the identification features of fungi and bacteria isolated from POME based on their microscopic/morphology, cultural and biochemical characteristics. The result showed that all the samples collected from various mills have similar microbial species. The bacteria isolated from the POME include Pseudomonas sp., Serratia sp., Bacillus sp., Staphylococcus sp. and Corynebacterium sp. While the fungi isolates are Aspergillus niger, Aspergillus flavus, Mucor sp., Fusarium sp. and Penicillium sp. Ugoji [25] identified E. coli, Flavobacterium, Desulfovibrio, Methanococcus sp., Bacillus subtilis, Pseudomonas, Fusarium moniliforms, Penicillium sp., Aspergillus sp., Botryodiplodia theobromae, Trichoderma viridae and Geotrichum candidum in POME.

The result from this study show that the microbial species isolated is similar to those found in areas polluted with petroleum hydrocarbons [27, 29]. However, Corynebacterium sp., Serratia sp., Bacillus sp. and Pseudomonas sp. and Staphylococcus sp. are lipase producing organisms associated with pathogenicity. Pseudomonas sp. was identified by Griffith et al. [17] as causing spoilage of dairy products and fats containing food. The production of the spores makes POME microbial species to be dormant and highly resistant to lethal effect of boiling, dry heating and ultra violet radiation [30]. POME is potential habitat for thermophilic lipolytic bacteria. Gowland et al. [16] isolated thermophilic lipase producing Bacillus from POME. Asikong [15] identified Aspergillus sp. as fungal species associated with lipase production. Lipase helps in hydrolysis of lipid causing subsequent breakdown into fatty acid and alcohol [15]. Aspergillus niger and Aspergillus flavus have been noted for their ability to survive in oily wastewater. The presence of Penicillium sp., Fusarium sp. and Mucor sp. in the POME shows that these fungi are able to survive in hostile environment [30]. These organisms are capable of biodegrading oily wastewater.

Microorganisms present in POME have been variously been mobilized for the treatment of the wastewater. Ahmed et al. [31] used Pseudomonas sp. culture in co-composting process of oil palm frond with POME anaerobic sludge. During composting, oil and grease in the POME is broken down and mineralized [32]. The bacteria found in POME are useful during upflow anaerobic sludge blanket system treatment [33]. The oily environment may provide a good environment for lipolytic microorganisms to flourish due to the unrecovered oil present in the effluent. However, the microbial content of POME is a good indicator of biodegradability of wastewater and it could also be used in bioremediation of hydrocarbon from crude oil spills. However, since most of these organisms are spore formers, it helps them to survive the harsh environmental conditions of POME such as anaerobiosis, high oil and grease content [3, 25], and acidity [34-36]. Under anaerobic conditions, methane and carbon dioxide are formed [25]. The
anaerobic microflora found in POME sludge may be useful for the production of biohydrogen and biogas generation by fermentation during treatment [37 - 39].

Table 3: Microscopic morphology and cultural characteristics of fungi isolates of POME

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Microscopic morphology</th>
<th>Cultural characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>Presence of septate hyphae; long and smooth conidiophores, long unbranded sporangiospores with large, round head.</td>
<td>Creamy to brownish-black mycelium with dark spores and often appears golden on the reverse side.</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>Presence of septate hyphae, colourless and rough conidiophores with swollen vesicles.</td>
<td>A greenish-yellow colour with a creamy edge. That appears golden in the reverse of the septate.</td>
</tr>
<tr>
<td>Fusarium species</td>
<td>Presence of dark pigment of macro and macro conidiophores and it is spherical in shape.</td>
<td>A creamy-yellowish powdery substance that appears yellowish-brown on the reverse side.</td>
</tr>
<tr>
<td>Penicillium species</td>
<td>Presence of septate and fruity mycelium and branched conidiophores. It has a red pigment, and the edge is surrounded by whith margin</td>
<td>A greenish filament is seen which changes to powdery greenish brown after days and it is yellow on the reverse side.</td>
</tr>
<tr>
<td>Mucor species</td>
<td>Presence of non septate hyphae with a visible spore and short sporangiospores.</td>
<td>A creamy colonies that covers the entire medium and they are irregular in shape.</td>
</tr>
</tbody>
</table>

Table 4: Biochemical test of bacteria isolates of POME

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Gram reaction</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Citrate</th>
<th>Coagulase</th>
<th>Uresae</th>
<th>Indole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp</td>
<td>Negative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serratia sp</td>
<td>Negative</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>Positive</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus sp</td>
<td>Positive cocci</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corynebacterium sp</td>
<td>Positive rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(= positive; - negative reactions)

CONCLUSION

In this study, the population of microorganisms found in POME was enumerated. The POME had a high population of heterotrophic bacteria and fungi. The proportion of hydrocarbon degraders to the heterotrophic bacteria and fungi population was quite high. The microbial isolates from study are the same in all the processing mills. These microbes have direct applications in industrial process such as bioremediation and biodegradation of oily wastewater.

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