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Isolation and purification of petroleum-decomposing bacteria from contaminated soils and identification the resulted compounds of degradation of such compounds

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Introduction

The most worrying environmental pollutants are hydrocarbons in different forms. Oil leaks into the environment due to the use of large amounts of oil as raw material, production of energy and chemicals from oil materials, as well as transportation, refining, storage and consumption (Gerhardt et al. 2009; Nogales et al. 2011). Bioremediation is one of the pollution removal technologies in which biological systems are used to destroy or change the harmful chemicals. The goal of biorefining of oil is the complete decomposition of hydrocarbons into water and carbon dioxide by micro-organisms. Among the biological methods, the use of bacteria has a very good efficiency in removing pollution (Khosravinodeh *et al.* 2012). Bacteria and fungi are the only biological species that have the metabolic ability to use petroleum carbon in their cell synthesis. But for reasons such as high abundance, rapid increase in growth rate and the ability to use a wide range of hydrocarbons, bacteria are used to clean contaminated soils (Doostaki *et al.* 2012). The biorefining is based on the action of microbial enzymes to convert or decompose harmful pollutants and is widely used to reduce hydrocarbon pollution (Wolicka *et al.* 2009).

Methods and Materials

Twenty soil samples were collected from the soil contaminated with petroleum substances around Tehran Refinery. After diluting in physiological serum (0.9% sodium chloride), the collected soil samples were cultured on nutrient agar medium and kept in a greenhouse at 35°C for 48 hours. Completely pure and contamination-free colonies were prepared from different colonies that grew on the culture medium.

In order to isolate the strains that had the ability to break down oil, CFMM with 2% kerosene was used as a minimum carbon-free culture medium. From each of the purified colonies, a quantity was picked up with a sterile loop and inoculated in this culture medium. After a week, the turbid environments were reported as oil-decomposing strains (Okerentugba & Ezerony 2003).

To evaluate the ability of the isolates to break down oil, the selected strains were cultured for two weeks in CFMM medium containing 2% kerosene.These culture mediums were used to check the decomposition ability of the strains. The ability to break down oil by isols was evaluated by infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC/MS) (Weisman & Group 1998).

Isolates with the ability to break down oil were identified using morphological (the appearance of colonies and gram staining), biochemical (oxidase, catalase, citrate, TSI, SIM and urease tests) and molecular (16SrRNA) identification methods.

Results and discussion

Bacterial colonies that grew in nutrient agar culture medium were carefully examined and finally 17 colonies that were different in appearance were selected and purified.

Ability of seperated bacterial isolates to break down oil was evaluated using CFMM medium containing 2% kerosene. This culture medium has no carbon source and only microorganisms that are able to use petroleum compounds as a carbon source can grow in it. Among the tested isolates, three isolates were able to use kerosene as a carbon source. These isolates were named HS1 to HS3 and used in the next steps.

Results of FTIR showed that the peaks related to aromatic O-H in the presence of all three strains have significantly decreased compared to the control sample; even HS2 isolate has completely eliminated the aromatic O-H. Also, all three isolates were able to completely eliminate aromatic C-C. Complex alkanes such as RCH2CH3 were converted to CH_2 in the presence of HS1 and HS3 isolates and completely disappeared in the presence of HS2. Carboxylic acids and amino compounds are produced in the presence of bacterial strains. The presence of carboxylic acid and amine groups in the samples containing the bacterial strain shows that these bacteria have converted the complex compounds of oil into simpler organic compounds, which is one of the characteristics of oil biorefining.

The analysis of the spectra obtained by GC/MS showed that complex hydrocarbon compounds were present in the control sample, but in the presence of bacterial strains, these compounds were either completely lost or greatly reduced. Finally, these isolates were identified, and the results showed that they included a strain of *Nocardia sp*., *Bacillus subtilis* and *Acinetobacter baumannii*.

The results of biochemical tests, like the morphological part, confirmed that isolate HS1 is from Actinomycetes and isolate HS2 from Bacillus. HS3 isolate was also identified as Acinetobacter.

The results of sequencing the PCR product of 16SrRNA gene showed that HS1 isolate with 100% similarity were identified as Nocardia sp., HS2 isolate with 95.44% similarity as Bacillus subtilis and HS3 isolate with 98.99. % similarity as Acinetobacter baumannii.

In the present study, one of the isolated strains was Actinomycete Nocardia. Actinobacteria belong to Actinomyceta family. This family of bacteria is well known due to the ability to produce numerous secondary metabolites against pathogenic microorganisms. These bacteria are mostly extracted from soil sources. This group of filamentous bacteria has adapted well to the environment and soil and can decompose complex biological polymers (Shahaby *et al.* 2015).

Another strain that was identified as an oil-degrading strain in the present experiment was Bacillus subtilis (isolate HS2). The ability of Bacillus subtilis to decompose petroleum compounds has been reported in many studies (Montagnolli *et al.* 2015; Darsa *et al.* 2014). The third identified isolate was Acinetobacter baumannii strain. Many strains of Acinetobacter have the ability to decompose aromatic compounds and use them as the only source of carbon, so that, by producing biosurfactant, they increase the solubility and speed of biodegradation. Therefore, Acinetobacter can be used in the biodegradation and purification of PAH compounds (Vanbroekhoven et al. 2004).

Conclusion

One of the most important oil pollutants is polycyclic aromatic hydrocarbons (PAH), which can cause major problems due to the possibility of their accumulation in plants and animals. (Jain et al. 2011). Microorganisms have the ability to convert hydrocarbon waste into carbon dioxide, water and cell mass, or converted them into less harmful substances (Souza *et al.* 2014). In the present research, the results showed that in the presence of bacterial strains, aromatic petroleum compounds were significantly reduced and complex hydrocarbon compounds were converted into organic compounds. So it may be possible to use them to purify petroleum contaminated soils.

Keywords: *Aromatic hydrocarbons, Bioremediation, Decomposing bacteria, Oil pollution.*