Changes in the Bacterial Community composition due to Petroleum Oil Exposure and Bioremediation

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ABSTRACT

The main goal of this study was to monitor the changes of marine bacterial communities due to different trends of oil exposures and after different bioremediation treatments in marine microcosms. Also to find the relationship between bacterial diversity and biodegradation potentials under different conditions. The best adaptation conditions to enhance Suez Gulf microflora (SGM) was examined. Nontraditional methods such as using immobilizing agents and special adapted inocula for bioremediation enhancement were tested. The changes in bacterial community composition were detected by analyzing the PCR amplified product of 16S rDNA via denaturing gradient gel electrophoresis (DGGE). DGGE profiles revealed remarkable changes in bacterial diversity due to changing the concentration of oil spell and the mode of exposure. Gradual exposure to oil was found to enhance the biodegradation potentials of SGM. Enhanced biodegradation by SGM in the presence of different immobilizing agents in marine microcosms polluted with 5000 ppm base oil revealed remarkable changes in bacterial diversity and enhanced the biodegradation potentials of SGM. On the other hand, application of biostimulation using inorganic fertilizer and bioaugmentation using different adapted biodegrading inocula revealed severe changes in the bacterial community composition. The biodegradation potentials of the adapted inoculum AB was as high as twice as that of the other adapted inocula. It was observable that AB structural fingerprints predominate when added to the oil polluted marine microcosms containing SGM. In order to achieve successful bioaugmentation results, gradual adaptation protocols are recommended to be applied on the natural consortia.

Key words: Biodegrading Bacteria, DGGE, Bioaugmentation, Hydrocarbons

INTRODUCTION

The study of microbial diversity and community dynamics is rapidly growing in microbial ecology. Interest in this area has been catalyzed by the rapid advancement of molecular ecological methodologies. Through the use of culture-independent molecular techniques, new insights into the composition of uncultivated microbial communities have been gained. It is now becoming possible to define the causes of time-dependent changes in the health of a stressed ecosystem on the basis of the structural composition of the ecosystem population (1).

Our seas, oceans and coastal zones are under great stress; and pollution, particularly by crude oil, remains a major threat to the sustainability of planet Earth. An estimated 1.3 million tons of petroleum enters the marine environment each year (2). A wide variety of micro-organisms are known to degrade petroleum hydrocarbons (3,4).

Microbial communities within contaminated ecosystems tend to be dominated by those organisms capable of utilizing and/or surviving toxic contamination. As a result, these communities are typically less diverse than those in nonstressed systems, although the diversity may be influenced by the complexity of chemical mixtures present and the length of time the populations have been
exposed. A method by which the shift in such a microbial community structure can be monitored in greater detail is denaturing gradient gel electrophoresis (DGGE). This method makes use of the 16S rDNA molecule carried by all bacteria, the sequences of which provide molecular markers for species identification (for historical reviews on the use of rRNA sequences for studying microbial communities (5,6). The method was originally used for profiling microbial populations in environmental samples by Muyzer et al. (7). Several examples of its application can be found in references (8-14).

Oil spills are treated as a widespread problem that poses a great threat to any ecosystem. Following first response actions, bioremediation has emerged as the best strategy for combating oil spills and can be enhanced by the following two complementary approaches: bioaugmentation and biostimulation. Bioaugmentation is one of the most controversial issues of bioremediation. Studies that compare the relative performance of bioaugmentation and biostimulation suggest that nutrient addition alone has a greater effect on oil biodegradation than the addition of microbial products because the survival and degradation ability of microbes introduced to a contaminated site are highly dependent on environmental conditions (15).

The marine environment is highly susceptible to pollution by petroleum, and so it is important to understand how microorganisms degrade hydrocarbons, and thereby mitigate ecosystem damage. The understanding about the ecology, physiology, biochemistry and genetics of oil-degrading bacteria and fungi has increased greatly in recent decades; however, individual populations of microbes do not function alone in nature. The diverse array of hydrocarbons present in crude oil requires resource partitioning by microbial populations, and microbial modification of oil components and the surrounding environment will lead to temporal succession. But even when just one type of hydrocarbon is present, a network of direct and indirect interactions within and between species is observed (2).

The aim of this study was to determine the changes of marine bacterial communities due to different trends of oil exposure and to determine the shifts in marine bacterial community structure after different bioremediation techniques.

MATERIAL AND METHODS

1- Base oil

The base oil used in this study is Balaeem light base oil which is characterized by the following: Specific gravity at 15/4°C = 0.88, pour point = 70°F, flash point = 345°F, viscosity at 100°C = 3-4 Cst, Distillation: Initial point = 325, at 5% = 369, at 95% = 341, according to data obtained from Petrobel oil company. The confirmed hydrocarbon type analysis of Balaeem Light base oil is: Saturated fraction, 59% and aromatic fraction, 41%.

2- Microbial inocula:

The Suez Gulf microbial consortium (SGM) used in this study were collected from the zone opposite to El-Nasr Petroleum Company on Suez Gulf. Different adapted SGM were obtained through different enrichments in the laboratory.

Design of oil polluted microcosms representing different exposure trends:

Oil-polluted microcosms contain 100 ml of Sigma-artificial sea water fertilized with inorganic sources of P and N, NaH₂PO₄ (3.5x10⁻⁴ M) and KNO₃ {10⁻² M}. Base oil (BO) was added in different concentrations and trends. After sterilization of microcosms, SGM was inoculated and the mixture was incubated in a shaking incubator at 30°C and 150 rpm for one week. SGM was exposed to different oil pollution concentrations and trends of exposure. Five groups of triplicate microcosms were designed:

O1: SGM + 1000 ppm of BO (one week)
O2: SGM + 5000 ppm of BO (one week)
O3: SGM + 10000 ppm of BO (one week)
O4: SGM + 1000 ppm of BO ► 5000 ppm of BO (one week for each exposure)
O5: SGM + 1000 ppm of BO ► 5000 ppm of BO ► 10000 ppm of BO (one week for each exposure).
In O4 microcosms, SGM were exposed to 1000 ppm of BO for one week, then one ml was transferred to fresh microcosms containing 5000 ppm and incubated as previously mentioned for one week. In O5, SGM were exposed to 1000, 5000 and 10000 ppm of BO subsequently, one week for each exposure.

After the five exposures mentioned above, bacteria in each microcosm were cultivated on MSO plates. SGM in microcosms and grown on MSO were analyzed via DGGE. Biodegradation in all the studied microcosms was determined.

**Oil biodegradation by SGM in the presence of immobilizing agents:**

The microcosms for this experiment contain 100 ml of sterile Sigma-artificial sea water in Erlenmeyer flasks + 5000 ppm BO + Inorganic fertilizer + 1g of immobilizing material.

Two immobilizing substrates were used separately, glass beads, 0.11mm in diameter (B) and artificial sponge (S). No immobilizing material was added to the control microcosms (N). The immobilizing agents were added to microcosms before autoclaving, and then they were inoculated with equal inocula of SGM and incubated for one week at 30°C and 150 rpm in shaking incubator. For each treatment, triplicate microcosms were used for each analysis. Bacterial diversity in each microcosm and the culturable bacterial diversity in the corresponding microcosm were DGGE analyzed. Also, the remained oil in microcosms was extracted for TPH determination.

**Seeding and Fertilization experiments:**

All marine microcosms contain 5000 ppm BO and Sigma-artificial sea water, four groups of microcosms were used for this experiment:

**Control microcosms:** Sea water + SGM

**Fertilized microcosms:** Sea water + SGM + inorganic fertilizer (F)

**Seeded fertilized microcosms:**

1- Sea water + SGM + F+ (BF)
2- Sea water + SGM + F+ N (NF)
3- Sea water + SGM + F+ S (SF)
4- Sea water + SGM + F+ AB (AB)
5- Sea water + F + AB (AB*)

**Seeded microcosms:**

1-Sea water + SGM + B (B)
2-Sea water + SGM + N (N)
3-Sea water + SGM + S (S)

The bacterial inocula B, S and N are different SGM inocula previously adapted in microcosms containing 5000 ppm of BO and glass beads, Sponge pieces and without immobilizing agent respectively.

AB is SGM inoculum adapted in series of oil polluted microcosms of increasing oil concentrations, 1000, 5000 and 10000 ppm respectively. The SGM was adapted for one week in each concentration.

**Changes in bacterial diversity in oil polluted microcosms:**

Bacterial pellets collected from different microcosms were collected by centrifugation for 5 min (10,000 x g) for DNA extraction, and then 16S rDNA was amplified using PCR. DGGE profiles of the PCR products reflect the changes in community structure due to different treatments. The numbers and positions of bands reflect the diversity in each microcosm.
The bands in DGGE profiles were counted accurately using a magnifying lens.

**Cultivation of Petroleum utilizing microorganisms:**

Sea water aliquots from all the studied microcosms were cultivated on the surface of (MSO) plates, and then incubated at 30°C. MSO is a developed medium for cultivation of culturable biodegrading bacteria by incorporating 0.5% of base oil which was previously adsorbed on 10 g activated silica gel into filtered sea water amended with and inorganic sources of P and N, NaH\(_2\)PO\(_4\) (3.5x10\(^{-4}\) M) and KNO\(_3\) (10\(^{-2}\) M)\(^{16}\). The bacterial growth was collected after 7 days in separate test tubes containing sterile saline solution and the bacterial pellets were separately collected by centrifugation for 5 min (10,000 x g) for DNA extraction, PCR amplification of 16S rDNA and DGGE profiling \(^{13}\).

**Extraction of hydrocarbons from sea water microcosms and gravimetric estimation.**

A modified method for base oil extraction from sea water was described by Zakaria\(^{16}\): 100 ml aliquots of the acidified sea water sample (from studied microcosms) were added to 50 ml CCl\(_4\) in 500 ml screw-capped conical flasks. The flasks were placed on shaking incubator at 300 rpm and 30°C for 1 hour. The contents of the flasks were poured in appropriate separating funnel. The CCl\(_4\) phase was allowed to pass through anhydrous sodium sulphate to a clean beaker. The same procedure was repeatedly carried out upon the remainder of the aqueous phase.

In order to remove polar materials, the solvent extract can be passed through activated silica-gel or alumina then the solvent was air dried in dark place and the oil sample was eventually accurately weighed.

**DNA Extraction from enriched sea water microcosms and MSO-grown mixtures:**

Direct extraction of total microbial DNA from water samples and from enrichment cultures was performed using a rapid bead beating protocol. Briefly, 2 g of the bacterial pellet from 50-ml enrichment culture plus 5 ml of 120 mM phosphate buffer and 3 g of glass beads (0.11 mm in diameter) were subjected to 90-s bead beating steps followed by the addition of 180µl of SDS (20%). The resulting suspensions were subjected to extractions with equal volumes of tris-buffered phenol and chloroform-isoamyl alcohol (24:1), precipitation with ethanol and resuspension of the pellet in TE buffer. Subsequent steps consisted of purification with CsCl to precipitate impurities, followed by the precipitation of the DNA with isopropanol, precipitation with potassium acetate (20µl of an 8M solution per 100 µl), precipitation with 0.6 volume of isopropanol and, finally purification over resin spin column (Wizard DNA cleanup system: Promega, Madison, Wis). Extracted DNA was visualized on 0.8% (wt/vol) agarose gels to check its purity. The final DNA extracts obtained from all enrichment flasks were amplified by PCR using 1µl of extract per 50-µl reaction volume \(^{12}\).

**Polymerase chain reaction (PCR):**

Microbial community DNA was extracted from enrichment microcosms as well as the growth mixture grown on MSO plates (as mentioned above) and amplified with eubacterial primers (8F-GC and 519R) in triplicate. A hot-start, touchdown program (annealing temperature of 65°C, touching down to 55°C in 10 cycles followed by 15 cycles at 55°C) was used to amplify community DNA \(^{17}\).

**Determination of bacterial community composition by DGGE:**

The reaction mixtures of PCR were concentrated by ethanol precipitation and were loaded into 6% polyacrylamide gel containing a 40 to 60% urea-formamide denaturing gradient. The denaturing gradient was prepared and allowed to polymerize for 1.5 h. A spacer gel (6% acrylamide, 0% denaturant, approximately 7.5 mm in height) into which the 20-well comb was inserted and applied on
top of denaturant gel to minimize denaturant gradient disturbance during comb insertion and was allowed to polymerize for 0.5 h. Wells were washed with buffer (40mM Tris-acetate, 1 mM EDTA, pH 8.0), and approximately 350ug of amplified PCR product was loaded per lane and run at 80 V for 16 h at 60ºC. The resulting gel was stained in 1 mg of ethidium bromide liter $^{-1}$ in Tris-acetate-EDTA buffer and was photographed. Comparisons of banding pattern were done for different samples to detect changes in bacterial community structures under different conditions $^{(12)}$.

RESULTS

Oil spills can alter the microbial diversity. The effect of different oil exposures on Suez Gulf microorganisms was studied. Fig. (1) represents the DGGE analysis of (A) biodegrading bacteria in different microcosms and (B) culturable bacteria of the corresponding microcosms. Each lane represents one microcosm. The bands represent the bacterial genera in the DGGE profiles.

The SGM in microcosms O1, O2 and O3 were exposed to 1000, 5000 and 10000 ppm respectively. The exposure to the high oil concentrations was sudden in the microcosms O2 and O3. The SGM were exposed to 5000, 10000 ppm gradually in O4 and O5.

In O4-microcosms SGM were exposed to 1000 ppm of BO for one week, then one ml was transferred to fresh microcosms containing 5000 ppm and incubated as previously mentioned for one week. In O5, SGM were exposed to 1000, 5000 and 10000 subsequently, one week for each exposure.

The different exposures to different oil concentrations and different modes of exposures resulted in changes in the bacterial community composition of SGM.

The number of DGGE bands in Fig.(1) were counted using a magnifying lens and represented in Table (1). Table 1 shows the bacterial diversity in microcosms after different oil exposures, the culturable bacteria in each microcosm and the corresponding biodegradation achievements by the affected SGM consortia in every microcosm.

Fig(2) shows the diversities in different oil exposure microcosms as well as their biodegradation potentials. No correlation was observed between the biodiversity and biodegradation potentials. SGM achieved high biodegradation of oil, 92.6% after one week incubation with 1000 ppm of BO. The biodiversity reflected in the DGGE profiles as the number of bands was 49. On exposure to 5000 ppm of base oil, the diversity was nearly the same (50 bands) the biodegradation percentage was 44.6%. In O3 microcosm which was exposed to 10000 ppm of base oil, the diversity was reduced after one week to 28 and only 21% of the oil was degraded. Surprisingly, SGM in microcosms O4 and O5 exposed to 5000 and 10000 ppm of BO achieved higher biodegradation results than O2 and O3 exposed to the same base oil concentrations without prior gradual exposure.

The exposure to 10000 ppm base oil resulted in a drastic reduction of bacterial diversity of SGM in case of both, gradual and sudden exposure trends.

OB, OS and ON are microcosms that contain glass beads, artificial sponge pieces and controls(no added immobilizing agent) respectively.

Clear shifts were observed in the community composition due to the addition of glass beads and sponge pieces to the bioremediation microcosms.
Fig. (1): DGGE analysis of 16S rDNA-PCR product of SGM in different microcosms after different oil exposures.

SW: Sea water, O1 to O3 represent the diversity SGM exposed to 1000, 5000 and 10000 ppm while 1, 2 and 3 represent the MSO grown bacterial diversity at the same exposures respectively. O4 and O5 represent bacterial diversity after gradual exposures to 5000 and 10000 ppm respectively, while 4 and 5 represent the MSO grown diversity at the same exposure trends.
Table (1): Diversity, Culturability and biodegradation potentials of SGM in marine microcosms of different oil exposures.

<table>
<thead>
<tr>
<th>microcosms</th>
<th>Total No. of DGGE bands</th>
<th>No. of culturable bands</th>
<th>Biodegradation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>38</td>
<td>-</td>
</tr>
<tr>
<td>O 1</td>
<td>49</td>
<td>38</td>
<td>29.6</td>
</tr>
<tr>
<td>O 2</td>
<td>50</td>
<td>38</td>
<td>44.6</td>
</tr>
<tr>
<td>O 3</td>
<td>28</td>
<td>38</td>
<td>21.2</td>
</tr>
<tr>
<td>O 4</td>
<td>42</td>
<td>32</td>
<td>53.4</td>
</tr>
<tr>
<td>O 5</td>
<td>33</td>
<td>30</td>
<td>68.5</td>
</tr>
</tbody>
</table>

Fig.(2): Biodegradation and bacterial diversity of SGM in marine microcosms after different oil exposures.

The DGGE profiles of SGM in fertilized microcosms containing 5000 ppm of base oil and different immobilizing substrates were shown in Fig.(3A). The DGGE profiles of MSO-grown SGM were represented in Fig.(3B).
Fig.(3): DGGE profiles of 16S rDNA PCR product of SGM in biodegradation microcosms containing 5000 ppm base oil and different immobilizing agents and the corresponding MSO growth. OB, ON and OS are microcosms containing glass beads, control (no immobilizing agent) and sponge pieces respectively. B, N and S represent the corresponding MSO growth.

Table (2) shows the diversity (the number of DGGE bands), the number of culturable bacteria and the biodegradation potentials of SGM in marine microcosms containing 5000 ppm of oil and different immobilizing agents. Fig.(4) shows that the addition of glass beads and sponge pieces increased the biodegradation potentials of SGM. The number of DGGE bands representing the bacterial diversity was 47 in OB microcosms which contain the glass beads, the bacteria in this microcosm was able to degrade 58.7% of the base oil but in OS microcosms which contain the sponge pieces, the diversity was 25 and the achieved biodegradation was 59.3%. The biodegradation in control microcosms (ON) was 44.6% and the diversity was 34.
Table (2): Diversity, Cultability and biodegradation potentials of SGM marine microcosms containing 5000 ppm of oil and different immobilizing agents.

<table>
<thead>
<tr>
<th>microcosms</th>
<th>Total No. of DGGE bands (Diversity)</th>
<th>No. of culturability bands</th>
<th>Biodegradation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>47</td>
<td>30</td>
<td>58.7</td>
</tr>
<tr>
<td>ON</td>
<td>34</td>
<td>25</td>
<td>44.6</td>
</tr>
<tr>
<td>OS</td>
<td>23</td>
<td>25</td>
<td>64.8</td>
</tr>
</tbody>
</table>

Fig (4): Diversity and biodegradation potentials of SGM in marine microcosms containing 5000 ppm of oil and different immobilizing agents.

Fig. (5) Shows the DGGE profiles of the 16S rDNA-PCR product of biodegrading bacteria in bioremediation microcosms polluted with 5000 ppm of base oil and inoculated with different inocula of adapted SGM in the presence and absence of inorganic fertilizer.

The addition of inorganic fertilizer to the marine microcosms which contain SGM lead to remarkable shifts in the bacterial community composition as shown in Fig. 5, lanes C and F.

The addition of previously adapted inocula to marine microcosms which contain SGM resulted in the occurrence of some shifts in the bacterial community structure. The DGGE profiles of bacterial communities in microcosms B, N and S were different in spite of the presence of SGM in all of them indicating the clear effects of the added inocula on the community structure.
Fig.(5): the DGGE profiles of the 16S rDNA-PCR product of biodegrading bacteria after 48 hours growth in bioremediation microcosms artificially polluted with 5000 ppm of base oil and inoculated with SGM in addition to different inocula of (adapted SGM) in the presence and absence of inorganic fertilizer. C: control - F: fertilizer, B, N and S: different adapted inocula- BF, NF, SF: different adapted inocula + fertilizer- AB is a super adapted inoculums + fertilizer - AB*: AB + fertilizer only (without SGM).
On the other hand, the variation in bacterial community structure in microcosms seeded with different adapted inocula disappeared in the microcosms to which inorganic fertilizer was added.

Lanes AB and AB* in Fig(5) represent the bacterial community structure of the fertilized contaminated microcosms seeded with the novel adapted inoculums with SGM (AB) and without SGM (AB*). It was found that AB* profile shows a specific finger print of bacterial diversity. This characteristic diversity fingerprint remained even in the presence of SGM.

The oil biodegradation potentials achieved by different adapted inocula in marine microcosms containing 5000 and 10000 ppm BO are represented in Fig. 6

![Biodegradation Chart](image)

**Fig. (6): Biodegradation of polluted marine microcosms by natural SGM and SGM adapted inocula**

The data in Fig. 6 shows the superiority of biodegradation potentials of the adapted inoculum AB. The base oil was degraded by AB as twice as the other adapted inocula at both concentrations, 5000 and 10000 ppm.

Fig. (7A) shows the biodegradation of 10000 ppm BO in marine microcosms before and after 48 hours biodegradation via SGM while Fig. (7B) shows the marine microcosms containing 10000 ppm base oil before and after biodegradation via the novel adapted inoculum AB after 48 hours.

The visual observations of Fig. (7B) shows the disappearance of base oil in marine microcosm containing 10000 ppm of base oil after 28 hours degraded via the adapted inoculum AB, unlike in the marine microcosms containing the same concentration of base oil and degraded by the original SGM (Fig. 7A).
Fig. (7A): The biodegradation of 10000 ppm BO in fertilized marine microcosms before and after biodegradation via SGM for 48 hours

Fig. (7B): The biodegradation of 10000 ppm BO in fertilized marine microcosms before and after biodegradation via AB for 48 hours
DISCUSSION

Changes in bacterial diversity in oil polluted microcosms:

In the present study, the SGM in microcosms O1, O2 and O3 were exposed to 1000, 5000 and 10000 ppm respectively. The exposure to the high oil concentrations was sudden in the microcosms O2 and O3 while the exposure to the same doses in microcosms O4 and O5 was gradual. The exposures to different oil concentrations and modes of exposure resulted in changes in the bacterial community composition of SGM.

No correlation was observed between the biodiversity and biodegradation potentials. SGM achieved high biodegradation of oil, 92.6% after one week incubation with 1000 ppm of BO, The biodiversity reflected in the DGGE profiles as the number of bands was 49. On exposure to 5000 ppm of base oil, the diversity was nearly the same (50 bands) while the biodegradation percentage was 44.6%. In O3 microcosm which was exposed to 10000 ppm of base oil, the diversity was reduced after one week to 28 and only 21% of the oil was degraded. Surprisingly, SGM in microcosms O4 and O5 exposed to 5000 and 10000 ppm of BO achieved higher biodegradation results than O2 and O3 exposed to the same base oil concentrations without prior gradual exposure.

The Prestige oil spill did not affect bacterial abundances in the areas studied but induced deep changes in the structure of bacterial communities (18). A similar situation was described after the Nakhodka oil spill, with a composition similar to that of the Prestige fuel, in the marine communities of the Japan Sea (19,20). Although the communities were qualitatively different from those in NOS, those affected by the oil spill still conserved high species richness and diversity. Community diversity was dramatically reduced just after the pollution event and progressively recovered to pre-oiling levels but with a different structure dominated by hydrocarbonoclastic bacteria (21,22).

In the present study, the sudden and gradual exposures to 10000 ppm base oil resulted in the reduction of bacterial diversity of SGM. Bioremediation treatments dramatically reduced the diversity of the bacterial community. The decrease in diversity could be attributed to a strong selection for bacteria belonging to the alkane-degrading bacteria. A rapid recovery of the bacterial community diversity to pre-oiling levels of diversity occurred. However, although the overall diversity was similar, there were considerable qualitative differences in the community structure before and after the bioremediation treatments (22).

Effects of adding immobilizing agents on community structure and biodegradation potentials of SGM in oil-contaminated microcosms:

Clear shifts were observed in the community composition due to the addition of glass beads and sponge pieces in the microcosms.

The addition of glass beads and sponge pieces increased the biodegradation potentials of SGM. Whereas, the number of DGGE bands representing the bacterial diversity was 47 in OB microcosms which contain the glass beads, the bacteria in this microcosm was able to degrade 58.7% of the base oil but in OS microcosms which contain the sponge pieces, the diversity was 25 and the achieved biodegradation was 59.3%. The biodegradation in control microcosms (ON) was 44.6% and the diversity was 34. This confirms the absence of certain correlation between bacterial diversity and biodegradation potentials.

A mixture of hydrocarbon-degrading bacterial strains was immobilized in alginate and incubated in crude oil-contaminated artificial seawater (ASW) (23). Analysis of hydrocarbon residues following a 30-day incubation period demonstrated that the biodegradation capacity of the microorganisms was not compromised by the immobilization. Removal of n-alkanes was similar in immobilized cells and control cells. The results confirmed that immobilized hydrocarbon-degrading
bacteria represent a promising application in the bioremediation of hydrocarbon-contaminated areas (23).

A new technique is presented for bioremediation by adding nutrient amendments to the oil spill using thin filmed minerals comprised largely of Fullers Earth clay. Together with adsorbed N and P fertilizers, filming additives, and organoclay, clay flakes can be engineered to float on seawater, attach to the oil, and slowly release contained nutrients (24).

Organic polymer materials (OPM) differing in sets of functional groups, fiber surface thickness and character, and density of fiber packing in fabric were synthesized. OPM were studied for assessing the possibility of their application as sorbents for oil spills in water bodies. The synthesized OPM were used for the creation of bio-hybrid materials as matrices for immobilization of bacteria of the genus *Rhodococcus* sp. capable of petroleum degradation. Actively dividing bacterial cells forming clusters were shown to be present at the surface of fibers. Active attachment of the cells to polymeric surface due to intrusion and/or excretion of extracellular biopolymeric matrix were detected. The modification of polymer sorbents was shown to influence bacterial immobilization. The peculiarity of growth and the specificity of cell morphology of bacterial culture were noted (25).

**Changes in Bacterial community structure during biostimulation and bioaugmentation**

The addition of inorganic fertilizer to the marine microcosms which contain SGM lead to remarkable shifts in the bacterial community composition.

In the present study, the addition of previously adapted inocula to marine microcosms which contain SGM resulted in the occurrence of some shifts in the bacterial community structure. The DGGE profiles of bacterial communities in microcosms B, N and S were different inspite of the presence of SGM in all of them indicating the clear effects of the added inocula on the community structure.

On the other hand, the variation in bacterial community structure in microcosms seeded with different adapted inocula disappeared in the microcosms to which inorganic fertilizer was added.

The bacterial community structure of the fertilized contaminated microcosms seeded with the novel adapted inoculum with SGM (AB) and without SGM (AB*). It was found that AB* profile shows a specific finger print of bacterial diversity. This characteristic diversity fingerprint predominated over the structure of the present SGM. The biodegradation achieved by the novel consortium AB of 5000 and 10000 ppm of base oil in the fertilized marine microcosms was as twice as the degradation achieved by the studied adapted consortia as well as the natural SGM.

A DGGE profiling is an attractive and dependable alternative despite some inherent uncertainties (26). Being faster, less-expensive and less labor-intensive than sequencing, it allows highly replicated analysis of bacterial communities (7). The PCR-DGGE is particularly suited to fingerprint the spatial and temporal differences in bacterial communities adapted inocula (27).

The biodegradation of buried oil is stimulated by addition of nutrients, either in a liquid form or in a solid form. The relationship between community structure and degradation appears to be complex since communities with similar structures showed different rates of degradation, while communities with different structures showed similar degrees of degradation (12).

The use of biostimulation additives in combination with naturally pre-adapted hydrocarbon-degrading consortia (bioaugmentation) has proved to be an effective treatment and is a promising strategy that could be applied specifically when an oil spill approaches near a shore line and an immediate hydrocarbon degradation effort is needed (15).

A marine microbial consortium obtained from a beach contaminated by the Prestige oil spill proved highly efficient in removing the different hydrocarbon families present in this heavy fuel oil (28).
Bioremediation treatments dramatically reduced the diversity of the bacterial community. The decrease in diversity could be accounted for by a strong selection for bacteria belonging to the alkane-degrading Alcanivorax/Fundibacter group. On the basis of Shannon-Weaver indices, rapid recovery of the bacterial community diversity to preoiling levels of diversity occurred. However, although the overall diversity was similar, there were considerable qualitative differences in the community structure before and after the bioremediation treatments (22).

There is an increasing evidence that the best approach to realize successful bioaugmentation is the use of microorganisms from the polluted area, an approach proposed as autochthonous bioaugmentation (ABA) and defined as a bioaugmentation technology that exclusively uses microorganisms indigenous to the sites (soil, sand, and water) slated for decontamination (15).

It has been suggested that restoration of the bacterial community structure to a state similar to that present prior to pollution could be used as a parameter for determination of the ecological end point of bioremediation (26).

CONCLUSION

Oil spills can drastically affect the bacterial community composition. In addition to the effects of exposure concentration on the community, also, the sequence of exposure is highly effective. Biodegradation in the presence of glass beads and sponge pieces alter the diversity and enhances biodegradation. In order to achieve successful bioaugmentation results, the convenient adaptation protocols should be applied on the natural consortia obtained from the polluted environment.

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