Enhanced Aerobic Biodegraradation of Some Toxic Hydrocarbon Pollutants

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List of abbreviations

ACOPS: Advisory Committee on Pollution of Sea

BOD: Biological Oxygen Demand

BTEX: Benzene Toluene Ethylbenzene Xylene

COD: Chemical Oxygen Demand

Cfu: Colony Forming Units

DMF: N,N,Dimethylformamide

EPA: Environmental Pollution Agency

FSW: Fertilized Sea Water

GESAMP: Group of experts on the Scientific aspects of Marine Pollution

HD: Hydrocarbon Degrader

HMW PAHs High Molecular Weight Polyaromatic Hydrocarbons

HPLC: High Performance Liquid Chromatography

MSO: Mineral Silica gel Oil medium

NRC: National Research Council

PAHs: Polyaromatic Hydrocarbons

PCP: Poly Chlrorinated Polyaromatics

REMIB: Regional Environmental Management Improvement Project

TGY: Tryptone Glucose Yeast extract medium

TPH: Total Petroleum Hydrocarbon

TSS: Total Suspended Solids

UCM: Unresolved Complex Mixture

UV: Ultraviolet

VOC: Volatile Organic Compounds

CONTENTS

	Page
Preface ……..	1
Literature Review …….	3
1-Petroleum Oil in Marine Environments…….	3
1.1-Definintion of Petroleum …….	3
1.2- Chemical Composition and Physical Properties of Petroleum Oils…….	3
1.3-Inputs of Oil and Hydrcarbon on The Marine Environment……	5
1.3.1- Accidentally…….	5
1.3.2- Loading and discharging activity……..	5
1.3.3-Natural seeps ……	5
1.3.4-Petroleum extraction……	6
1.3.5-Petroleum Transportation ……..	6
1.3.6-Petroleum Consumption……..	7
1.4- Hazards of Marine Oil Pollution…….	7
1.4.1- Effect of oil pollution on the marine ecosystem…….....	7
1.4.2-Effects of oil pollution on the human health……..	10
1.4.3- Effects of oil pollution on the man's use of the sea……...	11
1.4.3.1-Tainting of fish by oil……	11
1.4.3.2-Contamination of fishing gear and aquaculture facilities…	12
1.4.3.3-Impact on tourism…….	12
1.4.3.4-Impact on industrial uses……..	12
1.4.3.5- Costs of oil pollution……	13
1.5- Treatment of oil pollution……...	13
1.5.1- Natural Removal	13
1.5.2- Shoreline clean-up……..	13

1.5.3- Mechanical containment, recovery or removal …….. 14

1.5.4-Chemical dispersion……. 14

1.5.5-Other countermeasures…… 15

2- Petroleum Pollution in Gulf of Suez 16

3-Pollution sources in governorate of Suez…….. 17

3.1- Sea-based pollution sources……. 17

3.2- land-based pollution sources…… 18

4-Microbial Degradation of Petroleum Hydrocarbons 20

4.1- Interactions of Microorganisms with Oil…….. 20

4.1.1 – Protozoa…….. 20

4.1.2 - Actinomycetes yeasts and fngi…… 21

4.1.3 –Microalgae…….. 23

4.1.4-Bacteria …… 24

4.2-Distribution of hydrocarbon utilizing microorganisms… 26

4.3- Chemistry of petroleum biodegradation…….. 27

4.3.1- Biodegradation mechanism of paraffinic fraction……. 27

4.3.2- Biodegradation of Polyaromatic Hydrocarbons…….. 27

4.4- Physical and chemical factors affecting the biodegradation of hydrocarbons…….. 31

4.4.1- Chemical composition of the oil…… 31

4.4.2- Physical state of oil pollutants…….. 32

4.4.3- Concentration of the oil or hydrocarbons……. 34

4.4.4-Temperature…… 34

4.4.5-Oxygen……... 34

4.4.6-Nutrients…… 36

4.4.7- Salinity……. 37

4.4.8-Pressure…… 37

4.4.9- Water activity……... 38

4.4.10- pH ……. 38

4.5- Biological factors affecting the Biodegradation of hydrocarbons……. 39

4.5.1- Adaptation - effect of prior exposure…….. 39

4.5.2-Alteration of the genetic composition of the microbial community…….. 41

4.5.3-Seeding…….. 43

5- Bioremediation Technologies…… 46

5.1 EX situ methods……. 46

5.2-In situ methods…….. 46

BioAugmentation and BioStimulation…….. 47

6-Electromagnetic radiation…… 50

6.1-Definition and types of electromagnetic radiations….. 50

6.2-Types of radiation……. 51

6.3- Units of radiation……. 52

6.4-D_{10}-value…… 53

6.5- Radiation induced mutation…… 53

Materials and Methods 55

Materials …… 55

1- Base oil …….. 55

2-Sea water samples ……... 55

3-Bacterial isolates…… 55

4-Culture media……. 55

4.1- Medium used for isolation and enumertation of heterotrophic bacteria.Tryptone glucose yeast extract agar 55

4.2-Medium used for isolation and preservation of petroleum utilizing bacteria silica-gel-oil-agar (SOA)…….. 56
4.3-Medium used for studying kinetic of PAHs degradation (Fertilized sea water)……. 56
4.4-Medium used for adaptation of marine consortia on polyaromatic hydrocarbon …….. 56
Methods…… 57
1-Samples collection…….. 57
1.1-Samples for microbiological analysis…… 57
1.2-Samples for hydrocarbon analysis……. 57
2- Microbiological methods…….. 58
2.1-Enumeration of heterotrophic bacteria in polluted sea water samples …….. 58
2.2-Enumeration of petroteum utilizing bacteria in sea water samples…… 58
2.3- Determination of degradation kinetics of phenanthrene containing microcosms by Suez Gulf microbial consortia and other abiotic factors…….. 59
2.4-Isolation of PAHs Degrading microorganisms from…… 59
Enrichment of sea water …… 59
2.4.1-First Enrichment…… 59
2.4.2-Second Enrichment……. 59
2.5- Preservation of the mixed and pure isolated marine petroleum utilizing bacteria…….. 60
2.6 -Selection of the most promising hydrocarbon utilizing isolates…….. 60
2.7- Morphological characterization of PAHs degrading microorganisms from Suez Gulf…… 61
2.8-Examination of the bacterial isolates at a broad range of incubation temperatures…….. 61
2.9-Determination of tolerance of the bacterial isolates to different base oil concentration…….. 61
2.10- Determination of the incubation period for each bacterial isolate on MSO…….. 61

2.11- Determination of the biodegradation kinetics of the selected PAHs degrading bacteria……. 62
2.12- Characterization and identification of the most promising isolate…… 62
2.13- Gamma irradiation of the selected most promising biodegrading isolate……. 62
2.14- Examination of biostimulation efficiency for PAH bioremediation in marine environment…… 63
2.15- Examination of bioaugmentation efficiency for PAH bioremediation in marine environment…… 64
2.16-Comparative study between biostimulation and bioaugmentation for PAHs bioremediation in marine microcosms……. 64
2.17- A comparative semipilot study for PAH remediation in artificially polluted marine basins using biostimulation and bioaugmentation technologies …….. 65
3- Chemical methods…… 67
3.1-Extraction of hydrocarbons from sea water samples and gravimetric estimation………. 67
3.2-Ultraviolet spectraphotometric analysis…….. 67
3.3-Gas chromatographic analysis…… 68
3.4- High performance Liquid chromatographic (HPLC) analysis… 68
Results …….. 69
1-Impact of petroleum oil pollution on marine microorganisms……. 69
2-Biodegradation Potential Kinetics of Suez Gulf Consortia at different concentrations of Phenanthrene (PAH) in marine environment……. 72
3- Isolation of PAHs Degrading microorganisms from Suez Gulf…… 81
3.1- Incubation period…….. 83

3.2- Growth at different temperature…….. 83
3.3- Tolerance of the isoltes to different concentrations of base oil…… 84
3.4-Biochemical characterization of the most promising bacterial isolate…….. 89
4-Effect of γ-Irradiation on Growth And Biodegradation Potentials of HD20…… 95
5- Application of Bioremediation Technologies……. 103
5.1-Biostimulation for PAH Bioremediation…….. 103
5.2- Bioaugmentation for PAH Bioremediation……. 109
5.3-Comparative Study between Biostimulation and Bioaugmentation for PAHs Bioremediation in Marine Microcosms…….. 111
6-Efficacy of Biostimulation and Bioaugmentation in Artificially polluted basins…….. 113
Discussion …… 126
1-Impact of petroleum oil pollution on marine microorganisms…… 126
2-Biodegradation Potential Kinetics of Suez Gulf Consortia at different concentrations of Phenanthrene (PAH) in marine environment……. 128
3- Isolation of PAHs Degrading microorganisms from Suez Gulf…… 128
4-Effect of γ-Irradiation on Growth And Biodegradation Potentials of HD20…… 132
5- Application of Bioremediation Technologies……. 133
6-Efficacy of Biostimulation and Bioaugmentation in Artificially polluted basins…….. 138
Summary…… 141
Refereces…… 147
الملخص العربى…… 1

Abstract

Samples were collected from the same location in Suez Gulf during the period from June, 2004 to April 2006 then microbiologically and chemically analyzed. The TPH levels ranged from 55 to 86 ppm and exceeded the known permissible limits referring to a settled situation of chronic hydrocarbon pollution in the studied area. On the other hand the biodegrading bacterial counts cfu clearly reflected the great adaptation of endogenous bacteria to use hydrocarbons as a sole source of carbon. The ratio of biodegrading bacteria to heterotrophic ones ranged between 26 and 50 % over the period of collection.

The biodegradation potentials of Suez Gulf consertia were studied at different concentrations of phenanthrene as a sole carbon source. It was found that the degradation kinetics of phenanthrene either due to biotic or abiotic factors is affected with the initial concentration of PAHs.

Twenty PAHs degraders were isolated from Suez Gulf Consortia after different adaptation periods on phenanthrene. Ten isolates were selected to be promising due to their ability to tolerate high base oil concentrations, grow at wide range of temperatures and their short incubation period on MSO. The biodegradation kinetics of 200ppm phenanthrene by the selected isolates was monitored by HPLC.

Isolate HD20 was selected due to its fast and remarkable abilities to breakdown phenanathrene . It was then exposed to different doses of gamma radiation in order to increase their biodegradation potentials.

The biodegradation potentials of single selected irradiated colonies and the whole irradiated cultures were tested separately in marine microcosms containing phenanthrene. The selected single colony at dose of 0.7 kGy (S7) showed the maximum degradation potential and preserved on MSO for bioaugmentation experiments.

The optimal concentration of an inorganic fertilizers was used in marine microcosms containing phenanthrene and compared with different concentrations of organic fertilizer (Rice straw) for biostimulation of PAHs degradation by natural marine consortia. The superiority of the biostimulation action of rice straw over the inorganic alternatives has been proven. A direct relationship between the concentration of organic fertilizer on one side and both biotic and abiotic degradation potentials of phenanthrene on the other side .

Bioaugmentation study was applied using the promising isolate at 0.7 kGy (S7) in Different concentrations. Bioaugmentation microcosms presented high degradation potential of phenanthrene (31%) especially which contain the highest concentration of the augmented isolate.

On comparing biostimulating action using 1.0 gm/l of rice straw (the optimal chosen fertilizer) to the seeded fertilized microcosms using

the best inoculum concentration of HD20 on phenanthrene biodegradation in marine microcosms, it was found that there is no observable difference between both treatments on the bench scale.

In order to upscale the previous bench-scale results of biostimulation and bioaugmentation experiments, 25 litre capacity basins containing artificially polluted Suez Gulf water with 1000 ppm Balaeem base oil were used to represent biostimulation, bioaugmentation and untreated controls. A Remarkable increase in the total bacterial count and hydrocarbon degrading bacteria was observed in biostimulation basins and bioaugmentation basins in comparison to the control basins.

Total Petroleum Hydrocarbons (TPH) was determined gravimetrically while paraffins, isoparaffins and UCM were determined using gas chromatographic analysis of the residual hydrocarbons in each basin. The data show remarkable decrease in TPH (53%) , Paraffins, Isoparaffins and UCM of biostimulation basins while there was slight reduction in those of control and bioaugmentation (15%) basins which refer to higher microbial activity in biostimulation basins.

Polyaromatic hydrocarbons in extracted base oil samples from control , biostimulation and bioaugmentation basins were analyzed by HPLC technique. Phenanthrene disappeared completely in the extracted base oil samples from both biostimulation and bioaugmentation basins. On the other hand flourene and fluoranthene completely diapeared in biostimulation basins with the appearance of anthracene and pyrene .

المستخلص العربى

1- لقد تم دراسة العلاقة بين تركيز و نوع التلوث البترولى من ناحية و اتجاهات الكسير الحيوى لدى الكائنات الدقيقة و ذلك فى محطة على ساحل شركة النصر للبترول. ولهذا الغرض قد تم جمع عينات من نفس الموقع على خليج السويس خلال الفترة من يونيو 2004 الى ابريل و تم تحليلها ميكروبيولوجيا و كيميائيا. و لم يكن هناك اختلافات واضحة بين نتائج تحليل العينات التى جمعت خلال فترة الدراسة.

وقد تعددت الهيدروكربونات البترولية الكلية الحدود المسموح بها لكى تكون 10 جزء فى المليون فى العينات التى جمعت فى القترة من يونيو 2004 حتى ابريل 2006 و ذلك يشير الى استقرار الوضع كتلوث مزمن فى المنطقة تحت الدراسة.

من ناحية اخرى فان العد الميكروبى يعكس التكيف الكبير الذى يحدث للبكتريا الطبيعية لكى تستخدم الهيدروكربونات كمصدر وحيد للكربون.

2- لقد تم دراسة قدرات التكسير الحيوى للميكروبات فى منطقة خليج السويس فى اوساط تحوى تركيزات مختلفة من الفينانثرين كمصدر كربون وحيد. و اتضح من معدل التكسير للفينانثرين اما نتيجة النشاط الميكروبى او غير الميكروبى انه يتأثر بالتركيز الاصلى للهيدروكربونات متعددة الحلقات حيث انه حقق اعلى نسبة تكسير بعد 6 ساعات عند تركيز 100و 200جزء فى المليون بينما حقق اعلى معدل تكسير عند تركيز 150 جزء فى المليون بعد 12 ساعة. و تحقق اعلى تأثير بيولوجى او غير بيولوجى كان عند تركيز 200 جزء فى المليون.

3- كان الهدف من عزل السلالات البكتيرية التى تكسر المركبات الهيدروكربونية متعددة الحلقات هو التعريف و التوصيف لبعض السلالات التى تسهم فى عملية التكسير الحيوى و اختيار بعض السلالت التى لها القدرة العالية على التكسير و ذلك لاستخدامها فى عملية التكسير ودراسة عملية الحقن الميكروبى.

لقد نجحت 22 سلالة بكتيرية فى النمو على البيئة MSO و التى تحتوى على الزيت الاساسى كمصدر كربونى وحيد و هذه السلالات تم عزلها من مياه خليج السويس قبل و بعد استخدام الفينانثرين فى الوسط.

و لقد فشلت العزلات 12و13و15 ان تنمو على البيئات الصلبة MSO& TGY . وقد تم اختبار العزلات لتحديد فترات الحضانة على نطاق واسع من درجات الحرارة و قدراتها على احتمال التركيزات المختلفة من الهيدروكربونات و ذلك لكى نختار العزلة الواعدة التى يكون لها النشاط أكبر.

و قد اتضح ان 77,26 % من العزلات كانت لها القدرة على النمو بوفرة و بقوة على MSO المحتوى على تركيز من الزيت 5000 و 10000 جزء فى المليون بينما 54% فقط كان لها القدرة على النمو فى التركيز 20000 جزء فى المليون من الزيت.

و قد فشل 23 % من العزلات من النمو فى درجات حرارة للتحضين مختلفة على TGY.

100% من العزلات فشلت فى النمو بعد 24 ساعة عند درجة حرارة تحضين 15 ^{0}م بينما 54% و 59,1 % من العزلات كانت قادرة على النمو فى TGY بعد 24 ساعة عند درجة حرارة 25 ^{0}م , 25^{0}م و 42 ^{0}م .

فشل 25% من العزلات فى النمو على MSO بينما كلن هناك نمو سواء ضعيف او معتدل بعد 48 ساعة فى 75 % من العزلات و قد وصل النمو اقصاه بعد 72 ساعة حيث كان 50 % من العزلات كان قادرا بدرجة متوسطة على النمو فى MSO و قد اختيرت العزلات التى ربما تكون واعدة لقدرتها على مقاومة التركيزات العلية من الزيت و التى تنمو فى نطاق و اسع من درجات الحرارة و فترة تحضين قصيرة على وسط MSO و قد تم اختبار عشر سلالات منتخبة وهى HD2, HD6, HD10, HD14 , HD17, HD19, HD20, HD21 و HD22 فى اوساط تحتوى على 200 جزء فى المليون فينانثرين و قد تم تتبع التكسير الحيوى للحلقات بواسطة كل عزلة على حدة.