Bioremediation of Oily Based Drilling Waste Resulting From Oil & Gas Wells At Western Desert

BY

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B.Sc. Biochemistry
Faculty of Science, Ain Shams University, 2003

A Thesis
Submitted in partial fulfillment for the requirements of
M.Sc. Degree in Environmental Sciences

Department of Environmental Basic Sciences
Institute of Environmental Studies and Research
Ain Shams University

APPROVAL SHEET

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Metwalli M. El Noubi

Abstract

Bioremediation of Oily Based Drilling Waste Resulting From Oil & Gas Wells At Western Desert

This work examines the ability and efficiency of the bioremediation technique as an alternative, environmental friendly treatment process and a well-proven environmentally acceptable technology that uses microorganisms to biologically degrade the oily base mud (OBM) waste resulting from group of selected oil and gas drilling and exploration wells in Western Desert in Egypt.

Eleven samples from the waste of oily base mud were collected from different drilling locations at Western Desert, from (Abu Sennan, El Alamein, Qattara Depression, Burg El Arab, Marina, and El Hamra). The samples were analyzed for bacteriological examination to enumerate the total bacterial counts and to enumerate the Oil Degrading Bacteria (ODB), then isolation and purification of Oil Degrading Bacterial (ODB) isolates for further screening of ODB.

Phenotypic and genotypic identification of the most three potent oil degrading bacterial (ODB) isolates was performed after six screening steps. The three most potent oil degrading bacteria that were able to degrade the highest oil concentrations were utilized for the biodegradation of oil using these strains separately one by one. The degradation level was measured by using the three most potent isolates together at a fixed condition of temperature 30°C and pH= (7.0).

The results obtained showed a very promising ability and efficiency of the bioremediation process with the most three potent isolates. It was found that the biodegradation by using isolate number (H43- *Bacillus Subtilius*) is more efficient than the other isolates alone or even together.

Keywords and phrases: bioremediation, oil and gas, drilling and exploration, oily base mud (OBM), drilling waste, Oil Degrading Bacteria (ODB), biodegradation, western desert, Egypt.

SUMMARY

This thesis includes a study on the use of bioremediation technology and its effectiveness on selected samples of oil-based drilling mud residues from drilling oil and gas wells in some areas of the Egyptian Western Desert.

The purpose of this work is to isolate, identify, and determine the strongest oil-degrading bacterial isolates that isolated from the oil-based drilling mud waste samples and then identification of their metabolic fingerprints and determination of their ability to degrade the oil-based drilling mud residues.

In order to achieve these objectives, (11) samples of oil-based drilling mud samples were collected from various oil and gas drilling sites in Western desert in Egypt. Total bacterial counts in the collected oil base mud samples were enumerated at two different incubation temperatures (22°C and 30°C) for giving a full picture about the natural bacterial existence, by using pour plate method. A suitable dilution was plated on to plate count agar medium and after the incubation period, all plates were counted with the use of digital colony counter. The results showed that the average number of total bacterial counts at 22°C and 30 °C were ($1.4x10^5$) and ($1.6x10^4$) CFU/g, respectively.

The oil-degrading bacteria were then counted and determined by using surface plate method on oil agar (OA) media in the selected oil base mud samples. The number of oil-degrading bacteria ranged between (1.0×10^2) to ($6.1x10^3$) CFU/g in all collected samples.

After that isolation and purification of Oil Degrading Bacterial (ODB) Isolates on oil agar (OA) surface medium was performed where the isolates were picked and purified by re-streaking twice on the same culture agar medium (OA). Forty five (45) oil degrading bacterial isolates were purified and screened six times at different oil concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 (V/V).

- First screening step at oil concentration of 0.1% (V/V), **34** isolates out of total 45 (75.5%) been isolated.
- At concentration 0.2% (V/V), **30** isolates out of total 45 (66.6%) been isolated.
- At concentration 0.3% (V/V), **19** isolates out of total 45 (42.2%) been isolated.
- At concentration 0.4% (V/V), **11** isolates out of total 45 (24.4%) been isolated.
- At concentration 0.5% (V/V), **09** isolates out of total 45 (20%) been isolated.
- At concentration 0.6% (V/V), **03** isolates out of total 45 (6.6%) been isolated, and these are considered the most strong isolates to degrade and utilize the oil and identified as (H17, H32, and H43).

By using the application of the BIOLOG GEN III system, the three isolates were defined as below:

- (H17) as *Enterobacter hormaechei*
- (H32) as *Enterobacter cloacae.*
- (H43) as *Bacillus subtilis*

The metabolic fingerprints were then measured for the three isolates. It was observed that there was a difference in the metabolic fingerprints between the isolate (H17) and the isolate (H32), where (H17 "*Enterobacter hormaechei"*) are able to break and utilize the D-serine compound while the isolate (H32 "*Enterobacter cloacae"*) can't utilize the same compound.

Finally, the efficiency of the oil bioremediation technique was determined and measured by using the most potent three isolates separately one by one and then the degradation level was measured by using the three most potent isolates together. The experiment gave very promising results for the ability and efficiency of the bioremediation process with the most three potent isolates, and specially It was found that the biodegradation by using isolate number (H43- *Bacillus Subtilis*) is more efficient than the other isolates alone or even together.

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