Comparison of Aerobic and Anaerobic Bioremediation of Polluted Water Samples

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Abstract

The study of aerobic and anaerobic bioremediation of treated and raw crude polluted water samples each seeded with Aspergillus niger (fungi) and Pseudomonas aeruginosa (bacteria) has been investigated. It was found that the rate of aerobic bioremediation of the two polluted water samples was faster than that of anaerobic bioremediation. With bacteria, it took 35 days for aerobic bioremediation to significantly remediate the treated crude polluted water by 98.8% and 99.9% in 45 days, while with fungi the same samples biodegraded by 92.3% in 35 days and 95.8% in 45 days. In comparison, the anaerobic bioremediation of the raw crude polluted water seeded with bacteria, biodegraded by 55.32% in 35 days and by 76.3% in 45 days while with fungi, bioremediation was 39.2% in 35 days and 56% in 45 days. Anaerobic bioremediation of the two polluted water samples with either bacteria or fungi was only significant after 65 days.

Keywords: Aerobic, Anaerobic, Bioremediation, Treated crude, raw crude, Polluted water

1.0 Introduction

Bioremediation has been defined as "the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes, (Atlas, 1995; Hoff, 1993; Swannell et al., 1996). This technology is based on the premise that a large percentage of oil components are readily biodegradable in nature. The success of oil spill bioremediation depends on our ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment, Xueqing, Albert, Makram, and Kenneth (2001). There are two main approaches to oil spill bioremediation: The majority of bioremediation strategies for removal of petroleum hydrocarbon are aerobic respiration. Prior to the 1980s, it was accepted that microbial hydrocarbon degradation occurs mainly under aerobic conditions due to favorable energetic and that anaerobic hydrocarbon degradation was negligible. However, this approach has had limited success, because oxygen, an absolute requirement for aerobes is scarce in almost all contaminated environments.

The scarcity of oxygen in many contaminated sub surface environments has raised interest in the bioremediation potential of anaerobes. Anaerobic bacteria are present in soil and are a part of the normal flora of humans and other animals as well as the insects being investigated. This microbial life in the absence of oxygen is beginning to show significant potential for solving one of the important present day problems of environmental pollution and degradation. The key players in bioremediation are microorganisms that live virtually everywhere. They are ideally suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily. Bacteria in bio-remediation are prolific. Certain bacteria belonging to the Bacillus and Pseudomonas species have these desirable characteristics: they consume organic waste thousands of times faster than the types of bacteria that are naturally present in the waste, they grow and reproduce easily, are non-pathogenic, and do not produce foul odors or gas. Fungi have been used in the treatment of waste and waste waters and the role of fungi in the bioremediation of various hazardous and toxic compounds in soils and sediments have been established.

They have also shown the removal of metals and degradation and mineralization of phenols and other phenolic compound, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, poly chlorinated biphenyls, chlorinated insecticides and pesticides and other substances in various matrices. Saprophytic fungi degrade organic matter to release carbon, nitrogen, and other elements locked up in complexes, (Atlas, 1995**).** Often the normal alkanes in the range C_{10} to C_{26} are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by many marine microorganisms. More complex structures are more resistant to biodegradation, meaning that fewer microorganisms can degrade those structures. The greater the complexity of the hydrocarbon structure, the slower the rates of degradation (Atlas, 1995). There are several different bioremediation techniques. These refer to the introduction of specially selected or genetically engineered strains of microbes to a contaminated site. Indigenous populations of microbial bacteria can be stimulated through the addition of nutrients or other materials. Exogenous microbial populations can be introduced in the contaminated environment (Sugai et al., 1997, Radwan et al.). The addition of extra bacteria is known as bio augmentation. If necessary, genetically altered bacteria can be used. Once the bacteria are chosen, the engineer must carefully meet their nutritional needs by choosing the correct mix of fertilizer (Irwin, 1996). If site assessments reveal that species of indigenous microorganisms are unable to degrade target contaminants, exogenous microorganisms with the required biochemical capabilities can be introduced to successfully degrade specific waste compounds, Irwin, (1996) Generally, bioremediation of oil contaminated beaches was shown to be a safe clean-up technology (EPA, 1990). Results obtained from bioremediation study have demonstrated that the addition of nutrients and bacteria to oil has enhanced the biodegradation of the *n*-alkane fraction of the oil and microbial degradation of oil was not significant in the absence of nutrients or bacteria (Fayad et al, 1992). The complete biodegradation (mineralization) of hydrocarbons produces the non-toxic end products carbon dioxide and water, as well as cell biomass (largely protein) which can be safely assimilated into the food web (Atlas, 1995).

2.0 Materials and Methods

The anaerobic and aerobic conditions used for this study were carefully simulated under laboratory conditions to help in the investigation of the effects of bacteria and fungi on the bioremediation of the raw and treated crude oil samples. The kegs of the crude oil samples used for the aerobic study were perforated to provide enough holes for air (oxygen) supply while the kegs of the crude oil samples for anaerobic study were all air tight as there were no drilled holes or perforation in them.

The two crude samples (treated and raw) as used in this study were obtained from an oil company situated in the Niger Delta area of Delta State, Nigeria.

The fungi (*Aspergillus niger*) and bacteria (*Pseudomonas aeroginosa*) used for the study were cultured in the Microbiology Department of Covenant University using Nutrient Agar (NA) and Potato Dextrose Agar (PDA) respectively as feed.

Sample Preparation: The crude oil polluted water samples were made by adding 300ml of the two crude oil samples respectively to 3000ml of water in the ratio of 1: 10. Three different samples were prepared for each type of crude and stored in three different black plastic containers of 5 litres each until required. Before the experiment was started the crude oil polluted water samples were made to stand for 1 week to allow the indigenous microbes to grow and accustom to the medium. This action also allows toxins in the crude oil due to volatile fractions to evaporate before the microbes begin to use the oil to grow their population.

Then, 0.2M sodium nitrate was prepared by dissolving 56.1g of sodium nitrate in 3300ml (3.3L) litres of crude oil polluted water. The *Aspergillus niger* and the *Pseudomonas aeruginosa* were respectively inoculated into four containers, two each for the raw and treated crude oil polluted water and the last two kegs were left as controls.

2.1 Analyses Description

2.1.1 Biological Oxygen Demand

Reagents used: Winkler's solution A, Winkler's solution B and Starch solution

2.1.1 Procedure

Two 250 ml reagent bottles were first filled up completely with the crude polluted water samples and stoppered tightly. To one of the bottles, 1.5 ml each of Winkler's Solution A and B were added, and precipitant was formed. The precipitant was dissolved with 2 ml of concentrated sulphuric acid to form a golden brown solution.

50 ml of the resulting solution was poured into 250 ml conical flask and 3 drops of starch indicator were added and titrated against 0.2 M Sodium thiosulphate ($Na_2S_2O_3$) with initial blue black coloration and the volume of 0.2 M ($Na_2S_2O_3$) solution used was recorded and after titrating, it turned colourless detecting the endpoint. The second bottle was covered with black cellophane bag to prevent the penetration of light for 5 days. At the end of 5 days, the above procedure was repeated for the contents of the second bottle and the volume of 0.1N ($Na_2S_2O_3$) used for titration was recorded. This was done for the two raw crude polluted water samples, the two treated crude polluted water samples containing the two microbes respectively and the two control samples.

The BOD of the sample was calculated as follows:

$BOD_5 = DO_0 - DO_5$

Where;

DO_0 = Dissolved oxygen concentration at zero time
DO_5 = Dissolved oxygen concentration after 5 days incubation period
The above procedures were repeated for other samples

2.2 Total Hydrocarbon Content

The oil content of the polluted water was determined by shaking 5 g of a crude oil-water sample with 10 ml of toluene. The two phases formed (water phase and crude oil-toluene phase) were separated by using a 250ml separating funnel. A sample of the crude oil-toluene phase is poured in a glass cuvette and the absorbance was checked using a spectrophotometer at wavelength 420 nm. A standard curve of the absorbance of different known concentrations of crude oil in the toluene phase was first drawn. After taking readings from the spectrophotometer, oil concentrations in the polluted water sample were then calculated after reading the concentration of the oil in the extract from the standard curve. With reference to the standard curve, the hydrocarbon content of the oil was calculated by interpolating values on the concentration-absorbance curve.

3.0 Discussion of Results

3.1 The Effect of Bioremediation Method

The level of bioremediation obtained in the crude oil polluted water samples was observed to be a function of the type of bio-remediation used. As seen in figs 1-4, higher levels of bioremediation occurred with the aerobic than with the anaerobic bioremediation for both the raw and treated crude polluted water samples. From tables 1 and 2, the Biological Oxygen Demand (BOD) values which is a measure of the rate of bioremediation, reduced much faster for the aerobic than with the anaerobic bioremediation. These values tended to zero before 40 days for aerobic and more than sixty days for anaerobic. This is well reflected in figs 1 and 2, where the lower three curves in both figs 1 and 2 represent the aerobic bioremediation and the upper three curves in the two figures represent the anaerobic bioremediation. This means that, at any given time on the graph, BOD values are lower, that is, rate of bioremediation is fastest with aerobic than anaerobic bioremediation. From tables 3 and 4, the Total Hydrocarbon Content (THC) values obtained followed the same pattern of bioremediation as with the BOD values. The THC values were reduced much faster with aerobic than with anaerobic bioremediation, as seen also in figs 3 and 4 where it took about 40 days and more than 60 days for significant THC reduction for aerobic and anaerobic bioremediation respectively.

3.2 The Effect of Crude Oil Polluted Water Sample Type

The type of crude used in this study, affected the rate of bioremediation It was observed that bioremediation of the treated crude polluted water samples was much faster than the raw crude polluted water samples inoculated with both the *Aspergillus niger* (fungi) and *Pseudomonas aeroginosa* (bacteria) for both the aerobic and anaerobic conditions (see figs 1 – 4) and tables 1 -4. The raw crude taken directly from the well head had not undergone any form of treatment such as de-emulsification and removal of heavy metals such as lead, arsenic etc. These, in the raw crude affect microbial growth and activity which ultimately slows down the bioremediation process observed in the raw crude polluted water samples. Values of both the BOD and THC were relatively higher compared with values from the treated crude polluted water samples at any given time. The three upper curves in each of figs 1 – 4 depict slower rates of bioremediation for the raw crude polluted water samples, while the three lower curves in the same figs 1 – 4 depict faster rates of bioremediation for the treated crude polluted water samples.

3.3 The Effect of Microbes

The activities of the two microbes (*Pseudomonas aeroginosa* and *Aspergillus niger*) were observed to be different producing different levels of bioremediation in the two polluted water sample types. From figs 1- 4 *Pseudomonas aeroginosa* (bacteria) had the highest rate of bioremediation as measured by the BOD and THC values under the aerobic and anaerobic conditions imposed, The bacteria performed relatively better than the fungi (*Aspergillus niger*) in the two polluted water samples used. The first and fourth curves from the bottom in all the figures depict the performance of the bacteria while the second and fifth curves in the same figures show the performance of the *Aspergillus niger* (or fungi). This means that at any given time, the BOD and THC values of the bacteria are relatively lower (higher bioremediation) than the corresponding BOD and THC values of the fungi. However, samples of the polluted water were the two microbes (bacteria and fungi) were used recorded higher levels of bioremediation compared with controlled samples. Curves 3 and 6 from the bottom in figs 1 – 4, depict the lowest rate of bioremediation for the controlled samples. This means that at any given time, highest BOD and THC values (or lowest bioremediation) are obtained from the controlled samples than with bacteria and fungi seeded polluted water samples.

4.0 Conclusion

The bioremediation under aerobic and anaerobic conditions of two crude oil polluted water samples showed that

- Rate of aerobic bioremediation of the two crude oil polluted water samples was higher than anaerobic bioremediation
- Rate of bioremediation of two crude oil water polluted samples with *pseudomonas aeroginosa* (bacteria) was higher than that obtained with *Aspergillus niger* (fungi).
- The rate of bioremediation of the controlled samples (absence of microbes) were all generally lower than the bioremediation obtained when the bacteria and fungi were respectively used.
- The Treated polluted water sample was much easier to bioremediate than the raw crude oil polluted water.
- The presence of heavy metals in crude oil polluted water slows down the activities of microbes in the bioremediation process of such polluted water samples.

The seeding of crude oil polluted water samples with microbes generally enhances bioremediation of such samples. This practice should be encouraged to help abate crude oil spillages from the environment.

Table 1: BOD Values for Aerobic Bioremediation

	RAW CRUDE			**TREATED CRUDE**		
Time (days)	fungi	Bacteria	control	fungi	bacteria	control
0	1839.2	1839.2	1839.2	1746.6	1746.6	1746.6
5	1774.8	1683.3	1793	1606.3	1003.5	1643.2
10	1562.4	1451.5	1591.8	1163.6	615.7	1574.9
15	1153.4	1067.3	1382.4	894.3	374.1	1362.1
20	1072.2	883.7	1157.6	537.6	119.7	1015.4
25	803.6	564.4	963.5	378.2	84.4	892.6
30	690.4	378.2	714.4	220	55.9	738.4
35	531.68	134.2	567.1	126.1	26.7	587.1
40	341.3	76.3	408.2	93.2	13.1	351.4
45	196.1	38.5	289.5	79.8	2.4	116.5

Table 2: BOD Values for Anaerobic Bioremediation

	TREATED CRUDE			RAW CRUDE		
Time (days)	**fungi**	**bacteria**	**control**	**fungi**	**bacteria**	**control**
0	1638.7	1638.7	1638.7	1882.7	1882.7	1882.7
5	1589.3	1501.6	1611.3	1809.3	1762.7	1817.6
10	1237.7	1077.6	1553.2	1743.3	1699.4	1787.6
15	1052.1	848.7	1409.3	1684.7	1512.3	1725.8
20	928.3	719.5	1007.3	1609.1	1311.7	1652.2
25	811.7	497.3	933.8	1469.8	1101	1593.8
30	732.6	266.1	816.9	1300.7	986.2	1413.9
35	518.2	107.9	748.7	1117.6	821.7	1292.8
40	382.6	83.9	653	973.8	687.3	1017.6
45	278.1	67.7	570	809.3	435.9	923.7
50	166.5	39.3	421.6	692.6	299.7	793.1
55	105.3	25.8	389.1	413.9	173.9	614.4
60	88.6	17.2	215.8	315.8	92.5	501.7
65	67.1	8.9	136.5	243.6	64.7	470.2

Table 3: THC Values for Aerobic Bioremediation

	TREATED CRUDE			RAW CRUDE		
Time days	fungi	bacteria	control	fungi	Bacteria	Control
0	453.1	453.1	453.1	462.2	462.2	462.2
5	334.3	288.3	401.1	414.3	285.5	430.1
10	319.1	260.5	340.4	341.6	264.1	356.1
15	295.21	211.9	335.1	330.9	258.4	329.3
20	250.5	197	291.1	300.5	254.3	311.3
25	199.7	177.6	261.9	288.5	201.6	286.7
30	189.5	161.7	225	260	186.2	262.2
35	173.6	98.2	191.7	250.5	170.1	252.9
40	158	57.5	177.4	198.9	152.3	200.5
45	92.9	43.9	165.9	183.1	86.1	194.4

Table 4: THC Values for Anaerobic Bioremediation

Time	**RAW CRUDE**			**TREATED CRUDE**		
(Days)	**Fungi**	**bacteria**	**control**	**fungi**	**bacteria**	**Control**
0	9218.3	9218.3	9218.3	8634.8	8634.8	8634.8
5	8959.4	8322.4	8796.8	8558.8	8034.8	8453.4
10	8504.1	8004.5	8711.4	8318.6	6111.1	8387.3
15	7326	6995.3	8516.3	8223	3814.1	8169.2
20	6837.9	6092.4	8138.2	6833.3	3666.6	8088.2
25	5517.3	5148	7343.5	3809.6	3474.4	8002.5
30	5048.8	3836.2	7295.4	3610.7	3145.9	7187.5
35	3642.6	3022.7	7146.6	3264.4	2311.3	5339.1
40	3249.8	2673.8	6862.7	2695.1	1634.9	3655.4
45	2843.7	1706.2	4424.1	1333.8	988.8	3420.8
50	2560.3	1549.1	3894.1	963.2	625.2	2883.9
55	2259.5	1277.3	2982.7	887.1	416.4	1413
60	1973.2	895.43	2565.7	732.2	243.6	819.7
65	1248.6	372.19	1836.2	394.8	93	706.4

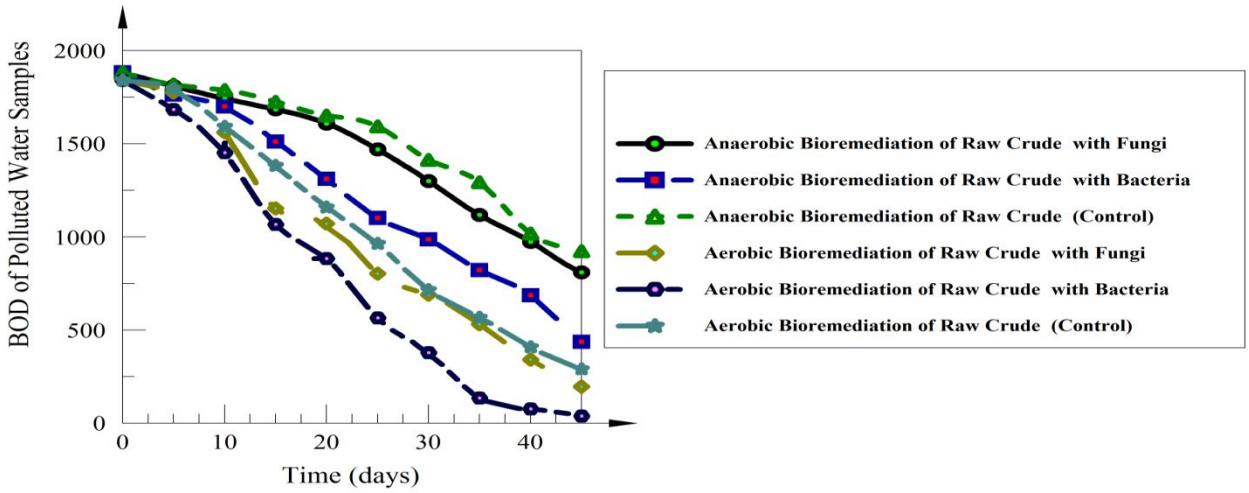

Fig 1: BOD from Aerobic and Anaerobic Bioremediation of Raw Crude Polluted Water Samples

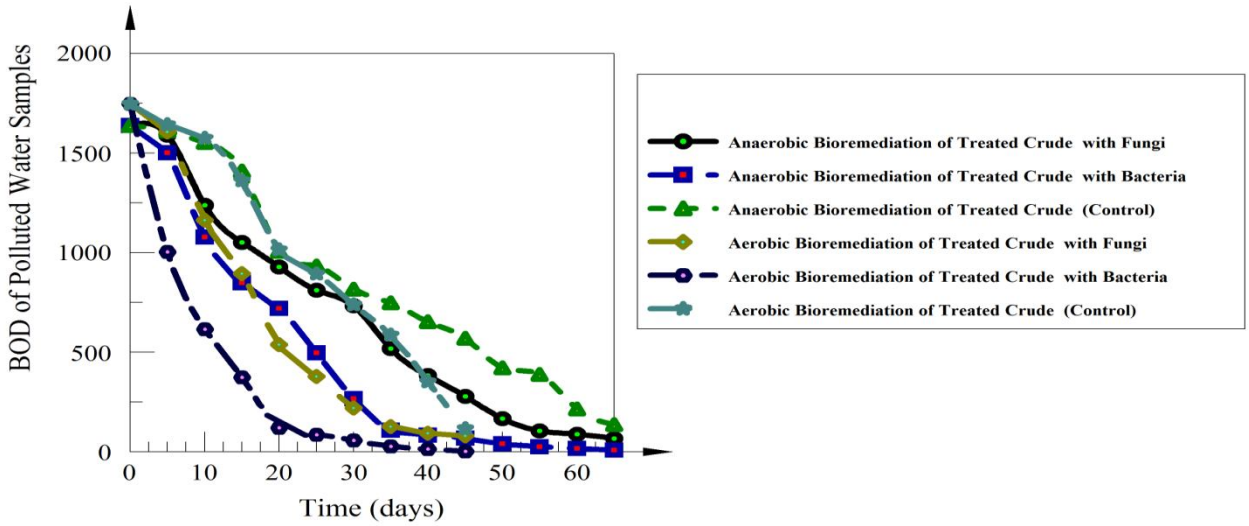

Fig 2: BOD from Aerobic and Anaerobic Bioremediation of Treated Crude Polluted Water Samples

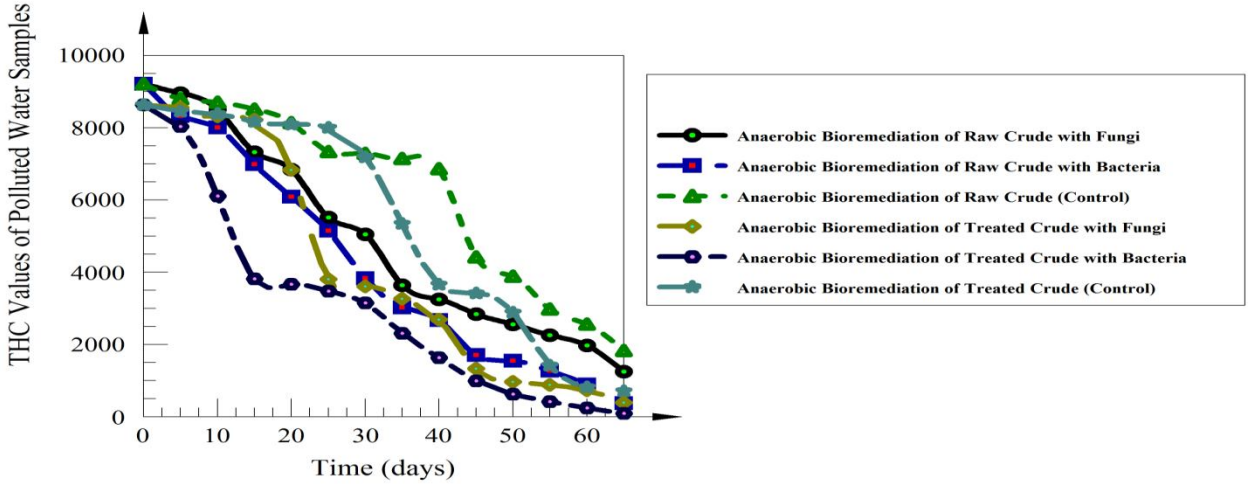

Fig.3: THC Values from Anaerobic Bioremediation of Raw and Treated Crude Polluted Water Samples

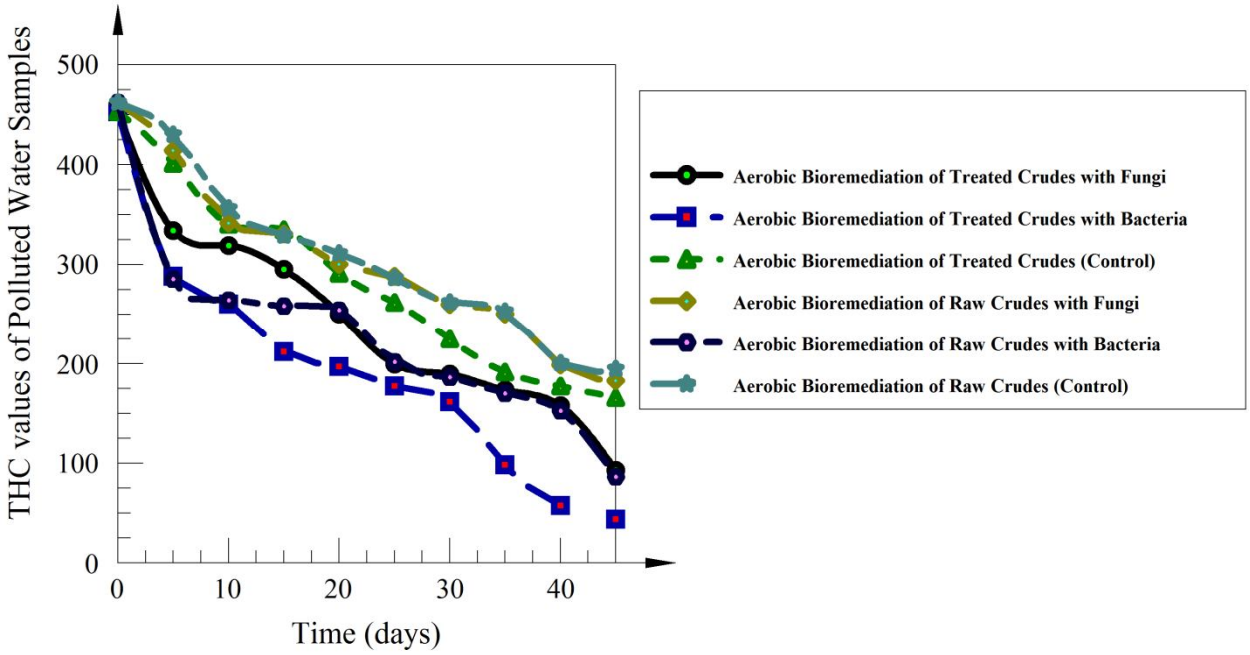

Fig.4: THC Values from Aerobic Bioremediation of Raw and Treated Crude Polluted Water Samples

References

Atlas, Ronald M. (1995). Petroleum Biodegradation and Oil Spill Bioremediation. *Marine Pollution Bulletin* **31**, 178-182.

Bioremediation for marine oil spills. (Viewed October 4, 2010). Available from: URL: http://www.fas.org/ota/reports/9109.pdf

Bioremediation methods for oil spills. (Viewed October 4, 2010). Available from: URL: http://www.pace.edu/.../bioremediation%20methods%20for%20oil%20spills.doc

Fayad, Nabil M.; Edora, Ruben L.; El-Mubarak, Aarif H.; Polancos Jr., Anastacio B. (1992). Effectiveness of a Bioremediation Product in Degrading the Oil Spilled in the 1991 Arabian Gulf War. *Bull. Environ. Contam. Toxicol.* **49**, 787-796

.Hoff, Rebecca Z. (1993). Bioremediation: an overview of its development and use for oil spill cleanup. *Marine Pollution Bulletin* **29**, 476-481

Irwin, Patricia (1996). To clean up environmental spill, know your medium. *Electrical World* 37-40.

Radwan, S.S.; Sorkhoh, N.A.; El-Nemr, I.M.; El-Desouky, A.F. (1997). A feasibility study on seeding as a bioremediation practice for the oily Kuwaiti desert. *Journal of Applied Microbiology* **83,** 353-358

Sugai, Susan F.; Lindstrom, Jon E.; Braddock, Joan F. (1997). Environmental Influences on the Microbial Degradation of *Exxon Valdez* Oil on the Shorelines of Prince William Sound, Alaska. *Environ. Sci. Technol.* **31,** 1564-1572.

Swannell, Richard P.J.; Lee, Kenneth; McDonagh, Madeleine (1996). Field Evaluations of Marine Oil Spill Bioremediation. *Microbiological Reviews* **60,** 342-365.

U.S. Enviromental Protection Agency (1990). Interim Report, Oil Spill Bioremediation Project. U.S. Environmental Protection Agency, Office of Research and Development, Washington