Rapid Screening of Crude Oil Degrading Bacteria Isolated from Oil Contaminated Areas

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Abstract

Environmental contamination by crude oil and its derivatives is a worldwide serious problem. Conventional physical and chemical methods are non economical, temporary and can generate residues which are toxic to biota of the ecosystem. Hence, biodegradation reveals an efficient biological ecofriendly and safe treatment process to remediate crude oil contamination. Naturally spread bacterial strains have capacity to degrade crude oil. Bacterial strains were isolated from oil contaminated areas and they were identified. A rapid and simple screening method was used for screening of efficient crude oil degrading bacteria by using redox indicator 2,6 dichlorophenol indophenol method and total plate count method. Bacteria having high crude oil degrading potential it turns the medium to become colorless. The efficiency was measured by optical density and total cell count and further screened by gravimetric analysis. In this present study the genera Pseudomonas and Bacillus selected as potential crude oil degraders and can be used to remediate petroleum wastes.

Keywords: Biodegradation; Redox indicator; Crude oil; Screening; Petroleum wastes.

Introduction

Pollution is the entry of contaminants in the natural environment; it leads to cause instability, harm or discomfort to the ecosystem. Soil pollution is caused by the presence of xenobiotic chemicals or other sources in the natural soil environment. These occurs when chemicals are released by spill or underground leakage. The most significant soil contaminants are hydrocarbons, heavy metals, herbicides and pesticides etc. All petroleum products are originated from crude oil whose major constituents are hydrocarbons. Petroleum components can be divided into four fractions: saturated, aromatic, resin and asphaltene fractions determined by absorption chromatography (Shigeaki et. al., 1999).

Many compounds of oily sludge are toxic, mutagenic and carcinogenic. Therefore, they are classified as priority environmental pollutants by the US Environmental Protection Agency (Liu et. al., 2010). Prolonged exposure of crude oil to the human body and high oil concentrations may cause the development of liver and kidney disease, possible damage to the bone marrow and increased risk of cancer (Mandri et. al., 2007). Many microorganisms have the ability to utilize hydrocarbons as a sole source of energy and carbon that are widely distributed in nature; such organisms are called as petrophiles. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons (Mandri et. al., 2007). Diverse bacterial population can metabolize the hydrocarbons found in the crude oil in to non toxic, non hazardous, biodegradable and ecofriendly end products (Bharti et. al., 2011). The biodegradation of crude oil by microorganisms is one of the primary ways to remove crude oil from contaminated area. It has been studied that bacterium that grows in oil contaminated soil are much capable of degrading oil when compare with those bacteria which are found on non-contaminated soil. The natural biodegradation process can be enhanced by addition of nutrients and optimizing the growth parameters. In order to remediate the crude oil pollution, crude oil biodegradation is necessary to isolate and characterize unique microbial species for evolution of their efficacy in utilization of crude oil before application of the contaminated sites. Therefore an attempt has made to examine the oil contaminated soil as a source of carbon utilizing bacteria to clean environmental contamination.

Materials and Methods

Isolation and Identification of bacteria

Crude oil contaminated soil samples were collected from motor vehicle workshops, water service stations and vehicle parking areas located in and around Salem, Tamil Nadu. Soil samples were collected randomly 5-10 cm beneath the surface using spatula and packed in sterile container. The samples were transported to the laboratory in an ice box and stored at 4°C for analysis. The collected soil samples were serially diluted from 10^1 to 10^10 dilution, and the diluted soil samples were spreaded on nutrient agar plates for plate count method. The obtained cultures were purified by quadrant streaking on sterile nutrient agar plates. At the strains were identified by various biochemical tests according to the Bergey's manual of determinative bacteriology. The isolated cultures were maintained on nutrient agar slants at 4°C for further studies.

Primary screening of crude oil degrading bacteria

Bushnell Hass broth media along with redox indicator 2,6-dichlorophenol indophenol was prepared. 1% of sterile crude oil was added. The isolated strains were inoculated into broth and incubated at 37°C for 7 days (Hanson et. al., 1993 and Joshi et. al., 2011). Five ml of broth was taken, centrifuged and the supernatant was used to measure the optical density at 540nm for degradation ability of the isolates. About 0.1 ml of each 7 days old BH broth culture was spread over to the nutrient agar plates for plate count method.

Secondary screening by gravimetric analysis

There are 6 bacterial strains showed more efficiency on crude oil degradation and increased in number. To obtain more potential strains for

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crude oil degradation, secondary screening was performed with 6 strains. About 100ml of Bushnell Hass broth media was prepared. To it one gram of crude oil was added in the broth. Oil degrading isolates were aseptically added as an inoculum. The flask was incubated at 30°C for 7 days in a rotary shaker at 120rpm. After incubation, the flask was added with Diethyl ether (C₂H₅O), a solvent for separation and mix well. Complete mixing; the broth was transferred to the separating flask. The setup was left for 20 minutes for oil and broth separation. The broth was separated in the lowest portion. Diethyl ether was added to the remove complete oil from separating flask. Oil along with solvent was collected in a preweighted petriplate. After the complete evaporation of the solvent the plate was weighed. The estimation of residual oil left after degradation was made by the amount of oil in a preweighted plate (Anupama et al., 2009). The percentage of oil degradation was calculated as \(1 - \left(\frac{X_o - X_1}{X_o}\right)\times 100\%\) where \(X_o\) initial amount of crude oil, \(X_1\) amount of crude oil after degradation (Chrzanowski et al., 2006 and Jayashree et al., 2012).

**Results**

There are 14 crude oil contaminated soil samples were collected for obtaining efficient crude oil degrading bacterial strain. Such strain has the capability to mineralize crude oil in crude oil contaminated site was proved by many scientists (Akhavan et al., 2008).

**Isolation of bacteria from soil sample**

Bacterial species were isolated from crude oil contaminated soil sample and mixed cultures were obtained. Pure cultures were isolated by quadrant streak method. There are 103 bacterial isolates were identified by staining and biochemical tests. Among these 103 bacterial isolates the predominant genera shown in Fig. 1. Among the isolates, 16 isolates have the potency to degrade oil was isolated by primary screening. They were identified based on optical density and colony forming units as shown in table 1 and Fig. 2.

**Gravimetric analysis**

The reduced optical density and increased colony forming unit, strains were used for secondary screening. Among this crude oil degrading isolates were further checked for potential degradation ability by using gravimetric method. Only 6 strains have the potency to degrade the crude oil (Table 2). The figure 3 showed secondary screening of crude oil degrading bacterial species. In this study there are 6 potentially degrading isolates identified belongs to the genera of Pseudomonas sp, Bacillus sp, Micrococcus sp and Enterobacteriaceae.

**Discussion**

Microorganisms are extremely diverse and are capable of utilizing the contaminants as energy and carbon source to survive in natural
Table 2: Gravitational Analysis of oil degrading Bacterial Strains

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Strain No.</th>
<th>Genus</th>
<th>Total weight (g)</th>
<th>Pre weight (g)</th>
<th>Residual Weight (g)</th>
<th>Degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CODB 9</td>
<td>Bacillus</td>
<td>42.26</td>
<td>41.51</td>
<td>0.75</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>CODB 10</td>
<td>Pseudomonas</td>
<td>52.91</td>
<td>52.24</td>
<td>0.67</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>CODB 42</td>
<td>Pseudomonas</td>
<td>44.06</td>
<td>43.25</td>
<td>0.81</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>CODB 61</td>
<td>Enterobacteriaceae</td>
<td>45.89</td>
<td>45.11</td>
<td>0.78</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>CODB 88</td>
<td>Micrococcus</td>
<td>49.59</td>
<td>48.79</td>
<td>0.80</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>CODB 99</td>
<td>Bacillus</td>
<td>46.87</td>
<td>46.09</td>
<td>0.78</td>
<td>22</td>
</tr>
</tbody>
</table>

Conclusion

The ability to metabolize oil is displayed by many different types of microbes. Some microbes highly prefer crude oil. Over their numbers will increase faster than others in community in reports to soil. For soil bioremediation, suitable microorganisms are necessary for an optimal treatment of soils contaminated with crude oil. Native predominant bacterial strains have more ability to degrade the crude oil which was proved by our study. Bushnel Hass medium is an excellent growth media for isolation of heterotrophic microorganisms which provide all nutrient sources except carbon source, by our study crude oil used as a sole source of carbon. A rapid primary screening procedure was performed to assess the indicator dye (2, 6-DCPIP) decolorization efficiency of selected strains for confirmation of crude oil biodegradation. The rate of degradation was further screened and confirmed by gravimetric method. 

References


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