**Prevention of Biodeterioration of Crude Oil in Tanks Using Anti-Microbial Agents**

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**Abstract.** The potential use of antimicrobial agents such as ethanol and extract from locally sourced *Mitracarpus scaber* for the prevention of biodeterioration of crude oil in storage was investigated in this study. Five crude oil samples under various treatment conditions (containing antimicrobial agents) and a control (without antimicrobial agents) were monitored for seven weeks for biodeterioration indicating parameters such as biochemical oxygen demand (BOD), total hydrocarbon content (THC), total microbial count (TMC) and pH. Results obtained for the control experiment indicated biodeterioration of the crude oil sample. This was evident from the variation in the values of BOD, THC, TMC and pH indicating the presence of microbial activity. Results obtained also showed that biodeterioration was mitigated when antimicrobial agents were introduced to the crude oil samples as seen in the stability in the values of BOD, THC, TMC and pH. Of all the samples analysed, sample D containing 100 mL of *Mitracarpus scaber* extract performed best as evident from the almost constant values of BOD, THC, TMC and pH, indicating the absence of microbial activity.

**Keywords:** Biodeterioration, crude oil, ethanol, *Mitracarpus scaber*, BOD, THC

**1. INTRODUCTION**

Crude oil is a complex biodegradable substance containing a large variety of hydrocarbons such as straight, branched and cyclic aliphatics, aromatic and heterocyclic compounds (Hidayat and Tachibana, 2012; Obahiagbon et al., 2009). The primary aim of crude oil extraction is to refine it into useable hydrocarbon fuels. These fuels are used all over the world for powering light and heavy vehicles, heavy duty machineries, electricity generating sets etc, making the refining industry one of the largest manufacturing industries in the world (Gaylarde et al., 1999; Yemashova et al., 2007). After extraction and prior to refining, crude oil is typically stored in large vessels (Adhiyia et al., 2007). Crude oil production companies spend a great amount of money annually on capital equipment, modernisation and maintenance, including prevention and treatment of microbial contamination that leads to biodeterioration (Gaylarde et al., 1999).

Biodeterioration has been defined as any undesirable change in the quality of a material caused by agents of microbiological origin with the change typically resulting from a breakdown in the structure of the material in question (Allsopp et al., 2004; Tucker, 2008; Sanyaolu et al., 2012). Biodeterioration of crude oil and its derivatives has been of great interest to scientists and researchers for the past sixty years (Murzaev, 1964; Odier, 1976; Rozanova, 1967). Even though most of the work in this area was done between the 1950’s and 1970’s when the world became aware of the effects of this problem, it is still taken as a matter of urgent importance in the present day world (Allsopp et al., 2004; Roling et al., 2003; Sanyaolu et al., 2012; Yemashova et al., 2007). Besides, most of the microbiological methods applied for enhanced oil recovery unwittingly resulted in the introduction of microbial agents which are responsible for crude oil biodeterioration (Sanyaolu et al., 2012).

Despite efforts to solve this problem such as the application of biocides, long term storage of crude oil and its derivatives in industrial tanks still leads to its deterioration (Chesneau, 2000). Various chemical substances used to combat this problem often lead to environmental pollution as these compounds are not readily susceptible to biodegradation and many of them are mutagenic and carcinogenic (Sanyaolu et al., 2012; Zhiglesova et al., 2000). Recent research efforts have been directed to the identification and application of the most effective biodeterioration inhibitors (Bonch-Osmolovskaya et al., 2003; Efremenko et al., 2005).

In developing countries like Nigeria however, there is a lack of research information regarding biodeterioration of crude oil and its derivatives in...
storage, and its concomitant negative consequences on the Nigerian oil industry and economy. *Mitracarpus scaber* is an annual plant commonly found in cultivated farmlands. It has been traditionally utilised for the treatment of skin infections resulting from microbial contamination (Imam et al., 2008). The plant is claimed to have both antibacterial and antifungal activities (Abere et al., 2007; Ali-Emmanuel et al., 2003; Bisignano et al., 2000). Hence this study investigated the potential use of extract from *Mitracarpus scaber* as an inhibitor for the prevention of biodeterioration of crude oil during storage. The study was aimed at identifying the best biodeterioration inhibitor amongst two antimicrobial agents; commercially available ethanol and extract from locally available *Mitracarpus scaber*.

### 2. MATERIALS AND METHODS

#### 2.1. Materials collection and preparation of plant extract

All chemicals used in this study were of analytical reagent grade and used without further purification. Ethanol and petroleum ether were obtained from British Drug Houses Ltd, England. The crude oil (Escravos light) used for this study was obtained from an Oil Producing Company located in the Niger Delta region of Nigeria. The properties of the crude oil sample were as follows: gravity API°; 35.3, gravity SG; 0.85, sulphur (wt%); 0.15, viscosity (cSt at 40°C); 3.28.

The plant was sourced locally in Benin City, Nigeria, identified and authenticated as *Mitracarpus scaber* by the plant curator of the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy University of Benin, Benin City, Nigeria, where a voucher specimen of the plant is deposited. Fresh leaves, whole stems and roots of the plant were sun dried and powdered using Moulinex mill after which it was stored in a dry and well stoppered bottle (Abere et al, 2007). Appropriate amounts of the milled plant material were extracted by soxhlet extraction method using petroleum ether. The solvent used in each batch was recovered under pressure until dry extracts were obtained and then stored in amber colored bottles.

#### 2.2. Biodeterioration inhibition studies

The antimicrobial agents (Ethanol and *Mitracarpus scaber* extract) formulated were tested for the ability to control microbial contamination in crude oil. Biodeterioration inhibition studies of the chosen antimicrobial agent were carried out in six closed vessels. The content of the vessels are as listed in Table 1. The samples were monitored for seven weeks for biodeterioration indicating parameters such as BOD, THC, TMC and pH.

### Table 1: Composition of crude oil samples for biodeterioration experiments

<table>
<thead>
<tr>
<th>Crude oil sample</th>
<th>Crude oil</th>
<th>Ethanol</th>
<th>Mitracarpus scaber extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample A</td>
<td>2000</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Sample B</td>
<td>2000</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Sample C</td>
<td>2000</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Sample D</td>
<td>2000</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Sample E</td>
<td>2000</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

#### 2.3. Analytical methods

Sampling was done on day zero (before the introduction of antimicrobial agents) and subsequently at intervals of seven days (one week) for a total of 49 days (seven weeks). The pH of the samples was measured using an electronic pH meter (Fisher Accruement pH meter). Winkler method was used in the estimation of the biochemical oxygen demand of the crude oil samples (Woodring and Clifford, 1988). Total hydrocarbon content of the crude oil sample was determined by mixing 20 mL of crude oil with 25 mL of hexane and the oil extracted was determined by the absorbance of the extract at 460 nm using a spectronic 70 spectrophotometer. Total microbial count was determined using the Thom's chamber (Zawierucha and Malina, 2006).

### 3. RESULTS AND DISCUSSION

The profile of BOD of crude oil samples at various conditions is shown in Figure 1. It can be observed that the BOD of most of the crude oil samples varied with no particular pattern indicating microbial activity. BOD is a measure of the amount of oxygen consumed by microorganisms in decomposing organic matter. It also measures the chemical oxidation of inorganic matter (i.e., the extraction of oxygen from water via chemical reaction). The greatest variation was recorded for the control experiment in which no antimicrobial agent was added. This shows that the
indigenous microorganisms present in the crude oil were actively degrading the organic content of the crude oil (Amenaghawon et al, 2013; Otokunefor and Obiukwu, 2010). When antimicrobial agents were added to the crude oil sample, some level of stability was observed as seen in the fairly stable values of BOD recorded for samples B, D and E. However, the greatest level of stability was observed for sample D (containing 100 mL of Mitracarpus scaber extract only) indicating that the Mitracarpus scaber extract was able to inhibit the action of the indigenous microbes initially present in the crude oil sample. Abere et al. (2007) reported that Mitracarpus scaber extract possesses antimicrobial activity that enables it to inhibit the action of microorganisms such as bacteria and fungi which are typically found in stored crude oil (Bento and Gaylarde, 2001).

The effect of antimicrobial treatment on TMC of crude oil for different treatment conditions is shown in Figure 3. The TMC expressed as the numbers of cells in 1 mL of a suspension is indicative of the concentration of microbial consortium present in the crude oil sample. TMC of the control sample increased progressively with time. This observation can be attributed to the absence of antimicrobial agents that would have inhibited the growth of these microbes hence they thrived under this condition. Oboh et al. (2006) reported that certain bacterial species such as Pseudomonas, Bacillus, Alcaligenes, Citrobacter and fungi such as Aspergillus sp., Penicillium, Rhizopus and Rhodotorula sp. possess the ability to grow and utilise crude petroleum as the sole carbon and energy source. Uzoamaka et al. (2009) also reported that some isolates of fungi including A. versicolor, A. niger, A.flavus, Syncephalastrum spp., Trichoderma spp., Neurospora sitophila, Rhizopus arrhizus and Mucor spp showed potentials for hydrocarbon biodegradation. The addition of antimicrobial agents to the crude oil samples resulted in a trend different to that of the control experiment as indicated in Figure 3. This trend indicated that TMC decreased with time with the greatest reduction

Figure 2 shows the effect of antimicrobial treatment on THC of crude oil for different treatment conditions. For the control experiment, it was observed that THC of the crude oil reduced consistently from the start of sampling to the last week of the study. The reduction in THC values is indicative of a reduction in hydrocarbon content which results from the mineralisation of the hydrocarbons by the indigenous microorganisms to less toxic substances such as CO₂ and H₂O (Alwan et al., 2013; Okoh, 2006; Otokunefor and Obiukwu, 2010). These results are similar to those obtained by Alwan et al. (2013) who investigated the bioremediation of the water contaminated by waste of hydrocarbon using Ceratophyllum and Potamogetonaceae plants. The introduction of antimicrobial agents resulted in stable values of THC of samples C, D and E. However, the highest level of stability was observed for sample D which contained crude oil and 100 mL of Mitracarpus scaber extract only. The high level of stability observed in the THC values of sample D could be attributed to the inactivity of microorganisms which could have resulted from introduction of the antimicrobial Mitracarpus scaber extract.

![Graph 1: Variation of BOD with time for different crude oil samples](image1)

![Graph 2: Variation of THC with time for different crude oil samples](image2)
recorded for sample D. Reduction in total microbial count can be attributed to the inhibition of microbial growth as a result of the action of the antimicrobial agents. Ahonkhai et al. (1999) reported that inhibition to growth of the microorganisms by the extracts can be attributed to the potency of active components of the extract.

Figure 4 shows the variation of the pH of crude oil samples with time for different treatment conditions. The general trend observed indicated that the pH of the crude oil samples tested increased with time. The variation in pH may be attributed to the effect of the antimicrobial agents on the growth of microorganism in crude oil. The presence of the antimicrobial agent typically prevents the degradation of the crude oil into other intermediate products which might have an influence on the pH of the crude oil sample (Obahiagbon et al., 2009). Amenaghawon et al. (2014) reported that the pH of the crude oil sample could actually increase with time if the indigenous microbial population is allowed to grow and thrive. According to them, the increase will result from the conversion of the crude oil to less acidic products. In this regard, the antimicrobial agents enhanced the stability of the pH of sample D. The stable pH values recorded for the case of sample D may be attributed to the activity of the antimicrobial agents which inhibits the growth of microbial cells and subsequent degradation of crude oil to other products.

4. CONCLUSION

The potential application of antimicrobial agents such as ethanol and extract from *Mitracarpus scaber* for the prevention of biodeterioration of crude oil was investigated. Ethanol and extract from *Mitracarpus scaber* enhanced the stability of crude oil in storage as observed from the fairly constant values of BOD, THC, TMC and pH. In addition, the total microbial count decreased when the antimicrobial agents were used. For best performance, 100 mL of *Mitracarpus scaber* extract corresponding to sample D is recommended based on the results obtained. This study has shown that extract from locally sourced *Mitracarpus scaber* can be used to mitigate biodeterioration in crude oil under storage, particularly Escravos light crude oil. However, the effect of the antimicrobial agents might be different for a different type of crude oil with different chemical composition and properties.

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