JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2011. Vol. **5**(1), p. 445-448

A Biostimulatory Efficacy on Oil Degrading Marine Bacteria

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(Received: 01 July 2010; accepted: 22 August 2010)

To day the cry pollution is heard from all the nooks and corners of the world and oil spills in marine environment has become a major menace to the environment as they severely damage the surrounding eco-system. Oil remediation with out any harmful side effects, time and cost effective is the major challenge of our times. The present study deals with the biostimulatory efficacy of seven different elements such as nitrogen, phosphorus, potassium (major nutrients) zinc, sulphur, magnesium and calcium (minor nutrients) in various combinations. The initial and final amount of oil and microbial population was estimated. The results revealed that there is a significant increase in oil remediation and microbial population in the experimental setup (sea water + crude oil + elements) and specific the NPK with trace elements combination showed a maximum remediation. Attempts were also made to identify the different microbial colonies in sea water and thus *Pseudomonas* sp was identified and characterized.

Key words: Bioremediation, biostimulation, oil remediation *Pseudomonas* sp.

Intensive sea traffic results in a constant danger of oil contamination in the sea environment. About 77 million tons of oil (mainly crude oil) were transported across the Baltic Sea in 1995, (Forsman and Magnusson) in 1996 - 133 million tons, and in1997 - 144 million tons (Magnusson, 1996 - 2001). Therefore, there is a high probability of oil appearing in the sea - in any case, several hundred illegal oil discharges in the Baltic Sea are registered by Helcom (1998) every year. A number of investigations which focus Large areas of earth's surface and the oceans and other water ways have already been contaminated with oil-derived compounds and toxic chemicals.

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An oil spill is the intentional or unintentional release of oil (generally petroleum) into the natural environment as a result of human activity. Bioremediation is the process of living microorganisms to clean up a contaminated site. Microorganisms do this by removing toxins from the materials. Psychotrophic *Pseudomonas putida* has removed a wide variety of phenolic compounds from waste water under aerobic and pH neutral conditions at temperature ranging from 1 to 35°C. The tragic Exxon valdez spill in 1989 in price William sound, Alaska provided an opportunity to field test these nutrient addition techniques in a cold climate. The study of identification of bacteria is important in microbial ecology, especially with molecular techniques (Watanabe and Hamamura ,2003) In particular, analysis of the microbial communities that take part in in-situ hydrocarbon biodegradation activities has been a challenge to microbiologist. Interest in this area has been catalyzed by the rapid advancement of molecular ecological methodologies (MacNaughton, 1999).

The biostimulation, in which there is an increase of the indigenous populations activity by adding nutrients and/or a terminal electron acceptor, and the bio-augmentation, in which there is an increase of the pollutant degradation potential by adding exogenous degrading microbial strains. In most cases, the treatment of oil contaminated environments has involved biostimulation – the addi-tion of nutrients to stimulate the indigenous microbial population (Bartha, 1986; Leahy and Colwell 1990; Morgan and Watkinson 1989). Rosenberg and Gutnick (1986) proposed that approximately 150 mg nitrogen and 30 mg phosphorus are required for metabolism of 1 g of hydrocarbon substrate. However, there has been considerable debate over the efficacy of bioaugmenta-tion (e.g. Atlas, 1991; Pritchard, 1992; Vogel, 1996), the addition of dried or liquid cultures of either indigenous or exogenous microorganisms to expedite the remedia-tion process.

MATERIAL AND METHODS

Sample collection

Sea water sample was collected from the Marina beach Chennai, Tamilnadu and was transported to the lab under aseptic conditions.

Biostimulation of natural microbes

The naturally existing oil degrading marine microbes was stimulated by the application of nutrients such as NPK, Ca, Mg ,S.

Determination of oil degrading efficiency

For this study, a control and experimental setups in triplicates were maintained. The container for control contained sea water and crude oil alone with out any nutrient. The experimental containers carried sea water, crude oil and the nutrients and the different treatment combinations are as follows.

T1 - Sea water+ crude oil+ NPK
T2 - Sea water+ crude oil+ calcium
T3 - Sea water+ crude oil+ magnesium
T4 - Sea water+ crude oil+ sulphur
T5 - Sea water+ crude oil +zinc
T6 - Sea water+ crude oil+ NPK+calcium
T7 - Sea water+ crude oil+ NPK+ calcium+ magnesium
T8 - Sea water+ crude oil+ NPK+ calcium+ magnesium + sulphur
T9 - Sea water+ crude oil+ NPK+ calcium+ magnesium +sulphur+zinc

The container was maintained under lab conditions with appropriate temperature and light conditions which to support the microbial growth. The containers were left for 7 days for the biodegradation of hydrocarbons (through stimulation) to take place. At fourth day the container were aerated in order to supply oxygen which is an essential requirement for the microbial growth. The amount of oil remediate from the sample containing combinations of nutrients was explaining method.

Identification of predominant isolates

After seven days of treatment the predominant colonies were isolated from the treated mixtures. The isolated colonies were identified based on the Gram's staining, Motility and biochemical characteristics.

RESULT AND DISCUSSION

The oil degradation capacity of naturally existing marine microbes was enhanced using various forms of nutrients such as nitrogen, phosphorous, potassium, zinc, sulphur, calcium, magnesium. The effects of these nutrients were tested by adding the nutrients individually and in combinations with sea water containing crude oil analysis (Fig. 1).

Sea water collected from Marina beach was treated with various inorganic nutrient sources. The treatment was maintained for a period of 7 days and at the end of treatment period the degradation efficiency was examined. Oil degradation efficiency results are given in Fig. 2.

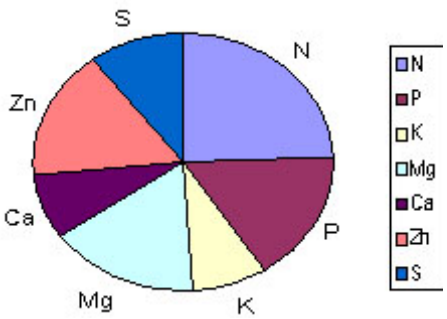
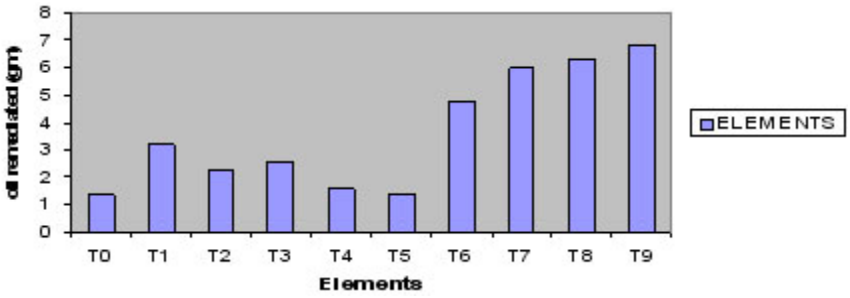


Fig. 1. Amount of elements used in the experiment (gms)



To – control
T1- Sea water+ crude oil+ NPK
T2- Sea water+ crude oil+ calcium
T3- Sea water+ crude oil+ magnesium
T4- Sea water+ crude oil+ sulphur
T5- Sea water+ crude oil +zinc
T6- Sea water+ crude oil+ NPK+calcium
T7- Sea water+ crude oil+ NPK+ calcium+ magnesium
T8- Sea water+ crude oil+ NPK+ calcium+ magnesium + sulphur
T9- Sea water+ crude oil+ NPK+ calcium+ magnesium +sulphur+zinc

Fig. **2.** Amount of oil remediated by various elements

The samples containing nitrogen, phosphorous, potassium remediated more amount of oil when compared with other elements (calcium, magnesium, zinc, sulphur). Higher oil remediation was obtained in the sea water sample supplemented with nitrogen, phosphorous, potassium as they are the major nutrients required by the microbial population and minor elements are calcium, magnesium, zinc, sulphur also facilitated the degradation process of hydrocarbon. Nutrients are one of the major factors limiting hydrocarbon biodegradation in sea. Addition of nutrients as fertilizers resulting in oil biodegradation has been reported by several workers. (Lee, K and Levy 1989). The presence of fertilizer stimulates the microbial growth. Microbes are the main degraders of petroleum hydrocarbons in contaminated ecosystems (Leahy and Cowell 1990).The capability of nutrients to increase microbial population (thereby increasing bioremediation activity) was tested.

The number of colonies was higher in major elements like nitrogen, phosphorous, potassium. Four crude oil-degrading microorganisms previously isolated and selected from the contaminated soil (SÁ, 2002).The microbes required nutrients for their growth and reproduction. Through the metabolism the hydrocarbon molecules can be broken down. For this break down nutrients act as catalyst thereby doubling the speed of natural oil removal. Biodegradation is prominently an oxidation process. Microbial enzymes will catalyze the insertion of oxygen into hydrocarbon so that the molecule can subsequently be consumed by cellular metabolisms (Bragg *et al.*, 1991).

ACKNOWLEDGEMENTS

The authors wish to acknowledge with gratitude the support for this work by Lady Doak college, Madurai.

REFERENCES

1. Atlas, R.M. Microbial hydrocarbon degradation – bioremediation of oil spills. *Journal of Chemical Technology and Biotech.,* 1991; **52**: 149-156.
2. Bartha, R. Biotechnology of petroleum pollutant bio-degradation. *Microbial Ecology,* 1986; **12**: 155–172.
3. Cornish, A., Battersby, N. S., & Watkinson, R. J. Environmental fate of mineral, vegetable and transesterified vegetable oils. *Pestic. Sci.,* 1993; **37**: 173–178.
4. Forsman B., Magnusson K. Transportation of oils in the Baltic Sea Area 1995. SSPA Maritime Consulting AB, Report 7596-1, 1996**.**
5. Helcom, Overview on activities. *Bait. Sea Environ. Proce*., 1998; **73.**

6. Leahy, J. and Cowell, R.R. Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, 1990; **54**: 305– 315.
7. Lee .K and Merlin.F.X, Bioremediation of oil an shoreline environments; development of techniques and guidelines. *Pure Appl.Chem.*, 1999; **71**: 161-171.
8. Lee.K and Leavy.E.M, Biodegradation of petroleum in marine environment, in Aquatic Toxicology and wastes Quality Mnagement, edited by J.A. Nriagu (John Wiley and sons, New York) 1989: pp 217-243.
9. MacNaughton S.J., Stephen J.R, Venosa A.D., Davis G.A., Chang Y.J., White D.C., *Applied and Env. Micro*., 1999; **65**: 8: 3566-3574.
10. Magnusson K. Oil handling in the Baltic Sea Area 1996 - 2001. SSPA Maritime Consulting AB, Report 7935-2, 1998**.**
11. Morgan, P. and Watkinson, R.J. Hydrocarbon biodeg-radation in soils and methods for soil biotreatment. *CRC Critical Reviews in Biotechnology,* 1989; **8**: 305–333.
12. Pritchard, P.H. Use of inoculation in bioremediation. *Current Opinion in Biotechnology*, 1992; **3**: 232-243.
13. Rosenberg Eugene, Rachel legmann, Ariel Kushmaro, Ran Taube, Ellik Adler and Eliora,Z.Ron. Petroleum bioremediation a multiphase problem. *Biodegradation*, 1992; **3**: pp 337-345.
14. Rosenberg, E., and Gutnick, D.L. The hydrocar-bon-oxidising bacteria. In: Starr, M.P., Stolp, H., Truper, H.G., Balows, A. and Schlegel, H.G. The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bac-teria, Springer-Verlag, Heidelberg, 1986; pp. 903-912.
15. SÁ, M. C. C. "Tratamento Biológico de Solo Contaminado com Hidrocarbonetos de Petróleo". Tese M. Sc., Universidade Federal do Rio de Janeiro, Escola de Química, Rio de Janeiro, Brasil, 2002: pp 116.
16. Vogel, T.M. Bioaugmentation as a soil bioremedia-tion approach. *Current Opinion in Biotechnology*, 1996; **7**: 311-316.
17. Watanabe K and Hamamura N. *Current Opinion in Biotechnology*, 2003; **14**: 289-295.