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Bioremediation of Oil Sludge using Pseudomonas Aeruginosa, Bacillus Subtilis and Brassica Juncea

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***Abstract*— The leakages of hydrocarbons into the environment in any form through any source, poses a serious threat to the environment. The oil sludge produced from the various operations during the drilling and refining of oil and other fuels need to be disposed properly to avoid any danger to the environment. Physical methods for proper disposal have been proven expensive. In order to degrade and dispose the sludge in an eco-friendly and efficient manner the use of microbes and plants has been implemented. The laboratory scale degradation of oil sludge in micro level, under controlled conditions using *Pseudomonas aeruginosa* and *Bacillus subtilis* has been carried and its efficiency has been determined. These organisms can be easily isolated from soil. Since the laboratory degradation is not possible in all the areas, the method of phytoremediation using *Brassica juncea* (Indian mustard) has been carried out and the possibility of degradation has been discussed. The complete analysis of efficiency of phytoremediation was not able to be done in a short span.**

Keywords*— Oil sludge, Hydrocarbons, Bioremediation, Microbial degradation, Phytoremediation, Pseudomonas sp., Bacillus sp.

I. INTRODUCTION

Oil is the principle source of energy as well as a principle source of environmental pollutants. Regardless of how they are released into the environment, hydrocarbons pose a serious threat to marine life and plant growth. Animals and birds have skin and fur protection and hence they are not affected directly by environmental pollutants where as it is not the same in case of plants and aquatic organisms. Petroleum or crude oil is one of the complex mixtures of hydrocarbons and contain more than 17,000 organic compounds, both volatile and polycyclic aromatic hydrocarbons. The volatile organic may be carcinogenic and evaporate easily which makes it dangerous. Polycyclic aromatic hydrocarbons have similar properties but they last much longer. In addition to accidental contamination of ecosystem due to oil spill, vast amount of oil sludge generated in the refineries from accumulated oily waste material pose great challenges because of the expense of disposal.

Bioremediation refers to the use of specific microorganisms or plants to metabolize and remove harmful substances from the environment. It can be done with a single species of plant or microorganism or plant or a synergy of microbes or a combination of plants and microorganisms based on the required application. The various types of microbial bioremediation include bio-augmentation and bio-stimulation. The types of phytoremediation include phytoextraction, phytodegradation, phytovolatilization and rhizosphere biodegradation. The success of bioremediation depends on a number of factors including the surrounding physical, chemical and biological parameters. The main aim of this research is to find an effective, efficient and an economic method for the disposal of oil sludge produced from the various operations during drilling and refining of various forms of oil and natural gas. In this research, the level of degradation of oil sludge from a fuel refinery by commonly available species of microbes and a plant has been studied and discussed.

II. METHODOLOGY

A. General

The main aim of the project is the degradation of oil sludge which was produced during the drilling and refining operations. The process adopted in this project is bioremediation. In bioremediation, the specifically chosen streams include Microbial degradation and Phytoremediation. These methods have been chosen due to the following reasons: Low cost, High efficiency, less space required, Results can be achieved in short span of time, easily available and precise.

B. Microbial Degradation

The first step in the studies is to isolate and identify the microorganisms from contaminated soil which are capable of crude oil degradation. Microorganisms have enzyme systems to degrade and utilize hydrocarbons as a source of energy. The proliferation of oil degrading microorganisms in polluted soil depends on the availability of nutrients and their hydrocarbonoclastic property. The microbial degradation will be carried out under different conditions with the isolated bacteria. The most commonly available hydrocarbon degrading bacteria from the soil sample are *Pseudomonas aeruginosa* and *Bacillus subtilis* and hence these species were chosen to carry out the study. The chosen species can be cultured under simple conditions with provision of commonly available nutrients.

1. Aerobic degradation

Hydrocarbons are easily degraded under aerobic conditions. Bacteria, fungi & algae are highly capable of degrading the hydrocarbons aerobically. The degradation rates may vary depending upon the source of the petroleum and age of the spill. For example, degradation may way from 5% to 30% in 28 days, where as it may go up to 100% with addition of nitrogen. One of the primary limiting factors is delivery of oxygen. Addition of oxygen can increase the level of

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degradation when compared to the naturally occurring rates. The degradation was carried out controlled aerobic conditions.

2. Culture and conditions

Bushnell Haas medium is prepared and the inoculum is introduced along with the sample to be degraded. The setup has to be maintained under suitable temperature and revolutions which is required for optimum growth and degradation. Different combinations have to be tested before choosing which set up will be best suited for efficient degradation. Then it is maintained for various periods of incubations. The level of degradation is studied using gravimetric analysis.

3. Biodegradation study

In order to study the level of biodegradation, gravimetric analysis is preferred in our study because the nature of sludge is very thick and black in color and hence the use of spectrometric methods is difficult. Hence the level of degradation is studied using weight analysis. The culture after the incubation period is mixed with petroleum ether and acetone and separated using separating funnel. The remaining quantity of sludge is measured and the percentage of degradation is to be determined.

C. Phytoremediation

Since the microbial degradation is not possible in a large scale and is also difficult for practical application, we decided to test an alternative method for the degradation as well. Hence we decided to study the results of phytoremediation using Indian mustard. The sludge was mixed with various quantities of sludge and planted with mustard and maintained in different combinations. The level of degradation is to be studied using the parameter Total Petroleum Hydrocarbon.

III. EXPERIMENTAL INVESTIGATION

A. Sample Collection

Sample was collected from the sludge collection pit from Gas Gathering Station (shown in Fig.1.) and the sludge collection pit from Effluent Treatment Plant. Sample was collected after mixing the sludge well in order to avoid improper distribution of waste matter. Sludge was collected from four different points in the tank at equal distance. Two and a half liters of sample was collected in the sample collection can with the help of a funnel. The cans were stored in the BOD chamber.

B. Culture Media and Incubation Condition

Bushnell-Haas Broth consisting of: Dipotassium Hydrogen Phosphate K_2HPO_4 1.0 g/L, Monopotassium Hydrogen Phosphate KH_2PO_4 1.0 g/L, Ammonium Nitrate NH_4NO_3 1.0 g/L, Magnesium Sulphate $MgSO_4$ 0.2 g/L, Calcium Chloride $CaCl_2$ 0.02 g/L and Ferric Chloride $FeCl_3$ 0.005 g/L was used for incubation. Flasks (120 ml) containing 10 ml of the nutrient broth were inoculated with 2 ml exponential phase culture inoculum of *Bacillus subtilis* and 2 ml exponential phase culture inoculum of *Pseudomonas aeruginosa* strains or their mixture, respectively and then the samples were added and incubated at 37˚C for 2, 4, 6 and up to 28 days. Controls (shown in Fig. 2.) without bacterial inoculation were prepared similarly for all incubations to evaluate the abiotic loss of hydrocarbons.

Fig.1. Sludge Pit

Fig.2. Control and Sample I & II Day-0

Flasks (120 ml) containing 10 ml of the nutrient broth were inoculated with 2 ml exponential phase culture inoculum of *Bacillus subtilis* and 2 ml exponential phase culture inoculum of *Pseudomonas aeruginosa* strains or their mixture, respectively and then the samples were added and incubated at 37˚C for 2, 4, 6 and up to 28 days. Controls without bacterial inoculation were prepared similarly for all incubations to evaluate the abiotic loss of hydrocarbons.

Initially, a test was performed by using three combinations including separate cultures of *Pseudomonas aeruginosa*, *Bacillus subtilis* and one combined with both.

C. Preparation of Bushnell-Hass Broth

1000 ml of broth was prepared by mixing the following ingredients in 1000 ml of distilled water: Dipotassium Hydrogen Phosphate -1 g, Monopotassium Hydrogen Phosphate - 1 g, Ammonium Nitrate - 1 g, Ferric Chloride- 0.005 g, Magnesium Sulphate- 0.2 g and Calcium Chloride- 0.02 g.

100 ml of Nutrient broth was prepared by adding the following ingredients in 100 ml of distilled water: Beef extract- 0.1 g, Yeast extract- 0.2 g, Sodium chloride- 0.5 g and Peptone- 0.5 g.

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10 conical flasks were filled with 100ml Bushnell-Haas medium each. 10 ml nutrient broth was added to each flask and kept in the autoclave. After the pressure was released the conical flasks were taken out and allowed to cool down to room temperature in the Laminar Air Flow chamber. Then the flasks were inoculated with 2 ml of exponential culture of *Bacillus subtilis* and 2 ml of exponential culture of *Pseudomonas aeruginosa*. Different concentrations (1 ml, 2 ml, 3 ml, 4 ml and 5 ml) of sample 1 & sample 2 were added to each flask. The flasks were kept in a shaking incubator at 37℃ and 150 rpm.

Fig.3. Sample-I 1g to 4 g flasks Day-4

Fig.4. Sample- II 1 g to 4 g Day-4

D. Biodegradation Study

Hydrocarbon degradation was studied by gravimetric analysis. After different incubation periods at 37˚C, the flasks (120 ml) were taken out and bacterial activities were stopped by adding 1% (v/v) 1N-HCL. For extraction of residual oil, 40 ml of culture broth was mixed with 40 ml petroleum ether/acetone (1:1) in a separating funnel and then shaken vigorously to get a single emulsified layer. Acetone was then added and shaken gently to break the emulsification, which resulted in three layers. The top layer was a mixture of petroleum ether, diesel oil and acetone; clumping cells make a middle layer and the bottom aqueous layer contains acetone and water in soluble form.

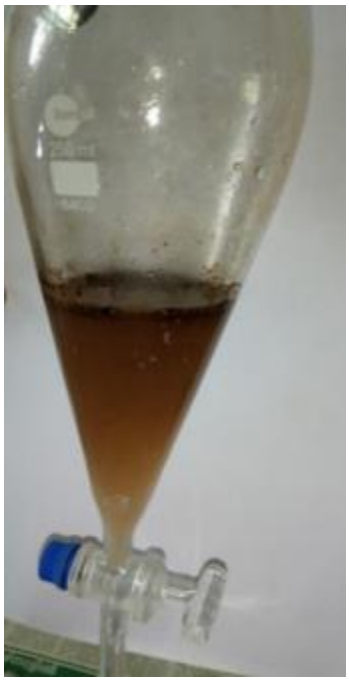

Fig.5. Separating flask with sample

The lower two layers were spread out while the top layer containing petroleum ether mixed with diesel oil or used oils and acetone was taken in a pre-weighed clean beaker. The extracted oil was passed through anhydrous sodium sulphate to remove moisture. The petroleum ether and acetone layer was evaporated on a water bath. The gravimetric estimation of residual oil left after biodegradation was made by weighing the quantity of oil in a tarred beaker. The percentage of biodegraded oil was then evaluated in comparison to the initial hydrocarbon amount in the control funnel.

E. Bioremediation using soil

Different amounts (1 g to 5 g) of samples were mixed in 200 grams of soil and Indian mustard (*Brassica juncea*) was grown in it with different combinations. The combinations included soil + sample + plant, soil + sample, soil + sample + plant + fertilizer, soil + plant + sample + culture & soil + sample + plant + fertilizer + culture. The soil samples were tested for Total Petroleum Hydrocarbon (TPH) initially and the content of TPH after 20 days.

Fig.6. Mixture of Soil & Sludge with mustard seed

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IV. RESULTS AND DISCUSSION

A. *Sludge Characteristics*

Sludge was collected from two different sources namely the sludge pits at the gas gathering station (GGS) and the effluent treatment plant (ETP). The sample from the GGS is sample-1 and the sample from the ETP is sample-2. The samples were given for test and the following results were obtained. On simple visual observation, sample-1 was found to be more watery than sample-2. This shows that the viscosity of sample-2 is greater than that of sample-1.

TABLE I. SLUDGE PROPERTIES

S. No	Parameters	Content in sample-1	Content in sample-2
1	pH	6.94	6.77
2	Density (g/cc)	1.005	0.9824
3	Viscosity (cSt)	23.8	25.1
4	Flash point (ºC)	72	89
5	Sulphur content %	5.93	7.12
6	Ash content %	2.08	3.79
7	Water content %	27.82	30.96
8	Organic matter %	75	68
9	Total petroleum hydrocarbon (µg/g)	8560	7498

The parameters of sample 1 & sample 2 were tested and the results have been given above. Sample 1 is found to be more concentrated than sample 2. But sample 1 is denser than sample 2.

B. *Initial comparison*

Initially, when the degradation results of *Pseudomonas aeruginosa* alone, *Bacillus subtilis* alone and the combination was tested, the results stated in TABLE II were obtained from degradation of one gram of sample.

TABLE II. INITIAL COMPARISON OF EFFICIENCY

Sample Number	Combination	Degradation	
		Grams	%
I	Bacillus sp. (4 ml) (NUMBER 1)	0.120	12
II		0.14	14
I	Pseudomonas sp. (4 ml) (NUMBER 2)	0.20	20
II		0.23	23
I	Combination (2+2) ml (NUMBER 3)	0.48	48
II		0.51	51

In both the combinations, the combination number 3 (*Pseudomonas aeruginosa* 2 ml + *Bacillus subtilis* 2 ml) was found to give the maximum level of degradation. So the combination number 3 was chosen for the further proceedings in the research.

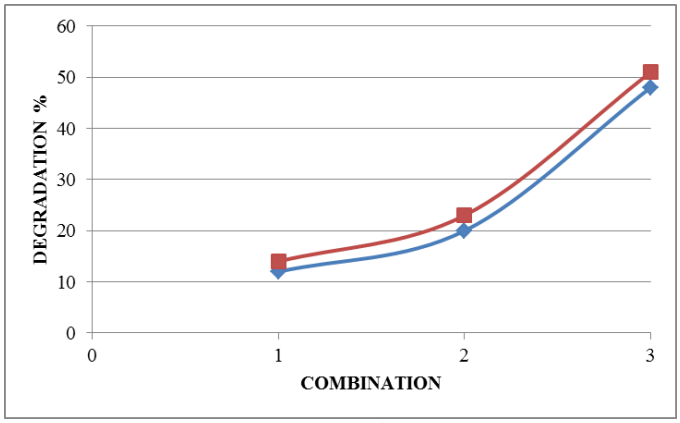

Fig 7. Comparison of degradation

The red line referred to the degradation in sample 1 and the blue referred to the degradation in sample 2.

C. *Results of microbial degradation*

The results of degradation after certain period of time was determined using gravimetric analysis and the following results were obtained. The tests were taken after 2 days, 8 days, 12 days and 20 days. After a period of 20 days the degradation of 1 gram sample was almost complete and degradation of 2 to 4 grams was comparatively satisfactory as well. But the 5 grams sample did not show much of the degradation without further augmentation.

So, the best suitable conditions for most efficient degradation in the laboratory scale must not be more than 3 grams for 100 ml broth and 4ml of combined culture of *Bacillus subtilis* & *Pseudomonas aeruginosa*. The results of degradation for the various combinations are given below.

TABLE III. DEGRADATION ON DAY-2

S. NO	Sample Number	Quantity (g)	Sludge Remaining (g)	Degradation	
				G	%
1	I	1	0.54	0.46	46
2	II		0.47	0.53	53
3	I	2	1.85	0.15	7.5
4	II		1.76	0.24	12
5	I	3	2.65	0.35	11.67
6	II		2.42	0.58	19.3
7	I	4	3.8	0.2	5
8	II		3.33	0.67	16.75
9	I	5	4.91	0.09	1.8
10	II		4.7	0.3	6

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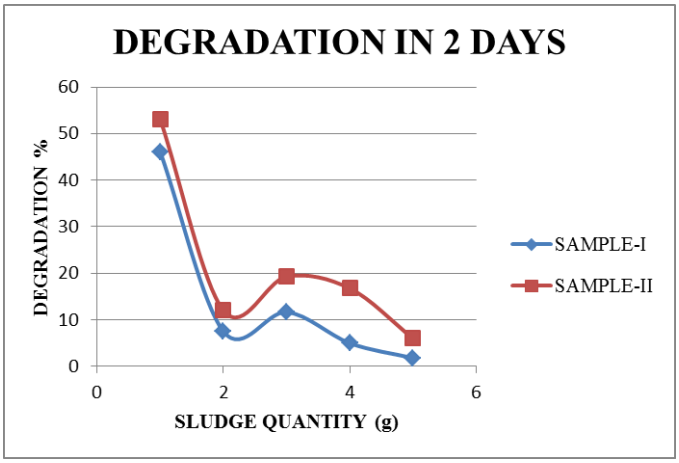

Fig.8. Degradation level in 2 days

TABLE IV. DEGRADATION ON DAY-8

S. NO	Sample Number	Quantity (g)	Sludge Remaining (g)	Degradation	
				g	%
1	I	1	0.18	0.82	82
2	II		0.08	0.92	92
3	I	2	1.8	2.2	55
4	II		1.33	2.67	66.7
5	I	3	1.45	1.55	51.6
6	II		1.42	1.58	52.6
7	I	4	1.15	0.85	42.5
8	II		1.12	0.88	44
9	I	5	3.7	1.3	26
10	II		3.91	1.09	21.8

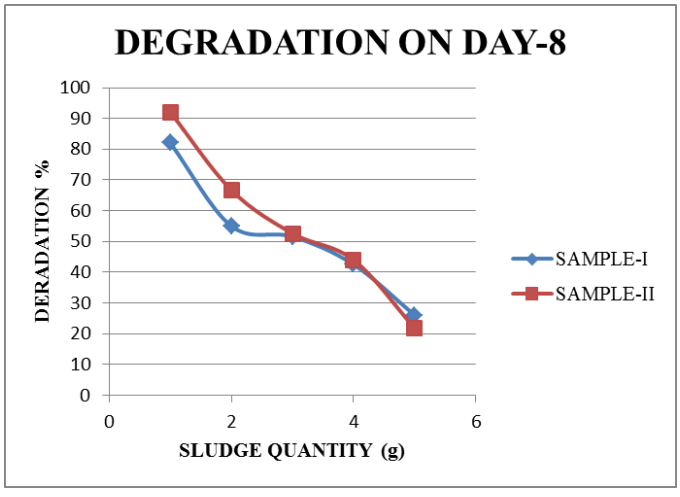

Fig. 9. Degradation level in 8 days

TABLE V. DEGRADATION ON DAY-12

S. NO	Sample Number	Quantity (g)	Sludge Remaining (g)	Degradation	
				G	%
1	I	1	0.01	0.99	99
2	II		0.08	0.92	92
3	I	2	0.15	1.85	92.5
4	II		0.12	1.88	94
5	I	3	1.05	1.95	65
6	II		1.02	1.98	66
7	I	4	2.8	1.2	30
8	II		2.33	1.67	41.8
9	I	5	2.91	2.09	41
10	II		2.7	2.3	46

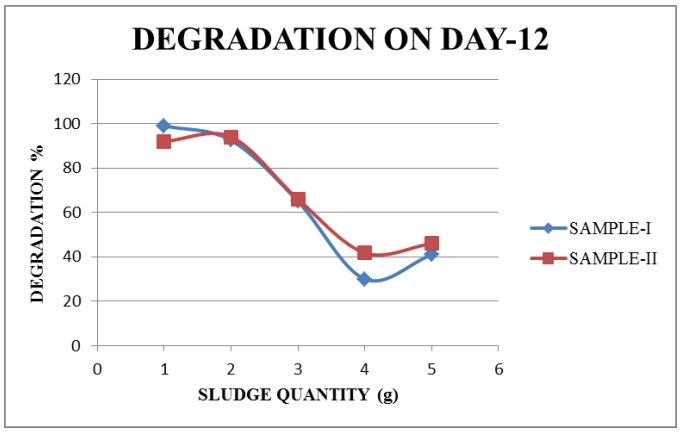

Fig.9. Degradation level in 12 days

TABLE VI. DEGRADATION ON DAY-20

S. NO	Sample Number	Quantity (g)	Sludge Remaining (g)	Degradation	
				G	%
1	I	1	0.008	0.992	99.2
2	II		0.002	0.998	99.8
3	I	2	0.75	1.25	62.5
4	II		0.62	1.38	69
5	I	3	0.95	2.05	68.33
6	II		0.82	2.18	72.67
7	I	4	1.2	2.8	70
8	II		1.03	2.97	74
9	I	5	2.82	2.18	43.6
10	II		2.5	2.5	50

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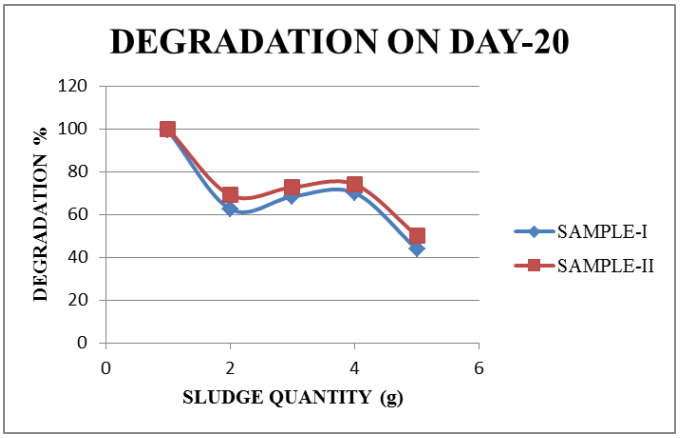

Fig.9. Degradation level in 20 days

D. Results of Phytoremediation

When the soil was tested for Total Petroleum Hydrocarbons after a period of plant growth of 20 days, the following results were obtained in the various combinations respectively.

TABLE VII. COMBINATION: CONTROL- SOIL + SAMPLE

Quantity	1 g	2 g	3 g	4 g	5 g
Sample-1 mg/kg	20	18	22	21	25
Sample-2 mg/kg	19	25	29	38	36

TABLE VIII. COMBINATION: SOIL + SAMPLE + PLANT

Quantity	1 g	2 g	3 g	4 g	5 g
Sample-1 mg/kg	37	41	46	55	67
Sample-2 mg/kg	44	49	53	59	72

TABLE IX. COMBINATION: SOIL + SAMPLE + PLANT + FERTILIZER

Quantity	1 g	2 g	3 g	4 g	5 g
Sample-1 mg/kg	15	18	20	28	32
Sample-2 mg/kg	20	17	24	33	42

TABLE X. COMBINATION: SOIL + SAMPLE + PLANT + CULTURE

Quantity	1 g	2 g	3 g	4 g	5 g
Sample-1 mg/kg	38	43	48	53	62
Sample-2 mg/kg	27	36	40	46	52

TABLE XI. COMBINATION: SOIL + SAMPLE + PLANT + FERTILIZER + CULTURE

Quantity	1 g	2 g	3 g	4 g	5 g
Sample-1 mg/kg	10	18	23	27	32
Sample-2 mg/kg	13	21	28	34	49

Fig.10. Sample-I – Combination S+P 1 to 5 grams

Fig.11. Sample-I – Combination C+S+P 1 to 5 grams

Fig.12. Sample-I – Combination F+C+S+P 1 to 5 grams

Fig.13. Sample-I – Combination F+S+P 1 to 5 grams

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Fig.14. Sample-II – Combination S+P 1 to 5 grams

Fig.15. Sample-II – Combination S+C+P 1 to 5 grams

Fig..16. Sample-II – Combination F+S+C+P 1 to 5 grams

Fig.17. Sample-II – Combination F+S+ P 1 to 5 grams

Fig.18. Growth of S+C+P in Sample-I in 20 days

Fig.19. Growth of F+S+P in Sample-I 20 days

Fig.20. Growth of S+P in Sample-I in 20 days

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Fig.21. Growth of C+S+P in Sample-II 20 days

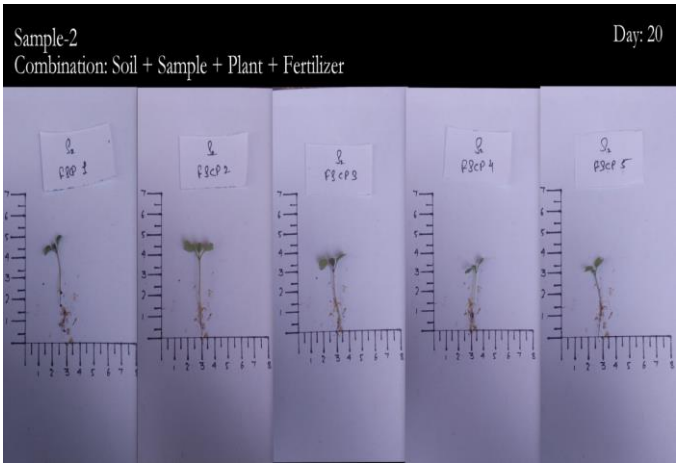

Fig.22. Growth of F+S+C+P in Sample-I 20 days

Figure No. 5.19 Growth of F+S+P in Sample-II 20 days

V. CONCLUSION

The above growth was recorded in each set after 20 days of proper care. The plants were maintained in room temperature without direct exposure to heavy sunlight. They were watered twice a day with 20ml of regular RO water. The measurements of the plant growth in each combination were done. The growth was found to be highest in the combination of Soil + Sample + Fertilizer and Soil + Sample + Fertilizer + Culture. But the level of degradation is not predictable and maximum efficiency is unable to be determined in a short period of 20 days. The results of microbial degradation after certain period of time was determined using gravimetric analysis and the following results were obtained. The tests were taken after 2 days, 8 days, 12 days and 20 days. After a period of 20 days the degradation of 1 gram sample was almost complete and degradation of 2 to 4 grams was comparatively satisfactory as well. But the 5 grams sample did not show much of the degradation without further augmentation.

REFERENCES

[1] Adebusoye S.A, M. O. Ilori, O. O. Amund, O. D. Teniola, and S. O. Olatope (2007) 'Microbial degradation of petroleum hydrocarbons in a polluted tropical stream,' World Journal of Microbiology and Biotechnology, vol. 23, no. 8, pp. 1149–1159,.

[2] Akpe, Azuka Romanus; Ekundayo, Afe Omolola; Aigere, Sandra Patrick; Okwu Grace Ifeoma (2015) 'Bacterial degradation of petroleum hydrocarbons in crude oil polluted soil amended with cassava peels.', American Journal of Research Communication, 3(7): 99-118.

[3] Amin Afifi, Hossein Motamedi, Bahram Alizadeh, Hadi Leilavi (2015) 'Isolation and identification of oil degrading bacteria from oil sludge in Abadan oil refinery', Environ Exp Biol 13: 13–18

[4] Arpita Gupte, Sheetal Sonawdekar (2015) 'Study of Oil Degrading Bacteria Isolated From Oil Contaminated Sites', Volume 3, Issue II, International Journal for Research in Applied Science and Engineering Technology (IJRASET) Page No: , ISSN : 2321-9653

[5] Asadirad, M., Mazaheri Assadi, M., Rashedi, H., Nejadsattari, T. (2016). 'Effects of Indigenous Microbial Consortium in Crude Oil Degradation: A Microcosm Experiment', International Journal of Environmental Research, 10(4), pp. 491-498

[6] Atlas R. and J. Bragg (2009) 'Bioremediation of marine oil spills: when and when not—the Exxon Valdez experience', Microbial Biotechnology, vol. 2, no. 2, pp. 213–221.

[7] Atlas R. M (1992) 'Petroleum microbiology' in Encyclopaedia of Microbiology, pp. 363–369, Academic Press, Baltimore, Md, USA.

[8] Amund O. O and N. Nwokoye (1993) 'Hydrocarbon potentials of yeast isolates from a polluted Lagoon', Journal of Scientific Research and Development, vol. 1, pp. 65–68.

[9] Barathi S. and N. Vasudevan (2001) 'Utilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from a petroleum-contaminated soil', Environment International, vol. 26, no. 5-6, pp. 413–416, 2001.

[10] Colwell R. R., J. D. Walker, and J. J. Cooney (1977) 'Ecological aspects of microbial degradation of petroleum in the marine environment,', Critical Reviews in Microbiology, vol. 5, no. 4, pp. 423–445, 1977.

[11] Cooney J. J., S. A. Silver, and E. A. Beck (1985) 'Factors influencing hydrocarbon degradation in three freshwater lakes', Microbial Ecology, vol. 11, no. 2, pp. 127–137, 1985.

[12] Darsa, K.V. & Joseph Thatheyus, A & Ramya, D. (2014). 'Biodegradation of Petroleum Compound Using the Bacterium Bacillus subtilis', Science International. 2. 20-25. 10.17311/sciintl.2014.20.25.

[13] Fusey, P. and Oudot, J. (1976) 'Comparison of Two Methods for the Evaluation of in Vitro Biodegradation of Hydro- carbons', Material Und Organismen, 11, 241-251

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
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[14] Guo-liang, Z., Yue-ting, W., Xin-ping, Q (2005) ' Biodegradation of crude oil by Pseudomonas aeruginosa in the presence of rhamnolipids', Zheijang Univ.-Sci. B 6: 725.
[15] Jalilzadeh Yengejeh, R., Sekhavatjou, M., Maktabi, P., Arbab Soleimani, N., Khadivi, S., Pourjafarian, V. (2014). 'The Biodegradation of Crude Oil by Bacillus subtilis Isolated from Contaminated Soil in Hot Weather Areas', International Journal of Environmental Research, 8(2), pp. 509-514.
[16] Jones D. M, A. G. Douglas, R. J. Parkes, J. Taylor, W. Giger, and C. Schaffner (1983) 'The recognition of biodegraded petroleum-derived aromatic hydrocarbons in recent marine sediments,' Marine Pollution Bulletin, vol. 14, no. 3, pp. 103–108, 1983.
[17] Kaimi, Etsuko, Mukaidani, Tsukasa, Tamaki, Masahiko. (2007). 'Screening of Twelve Plant Species for Phytoremediation of Petroleum Hydrocarbon-Contaminated Soil', Plant Production Science - PLANT PROD SCI. 10. 211-218. 10.1626/pps.10.211.
[18] Kim Ji-Young; Cho Kyung-Suk (2006) 'Bioremediation of Oil-Contaminated Soil Using Rhizobacteria and Plants', Microbiology and Biotechnology Letters Volume 34, Issue 3, 2006, pp.185-195
[19] B. Lal and S. Khanna (1996) 'Degradation of crude oil by Acinetobacter calcoaceticus and Alcaligenes odorans', Journal of Applied Bacteriology, vol. 81, no. 4, pp. 355–362, 1996
[20] I. Lazar, S. Dobrota, A. Voicu, M. Stefanescu, L. Sandulescu, I.G. Petrisor (1999) 'Microbial degradation of waste hydrocarbons in oily sludge from some Romanian oil fields', Journal of Petroleum Science and Engineering, Volume 22, Issues 1–3,1999,Pages 151-160,ISSN 0920-4105
[21] Lesley C. Batty & Mark Anslow (2008) 'Effect of a Polycyclic Aromatic Hydrocarbon on the Phytoremediation of Zinc by Two Plant Species', International Journal of Phytoremediation, 10:3, 236-251
[22] Liu, Wuxing, Luo, Yongming, Teng, Ying, Li, Zhengao and Ma, Lena. (2009) 'Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes', Environmental geochemistry and health. 32. 23-9. 10.1007/s10653-009-9262-5.
[23] Marquez-Rocha, F.J., Hernandez-Rodriguez, V. and Lamela, M.T. (2001) 'Biodegradation of Diesel Oil in Soil by a Microbial Consortium. Water, Air, and Soil Pollution', 128, 313-320.
[24] Nalinee Kumari, Abhishek Vashishtha, Pooja Saini and Ekta Menghani (2013) 'Isolation, Identification and Characterization of Oil Degrading Bacteria Isolated from the Contaminated Sites of Barmer, Rajasthan', International Journal of Biotechnology and Bioengineering Research. ISSN 2231-1238, Volume 4, Number 5 (2013), pp. 429-436
[25] Napoleon, A and Probowati, D.S. (2014). 'Exploration of hydrocarbon degrading bacteria on soils contaminated by crude oil from South Sumatera', Journal of Degraded and Mining Lands Management. 1. 10.15243/jdmlm.2014.014.201.
[26] M. A. Nogo ole, A. Tuhuloula, S.R. Juliastutiv (2016) 'Bioremediation by Single Culture of Pseudomonas Putida in Petroleum Contaminated Soil', 88th The IIER International Conference, Singapore, 16th-17th December 2016, ISBN: 978-93-86291-56-1
[27] Panda, S.K., Kar, R.N. and Panda, C.R. (2013) 'Isolation and Identification of Petroleum Hydrocarbon Degrading Microorganisms from Oil Contaminated Environment', International Journal of Environmental Sciences, 3, 1314-1321.
[28] Palmroth, Marja & Pichtel, John & Puhakka, Jaakko. (2002). 'Phytoremediation of sub-arctic soil contamination with diesel fuel', Bioresoure Technologies 84. 224-228.
[29] J. J. Perry (1984) 'Microbial metabolism of cyclic alkanes', in Petroleum Microbiology, R. M. Atlas, Ed., pp. 61–98, Macmillan, New York, NY , USA, 1984.
[30] Prakash B, Irfan M (2011) 'Pseudomonas aeruginosa is Present in Crude Oil Contaminated Sites of Barmer Region (India)', J Bioremed Biodegrad 2:129. doi:10.4172/2155-6199.1000129
[31] Sawadogo, A., Otoidobiga, C.H., Sawadogo, J.B., Kaboré, A., Traoré, A.S. and Dianou, D. (2014) 'Isolation and Characterization of Hydrocarbon-Degrading Bacteria from Wastewaters in Ouagadougou, Burkina Faso', Journal of Environmental Protection, 5, 1183-1196.
[32] Sumia khan, Muhammad Afzal, Samina Iqbal, Qaiser M. Khan (2012) 'Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils', Chemosphere 2013 Jan 8;90(4):1317-32. Epub 2012 Oct 8.
[33] Tyagi, M., da Fonseca, M.M.R. & de Carvalho, C.C.C.R. Biodegradation (2011) 22: 231
[34] N. Vasudevan and P. Rajaram (2001) 'Bioremediation of oil sludge-contaminated soil', Environment International, vol. 26, no. 5-6, pp. 409–411, 2001
[35] W. Ulrici (2000) 'Contaminant soil areas, different countries and contaminant monitoring of contaminants', in Environmental Process II. Soil Decontamination Biotechnology, H. J. Rehm and G. Reed, Eds., vol. 11, pp. 5–42, 2000.