Microbial treatments of certain non-hydrocarbon pollutants in lubricating oil wastes

Thesis
Submitted for the award of the degree of doctor philosophy in microbiology
By

Amal Salah Mohamed Hassan
(M.Sc. Microbiology, 2008)

Supervisors

Prof. Nagwa Ahmed Abdallah
Professor of Microbiology,
Microbiology Department,
Faculty of Science,
Ain Shams University.

Prof. Ali Ahmed Ebraheem Hammad
Professor of Microbiology,
National Center of Radiation Research and Technology (NCRRT).
Atomic Energy Authority.

Prof. Abd El Fattah I. Helal
Professor of Atomic Physics,
Former Vice Chairman Atomic Energy Authority.

Dr. Khaled Zakaria El-Baghdady
Associate Prof. of Microbiology,
Microbiology Department,
Faculty of Science,
Ain Shams University.

Dr. Abeer E. M. Zakaria
Associate Prof. of Microbiology,
Microbiology Department,
National Center of Radiation Research and Technology (NCRRT).

Microbiology Department,
Faculty of Science,
Ain Shams University.
2015

Approval sheet

Microbial treatments of certain non-hydrocarbon pollutants in lubricating oil wastes

By

Amal Salah Mohamed Hassan

M.Sc. In Microbiology

Ain Shams University

2008

Supervisors **Approved**

Prof. Nagwa Ahmed Abdallah
Prof. of Microbilogy,
Faculty of Science,
Ain Shams University.

Prof. Ali Ahmed Ebraheem Hammad
Prof. of Microbilogy,
National Center of Radiation
Research and Technology (NCRRT),
Atomic Energy Authority.

Prof. Abd El Fattah I. Helal
Prof. of Atomic Physics,
Former Vice Chairman Atomic Energy Authority.

Dr. Khaled Zakaria El-Baghdady
Ass. Prof. of Microbiology,
Faculty of Science,
Ain Shams University.

Dr. Abeer E. M. Zakaria
Ass. Prof. of Microbiology,
National Center of Radiation
Research and Technology (NCRRT).

Examination committee

Prof. Mamdouh Salem El- Gamal
Prof. of Microbilogy,
Faculty of Science,
Al-Azhar University.

Prof. Azza Abdel Azziz Mhana
Prof. of Microbilogy,
Faculty of Science,
Zgazeg University.

Prof. Ali Ahmed Ebraheem Hammad
Prof. of Microbilogy,
National Center of Radiation
Research and Technology (NCRRT),
Atomic Energy Authority.

Dr. Khaled Zakaria El-Baghdady
Ass. Prof. of Microbiology,
Faculty of Science,
Ain Shams University.

Approval date 2/ 2016 University Council approved /2016

This dissertation has not been previously submitted for any degree at this or at any other university

Amal Salah Mohamed Hassan

بِسْمِ اللهِ الرحْمَنِ الرحِيم

قَالُوا سُبْحَانَكَ لاَ عِلْمَ
لَنَا إِلا مَا عَلمْتَنَا إِنكَ
أَنْتَ العَلِيمُ الحَكِيمُ

صدق الله العظيم

(البقره آيه: 32)

ACKNOWLEDGEMENT

First and foremost, I feel always indebted to Allah, the most beneficent and merciful

A great thank to Professor ***Nagwa Ahmed Abdullah,*** *Professor of Microbiology, Faculty of Science, Ain Shams University for her continuous helps and encouragement. She was always patient, perfect in work organization and the best advisor and she is a good leader to our team work.*

I would also like to show my gratitude to Professor ***Ali Ahmed Ebraheem Hammad*** *Professor of microbiology, National Center of Radiation Research and Technology (NCRRT), Atomic Energy Authority for his help*

I want to express my thanks to Professor ***Abd El Fattah I. Helal,*** *Professor of Atomic Physics, Former Vice Chairman Atomic Energy Authority for his continuous helps, valuable advices and encouragement.*

I would like to express my deep gratitude and thanks to my dear supervisor Dr. ***Khaled Zakaria El-Baghdady,*** *Associate Prof. of Microbiology, Faculty of Science, Ain Shams University for his help, encouragement, continuous advice, valuable suggestion in all steps of this thesis and his expert supervision. I am proud to be one of his students and I hope that he is satisfied with me.*

I gratefully and sincerely thank my dear supervisor Dr. ***Abeer E. M. Zakaria,*** *Associate Professor of Microbiology, National Center of Radiation Research and Technology (NCRRT) for her help, continuous support, valuable instructions and guidance from the start of the work. She was always patient, perfect in work organization and the best advisor.*

A deep thank to ***Microbiology Department*** *and all* ***my Colleagues*** *in Microbiology department for their assistance and support.*

Deep thanks to ***MY Son*** *for their patience. Great thanks to* ***My BROTHERS And SISTERS*** *for their support, their prayers and continuous help from the start of my study.*

And Finally, My deep appreciation goes to ***My ADORABLE PARENTS*** *who learned me the first of everything in my life and without them, this work would not have seen light.*

AIM OF WORK

AIM OF WORK

This work aims to recycle used lubricant oil by reducing its heavy metals content using bioremediation strategy.

ABSTRACT

ABSTRACT

Used lubricant oils are the most frequent environmental pollutants which contain contaminants such as heavy metals. They massively discharged to the environment due to their large scale use. This study attempted to apply an environmentally friendly and effective technology to remove heavy metals and degrade hydrocarbons of used lubricant oil.

Caltex lubricant motor oil (1000 Km) was selected as a model of used motor oil for the present study. The used motor oil was chemically analyzed by Inductively Coupled Plasma Optical Emission Spectrometer (ICP -OES**).** Different metals were found with different concentrations. The most abundant metals were Zn, Ca, Ni and Fe in concentrations 1139.5 ±1.7, 382 ±1.4, 202 ±1.4 and 195 ± 0.18 ppm respectively. According to the previous results and previously known health hazards of heavy metals, Zn and Ni were targeted for removal from used oils in the present study.

In this study, five soil samples (S1, S2, S3, S4, S5) were collected from different locations in Egypt. Soil samples: S1, S2 and S3 were collected from mechanical workshops from different areas in Cairo, while S4 and S5 were collected upon Maize field

and Clover field soils from El –kalubia respectively. Soil samples were chemically and microbiologically analyzed**.** They contained different heavy metals in different concentrations. The concentrations of Ni in the collected soil samples ranged from 6.7 ± 0.28 (S5) to 40.95 ± 1.2 (S1) ppm and the concentrations of Zn ranged from 68.2 ± 1.3(S5) to 113.5 ±1.2 (S1) ppm respectively. While, the total petroleum hydrocarbons in samples ranged from 1570 ± 1.1 (S5) to 103240 ± 1.6 (S1) µg/g, the total heterotrophic bacterial counts (HC) in all samples ranged from 1.6 x 10^7 ± 0.08 to 3.2 x 10^7 ± 0.04 CFU/g, while for heavy metals resistant bacterial counts (HRB) grown on MSO plates containing 5mM of Ni and Zn separately, Ni resistant bacterial counts ranged 1.8x 10^4 ± 0.03 to 3.5 x 10^5 ± 0.02 CFU/g, while Zn resistant bacterial counts ranged from 1.6 x 10^5 ± 0.06 to 3.7 x 10^6 ± 0.02 CFU/g. The ratio (HRB/HC) % ranged from 0.09 to 10.8 % for all samples.

25 bacterial isolates were isolated from the five soil samples on MSO plates. Only 15 bacterial isolates were selected as promising isolates for Ni and Zn removal and TPH biodegradation of used lubricant oil due to their short incubation periods required to grow on MSO plates which contain used lubricant oil as a sole source of carbon and energy. Also due to

their abilities to grow at broad range of incubation temperatures (15 to 42 ^{o}C) in addition to their special ability to grow well at very high concentrations of used lubricant oil (up to 50000ppm).

All the selected (15) isolates were able to remove Ni, Zn and degrade TPH of used lubricant oil. The maximum removal of Ni and Zn as well as TPH biodegradation was recorded by HM16, HM24 and HM7. Also, the consortia from soil sample (S1) showed the highest percentage of Ni and Zn removal and TPH biodegradation of used lubricant oil.

The optimal environmental conditions for Ni and Zn removal as well as TPH degradation by HM16, HM24 and HM7 were found to be 10 days incubation period, 37 ^{o} C incubation temperature, pH 7, aeration condition 150 rpm and fertilizing system W4.

The most promising bacterial isolates HM16, HM24 and HM7, respectively, were exposed to increasing doses of gamma radiation 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5kGy. The calculated D_{10}-values of HM16, HM24 and HM7 were found to be 0.45, 0.54 and 0.37 kGy, respectively.

The irradiated bacterial cell mixtures of each of the most promising isolates HM16, HM24 and HM7, showed higher

percentage in Ni, Zn and TPH loss than the control (non-irradiated). On the other hand, The highest percentages of Ni and Zn removal and TPH biodegradation (47.5 ± 0.51, 39.2 ± 0.47and 39.5 ± 0.98 %), (46.5 ± 0.48, 33.6 ± 0.46 and 35.6 ± 0.78 %) and (43.3 ± 0.46 , 27.2 ± 0.63 and 31.7 ± 1.1 %) respectively have been obtained by HM16, HM24 and HM7 isolates, respectively, exposed to 0.7 kGy.

Bacterial isolates HM16 and HM24 which exhibited the highest percentages of Ni and Zn removal and TPH biodegradation of used lubricant oil were identified according to 16Sr DNA sequences of both isolates. HM16 and HM24 were identified as two different strains of *Bacillus megaterium*.

The effect of bioaugmentaion and biostimulation using different bacterial inocula which were HM16 , HM 24, consortia S1,S2 and S3, mixed isolates (HM16 + HM 24) as well as LAB (commercial product) for Ni and Zn removal and TPH biodegradation of used lubricant oil was studied. It was observed that using the mixed isolates (HM16 + MH24) showed the highest percentage of Ni, Zn removal and TPH degradation of used lubricant oil compared

to the other inocula which were 57.3 ± 0.55, 55.1± 0.73 and 49.4 ± 0.69, respectively

LIST OF TABLES

Table no.	Title	Page no.
1	Sampling sites	64
2	Different concentrations of nitrogen and phosphorus supplemented in W-media.	77
3	Operation condition of ICP-OES.	83
4	Quantitative detection of metals in used lubricant oil.	87
5	Concentration of metals in collected soil samples.	89
6	Total petroleum hydrocarbon and microbiological analysis of soil samples.	91
7	Microscopic characterization and Gram-reaction of selected isolates.	93
8	Growth response of isolates on MSO medium incubated at different temperatures for different incubation periods.	95
9	Growth response of isolates on MSO medium supplemented with different concentrations of caltex oil incubated for different periods.	97
10	The capacity of bacterial isolates to remove Ni, Zn and TPH biodegradation of used lubricant oil.	102