Isolation and identification of hydrocarbon degrading bacteria from oil contaminated soil samples

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Abstract:
The present study was undertaken to isolate and describe hydrocarbon degrader bacteria from oil contaminated soil samples in of Misurata city-Libya. Totally 85 bacterial isolates were obtained from 64 soil samples from automobile work shop and petrol station source (57 Klebsilla pneumonia;14 Escherichia coli;11 Bacillus cereus and 3 Pseudomonas aeruginosa). The incidence of bacterial isolates in collected samples were depended on season of collection. Isolates growth in solid and liquid carbon free minimum media CFMM reflected their ability to degrade and utilize the tested hydrocarbon type (benzene; engine oil; cured oil) as sole carbon source. Finally, three isolates (Klebsilla pneumonia, Escherichia coli, Bacillus cereus) were selected through secondary screening by an agar well diffusion method and culture turbidity reading at D 600. Klebsilla isolate had the highest degradation potential for the three-tested carbon source. Rates of hydrocarbon oils consumption and bacterial growth was limited by bacteria activity in carbon free media. Data clearly shows that gram-negative bacteria are dominant in oil contaminated soil samples and they are more effective in decomposition of hydrocarbons used in this study.

Key words: Biodegradation, Hydrocarbon decomposition, Crude and Engine oil soil contamination.

Introduction
In most countries of world, oil spills everywhere including automobile workshops and gas stations. Oils spills are source of environmental contamination that threaten human health (Anupama and Singh, 2008; Boonchanet al., 2000; Keith and Telliard, 2003; Navdeeper et al., 2013). All petroleum derivatives are emerging of crude oil, which are main components of hydrocarbons; crude oil consists of complex mixtures of paraffin and epoxide aliphatic and aromatic hydrocarbons (Forcanet et al., 2010). Prolonged exposure to oil or petroleum derivatives such as Benzene, Toluene and Xylene may cause cancer, lung, heart, and kidney diseases by direct contact with contaminated vapors and secondary contamination or by water within the soil supply (Bijay et al., 2012; Mandri and Lin, 2007). Many techniques were used to solve the problem of oil pollution including physical and chemical ways to reduce the pollution of hydrocarbons. Among these measures is biological treatment by presence of some species of microorganisms such as bacteria and fungi that able to degrade hydrocarbons and used it as a source of carbon and energy (Esinet al., 2011; Anitha et al., 2009; Navdeesper et al., 2013; Maruthiet al., 2013; Stelige,2011). Bioremediation is the naturally occurring process by which microorganisms can change environmental contaminants such as oil to harmless products, in order to obtain carbon and energy sources. This method is the most effective because it does not have irreversible negative effects on soil characteristic and low cost. Hydro carbon–transforming bacteria have been isolated from different oil contaminated soil samples and areas (Mandri and Lin, 2007; Esinet al., 2011; Anupmmama et al., 2013; Geetha et al., 2013;
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Gupte and Sonawdekar, 2015; Mamitha et al., 2013; Jayanthi et al., 2015; Al Oraibi an El Grabulli, 2017). The present study aimed to identify and isolate oil degrader bacteria from automobile workshops and gas stations contaminated soil samples in Misurata city-Libya.

Material and method

Preparation of Free carbon media (FCMM):
One-gram K$_2$HPO$_4$, 0.5g Mg SO$_4$, 7H$_2$O, 0.5 NaCl, 0.001g FeSO$_4$. 7H$_2$O, 0.01g MnSO$_4$. 4H$_2$O, 0.05g CaCO$_3$ were added to distil water. The volume was completed to liter one-liter distilled water. The flasks containing media were autoclaved for 20 minutes at 121 °C, allowed to cool at room temperature and kept at 5 °C (Tepper et al., 1994).

Collection of soil samples and screening of bacterial isolates from oil contaminated soil:
74 Soil samples contaminated with hydrocarbons were collected from automobile workshops and petrol station. Sequential samples dilution of soil samples was incubated on CFFM agar plates with 100µl hydrocarbon (Benzene- Crude oil- Engine oil) at 25 °C for 14 days. Positive growth of individual bacterial culture was purified and stored for farther investigation.

Screening for bacterial biodegradation activity:
Soil isolates were incubated in CFMM broth media with addition hydrocarbon source and incubated in 25 °C for 7 days. The grown bacteria cells recovered by centrifugation, washed and re-suspended in FCMM. Inoculums (0.5 Mckm&d) of each isolated bacterium was used for biodegradation assessment of studied hydrocarbon in liquid and solid media. In prepared 8mm wells,50µl of conformed biodegradation active bacteria broth culture was inculcated on CFMM agar plates with 100µl of hydrocarbon (Benzene- crude oil- Engine oil) at 37°C for 48 hrs. The diameter of clear zone around each well was measured.

Secondary screening by spectrophotometer device:
100µl of bacterial isolates were inculcated in 20ml of CFFM broth with 100µl of hydrocarbon oils as the source of carbon and energy at 25 °C for 14 days. Turbidity reading was taken at 600nm wavelength (OD600) for different source of hydrocarbons.

Results and discussion
The ability of many microorganism to biodegrade hydrocarbon has been studied and confirmed to be genetically inherited character by many researchers (Mandri and Lin, 2004; Wang et al., 2008; Forkan et al., 2010; Bijai et al., 2012; Amitans and Rashmi, 2013). In present study 85 isolates were obtained from 64 soil oil contaminated samples table (1).

Table (1) Hydrocarbon decomposition activity of isolated bacteria.

<table>
<thead>
<tr>
<th>Type and number of isolates</th>
<th>Carbon sources</th>
<th>Benzene</th>
<th>Crude oil</th>
<th>Engine oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>Activity</td>
<td>No. of isolates</td>
<td>Activity</td>
</tr>
<tr>
<td>Klebsilla pneumonia (57)</td>
<td>24</td>
<td>-</td>
<td>28</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>+</td>
<td>26</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>+++</td>
<td>3</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+++</td>
<td>7</td>
<td>+++</td>
</tr>
<tr>
<td>Escherichia coli (14)</td>
<td>3</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+++</td>
<td>5</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+++</td>
<td>1</td>
<td>+++</td>
</tr>
<tr>
<td>Bacillus cereus (11)</td>
<td>4</td>
<td>-</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+</td>
<td>4</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+++</td>
<td>2</td>
<td>+++</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa(3)</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>1</td>
<td>+++</td>
</tr>
</tbody>
</table>

- Inability to decompose. + Weak decomposition.
++ Moderate decomposition. +++ Strong decomposition.
The bacteria were isolated by serial dilution method. Cultures were purified by streaking technique and the purity was cross examined by Gram stain. The isolates included *Klebsilla pneumonia; Escherichia coli; Bacillus cereus* and *Pseudomonas*. Incidence of each bacterial type in collected samples was variable depend on bacteria species and season of collection table (2).

**Table (2) The percentage of isolates / season.**

<table>
<thead>
<tr>
<th>Type of isolation</th>
<th>Total of isolation</th>
<th>Autumn %</th>
<th>Winter %</th>
<th>Spring %</th>
<th>Summer %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsilla pneumonia</em></td>
<td>57</td>
<td>8.77%</td>
<td>19.29%</td>
<td>56.14%</td>
<td>15.78%</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>14</td>
<td>14.28%</td>
<td>28%</td>
<td>14.28%</td>
<td>42.85%</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>11</td>
<td></td>
<td>54.54%</td>
<td>36.36%</td>
<td>9.09%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>3</td>
<td>/</td>
<td>33.33%</td>
<td>66.66%</td>
<td>/</td>
</tr>
</tbody>
</table>

The total number isolates in each season 85 8.23% 25.88% 47.05% 18.82%

Most of *Klebsilla* and *Pseudomonas* isolates obtained in spring while *Escherichia coli* isolates found in summer. Data in this study showed that the biodegradation activity to decompose hydrocarbons depend on bacterial type and source of utilized carbon. Oil compound consist of complex mixture of different hydrocarbon. Bacteria species have only limited ability to degrade all the fraction of hydrocarbon (Weissenfeldset al., 1991; Li et al., 2000) because some hydrocarbon is resistant to biodegradation especially that contain benzene ring (Shaifee et al., 2006; Suseo et al., 2009). *Klebsilla pneumonia* in this study raveled high ability to decompose crude oil and engine oil (100% and 98% respectively) with potential ranged from weak to strong decomposer. Most of isolates showed less ability to degrade benzene. Higher utilization of crude oil compared to benzene might explained by their structure and susceptibility to microbial attack (Li et al., 2000).

Potential to compose hydrocarbon was also measured by well diffusion method. Data in figure (1) showed that *Klebsilla* isolate has the highest clear zone diameter followed for by *Esch. coli*. Different reported diameter means of clear zone indicate the potential of bacteria to compose tested hydrocarbon (Ting el., 2009).

**Figure (1) Determination of degradation potential by ager-well diffusion method.**

Growth profile of individual bacterial culture of selected isolates *Klebsilla, Esc. coli* and *Bacillus cereus* in CFMM supplemented by different carbon source confirm their
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hydrocarbon degradation activity and revealed the level of hydrocarbon utilization potential figure (2).

![Figure (2)](image)

**Figure (2)** Growth of isolated bacteria (turbidity) in carbon free media with different hydrocarbon source for 14 days.

OD600 value indicate that *Klebsilla* isolates had the strongest degradation activity of crude oil and engine oil comparing to the negative control (bacteria have no biodegradation ability). Optical density of bacterial growth was used as indicator for biodegradation by many researchers (Kirimura *et al.*, 1999; Emtiaziet *et al.*, 2005; Ebrahimiet *et al.*, 2012; Sawadogoeot *et al.*, 2014).

**Conclusion**

In this study four types of bacteria isolates were obtained from soil contaminated with oil in Misurata city- Libya. Biodegradation activity of the isolates was conformed. Highest degradability of tested hydrocarbon (crude oil, engine oil and benzene) was observed by *Klepcilla pneumonia*.

**References**