IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 12 Ver. III (Dec. 2014), PP 33-35
www.iosrjournals.org

Bioremediation of oil contaminated soil by Pseudomonas putidaP11 and tests on efficacy of plant growth

J.Sureka[1], M.Vijayakumari[2]
[1] *(Department of Biochemistry, Mother Teresa women,s university, India)*
[2] *(Department of Biochemistry, Mother Teresa women,s university, India)*

Abstract: *In this present study contaminated soil was degraded using Pseudomonas and NPKFertilizer. Normal soil and contaminated soil were degraded by using Pseudomonas and then Contaminated soil was degraded by using Pseudomonas and NPK fertilizer. All the samples were incubated for 18 days. As a result microbial degradation was increased, pH was decreased and also macro nutrients, microbial count, The growth of Phaseolus vulgaris was tested, phytochemical, chlorophyll content was increased. Pseudomonas has the ability to degrade hydrocarbon present in the contaminated soil by the addition of NPK fertilizer. The rate of degradation of contaminated soil was enhanced by Pseudomonas putidaP11.*
Keywords: *Bioremediation, Oil contaminated soil, Pseudomonas putida, Phaseolus vulgaris*

I. Introduction

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Accidental release of petroleum products are of particular concern in the environment .Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can expensive [4].

Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Bioremediation functions basically on bio degradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds by biological agents like microorganisms. Many indigenous microorganisms in water and capable of degrading hydrocarbon contaminants [10].The hydrocarbon degrading microbes have an inherent capacity to assimilate hydrocarbons and or its products [1].

The process is therefore regarded as a complex biological oxidation process involving mostly aerobic organisms which may be enhanced by supplementation with fixed Nitrogen, Phosphate and other Nutrients [6].

Microbial degradation is the major and ultimate natural mechanisms by which one can clean up the petroleum hydrocarbon pollutants from the environment.The recognition of bio degraded petroleum –derived aromatic hydrocarbon [11]. The microorganisms include bacteria of the *Genera, Klebsiella, Proteus, Bacillus, Escherichia Coli, Pseudomonas, Streptomyces, Nocardia, Seratia, Xanthomonas, Micrococcus* etc…and *Fungi of the Genera- Rhizopus, Fusarium, Pencillium, Cladosporium* and *Aspergillus e*tc. .Bio surfactants are heterogeneous group of surface active chemical compounds produced by a wide variety of microorganisms. Surfactants enhance solubilization and removal of contaminants. Biodegradation is also enhanced by surfactants due to increased bio availability of pollutants [5].

II. Materials And Methods

1.EXPERIMENTAL SET UP

Contaminated soil was converted into degraded soil using *Pseudomonas* and NPK fertilizers. Normal soil and contaminated soil were taken and used as control I and II. .250 g of Contaminated soil was taken and to this 10 ml of stock solution of *Pseudomonas* was mixed and taken as sample I. Then 250 g of contaminated soil, 10ml of *Pseudomonas* and 25 g of NPK fertilizer were taken as sample II. Totally four different soil samples were incubated for 18 days. After incubation the samples were used for pH analysis, nutrient analysis, and microbial count. The *Phaseolus vulgaris* plants were grown in thesefour soil samples and the Morphological and biochemical characters were analyzed.

2.ANALYSIS OF CONTAMINATED SOIL

The pH of the given contaminated soil sample was determined using the method of [9]. The nitrogen was estimated by Microkjeldhal method.. The phosphorous was estimated by the method of[2]. The potassium was estimated using Flame photometric method. The total petroleum hydrocarbon content was determined .The microbial population studies was carried out by using [3].

3.GROWTH PARAMETERS

The measurement of various morphological growth parameters such as root length, shoot length was taken within the time interval of 10, 20 days and biochemical constituents such as chlorophyll content was estimated by the methods of [7]. and phytochemical contents were evaluated by the methods of[8].

III. Results And Discussion

In the present study contaminated soil with the help of *Pseudomonas* and NPK was converted into less contaminated soil. The physical, chemical and biological factor was determined.

ANALYSIS OF CONTAMINATED SOIL

The pH of the soil was determined using pH meter. Initial pH was high and after microbial degradation of contaminated soil. The pH was reduced.The pH decreased from alkaline to acidic. The pH shift toward acidic condition was attributed to mineralization of Nitrogen, Phosphorous and potassium in to nitrites and ortho-phosphates; bioconversion of organic material in to intermediate species of organic acid production of CO_2 and organic acid by microbial degradation during degraded soil lowers the p^H of degraded soil .

The total Nitrogen, Phosphorus and potassium shows that it was low in initial level. After degradation process the nitrogen, phosphate, and potassium concentration increased**.** Nitrogen, Phosphorous and Potassium contents were higher in microbial degraded soil. The reason could be due to higher organic matter content in microbial degraded soil [13]. The present study shows that there was an increase in level of bacterial and fungal count in degraded soil than contaminated soil. The bacterial and fungal population count was increased as the microbes utilized hydrocarbon for carbon and energy sources the increased in population count and degradation of the hydrocarbon was stimulated by the fertilizer. It was observed that as the population count increased due to hydrocarbon utilization for carbon and energy residual hydrocarbon decreased and percentageof degradation increased [12].

GROWTH PARAMETER

The shoot lengths of the plants were measured at 10 and 20 days intervals.

According to the experiments maximum growth of *Phaselous Vulgaris* was recorded in contaminated soil treated with *Pseudomonas* and NPK. The degraded soil have shown great ability to assimilate essential macro, micro nutrients that results in shoot development(Table 3).The Phytochemical contents of plants increased in plants treated with degraded soil than the control. Phytochemicals form a part of the natural plant defense system against infection and Microbialinvasions

IV. Figures And Tables

TABLE.1: THE LEVEL OF MAJOR ELEMENT OF VARIOUS SOIL SAMPLE:

S.NO	SOIL SAMPLE	N(%)			P(%)			K(%)		
		0 Days	9 Days	18 Days	0 Days	9 Days	18 Days	0 Days	9 Days	18 Days
I	Normal soil	**0.6**	**0.6**	**0.6**	**0.16**	**0.16**	**0.16**	**0.31**	**0.31**	**0.31**
II	Contaminated soil	**0.4**	**0.4**	**0.4**	**0.09**	**0.09**	**0.09**	**0.18**	**0.18**	**0.18**
III	Contaminated soil + *Pseudomonas*	**0.4**	**0.47**	**0.53**	**0.09**	**0.13**	**0.18**	**0.18**	**0.21**	**0.24**
IV	Contaminated soil + *Pseudomonas* +NPK fertilizer	**0.5**	**0.58**	**0.65**	**0.22**	**0.29**	**0.37**	**0.38**	**0.42**	**0.48**

TABLE.2: THE LEVEL OF MICROBIAL COUNT IN VARIOUS SOIL SAMPLE:

S.No	SOIL SAMPLE	BACTERIA X 10^6 CFU		FUNGAI X 10^3 CFU	
		Initial	Final	Initial	Final
I	Normal soil	21	21	7	7
II	Contaminated soil	13	13	4	4
III	Contaminatedsoil +*Pseudomonas*	13	19	4	10
IV	Contaminated soil+*Pseudomonas* +NPK fertilizer	13	24	4	15

TABLE.3: THE LEVEL OF SHOOT LENGTH IN VARIOUS SOIL SAMPLE

S.NO	SOIL SAMPLE	SHOOT LENGTH	
		Initial	Final
I	Normal soil	5.8	12.5
II	Contaminated soil	4.7	8.2
III	Contaminatedsoil +*Pseudomonas*	4.9	8.5
IV	Contaminated soil+*Pseudomonas* +NPK fertilizer	5.6	11.2

TABLE.4: THE LEVEL OF CHLOROPHYLL IN VARIOUS SOIL SAMPLE

S.No	Treatment	Chlorophyll **(mg/g fresh weight)**
1.	Normal Soil	2.8
2	Contaminated soil	1.7
3	Contaminated Soil + *Pseudomonas*	2.1
4	Contaminated Soil + NPK + *Pseudomonas*	2.6

V. Conclusion

It may be concluded that microbial degradation can be considered as a key component in the clean up strategy for hydrocarbon remediation. Microorganism degrades hydrocarbon helps to reduce the polluted environment to regain its natural characters. Microbial degradation process aids the elimination of spilled oil from the environment. This is possible because microorganisms have enzyme system to degrade the utilize different hydrocarbon as a source of carbon and energy.

Acknowledgements

We thank the authorities of Sri Adi Chunchanagiri Womens College Cumbum for providing lab facilities and to the Department of Biochemistry, Biotechnology.

References

[1] R.M.Atlas ,petroleum microbiology, Macmillion ,New York,(1984)
[2] Tandon.H.Z., "Methods of analysis of soils, plant, water and Fertilizers". Fertilizer Development and Consultation Organization, New Delhi., (1993), 1-148.
[3] Baron.J.E., Peterson.R.L and Finegold.M.S., "Cultivation and isolation of viable pathogens". In: Diagnostic microbiology, (1994), 9th Edn., Mosby, London.
[4] C.Holliger, S.Gaspard,Contaminated environment in the surface and bioremediation: Organic contaminants,(1997) 517-523.
[5] G.M.Bai,L.Brusseauand R.M.miller, Biosufactants-enchanced removal of residual hydrocarbon from soil,(1997) 157-170.
[6] W.N.Eugene, Microbiology human respective, Mc-Graw-Hill London(1998).
[7] D.I.Arnon,Copper enzyme in isolated chloroplast polyphenol oxidase in Beta vulgaris, (1999),1-15.
[8] Harborne.J.B., "Phytochemical methods: A guide to modern techniques of plant analysis". 3rd ed. Chapman and Hall,London,(1998),302.
[9] S.K.Sujatha, A.Mahalakshmi and R.Shenbaga rathi, Effect of indigenous plants of soil samples, *Indian.J. Biotechnology in agriculture and Environment*, (2003),345-353.
[10] K.A. Kvenvolden and C.K.Cooper, Natural seepage of crude oil the marine environment, *Geo- Marine Letters*, 23, 3, (2003) 140-146,
[11] D.M Jones ,The recognition of biodegraded petroleum derived hydrocarbon in marine sediments, 14,(2003).
[12] John Kanayochuku, Utilisation of different microbes in bioremediation of hydrocarbon conjtaminated soil with inorganic and organic fertilizer, (2012)2157-7463.
[13] E.Stepha. and Egene ,Microbiology and physicochemical properties of soil polluted with Lubricating oil,(2012) 49-52.