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The Effects of Pseudomonas Aeroginosa and Aspergillus Niger on the Bioremediation of Raw and Treated Crude Oil Polluted Water

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ABSTRACT

The biodegradable activities of Aspergillus niger (fungi), and Pseudomonas aeruginosa (bacteria) on two crudes were investigated. Treated crude oil polluted water (TCOW) and the raw crude oil polluted water (RUCOW) containing the microbes and the control were monitored for Biological Oxygen Demand (BOD) and Total Hydrocarbon Content (THC). At the end of the bioremediation period, the results obtained showed that TCOW were more easily bio-remediated than RUCOW. Also the highest bio-remediation for both TCOW and RUCOW occurred using bacteria compared with the other microbes. For bacteria BOD values were 99.8% in the TCOW and 97.9% in the RUCOW. For fungi, BOD values were 95.4% and 89.3% whiles for the combination of bacteria and fungi the values were 97.9% and 93.8%. The control samples were 93.3% and 84.5% bio-remediated for the TCOW and RUCOW samples respectively. Similar variation of THC values were observed for the three microbes.. The TCOW remediated with bacteria showed the highest bioremediation of 95.3% followed by the combined fungi - bacteria sample 90%, then the fungi sample 86.5% and the control sample 63.4%, For RUCOW THC values were 91.3% for bacteria, 84.5% for the combined fungi - bacteria, 76.4% for fungi and 70.7% for the control samples.

Keywords: *Bioremediation, Polluted water, Microbes, Treated crude, Raw crude.*

1. INTRODUCTION

Oil spillages have been the negative fallout of oil exploration. Since the discovery of oil in Oloibiri, Bayelsa State Nigeria in 1956, there has been increase public concern on the adverse effects of oil exploration on the environment. [1] The magnitude of crude oil pollution and damage occasioned by Multinational Oil Companies operation in the Niger Delta region of Nigeria is incredible. It is noteworthy that, the devastating consequences of oil spills in the Niger Delta region with its eventual hazards on both aerial and terrestrial environs are tantamount to an irreversible chain effect on both the bio-diversity and human safety.[2] In Nigeria, there have been a number of oil spillages. Examples are Shell oil spill in Ogbudu (2001), Bille (1999) and Chevron oil spill in Ilaje (1998) [1] to state just a few. A top 10 oil exporter with proven reserves of 36 billion barrels, Nigeria today also ranks among the world's worst in petroleum safety. According to reports, Nigeria recorded 2,097 oil spill incidents between 1997 to 2001.[3] Oil spillage is also a major problem affecting a large part of the world. A blow out from a well in Gulf of Mexico gushed millions of barrels of crude oil into the sea.[4] It was the largest accidental marine oil spill in the history of the petroleum industry.[4] Meanwhile, Nigeria reportedly leaks as much oil as the Valdez - which spewed nearly 11 million gallons of crude into Alaskan waters in 1989 - *every year*, with little attention paid.[5]

These spillages contaminate the soil, water, air and in turn affect human, animal and plant life. When there is an oil spill on water, spreading immediately takes place. The gaseous and liquid components evaporate. Some get dissolved in water and even oxidize, and yet some undergo bacterial changes and eventually sink to the bottom by gravitational action. The soil is then contaminated with a gross effect upon the terrestrial life. As the evaporation of the volatile lower molecular weight components affect aerial life, so the dissolution of the less volatile components with the resulting emulsified water, affects aquatic life [2].

The impact of oil spillage is phenomenal- affecting human, biodiversity and the environment. The marine environment is subject to contamination by organic pollutants from a variety of sources. Organic contamination results from uncontrolled releases from manufacturing and refining installations, spillages during transportation, direct discharge from effluent treatment plants and run-off from terrestrial sources. In quantitative terms, crude oil is one of the most important organic pollutants in marine environments with current global rate of natural seepage of crude oil at 600,000 tonnes per year, with a range of 200,000 to 2,000,000 tons per year. [6] 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. 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. There are several different bioremediation techniques. These refer to the introduction of

specially selected or genetically engineered strains of microbes to a contaminated site. 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.[7-8] 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. [9-10] In this research study, Aspergillus Niger (a fungus), Pseudomonas Aeroginosa (a bacterium) and a combination of both were used separately to inoculate the raw and treated crude oils polluted water samples. The performances of these microbes in the degradation of the hydrocarbon were then monitored.

2. MATERIALS AND METHODS

The crude oil (Escravos light) used in this study was sourced from Shell Petroleum Development Company (SPDC) in Warri, Delta State Nigeria, October 2010. The fungi (Aspergillus niger) and bacteria (Pseudomonas aeroginosa) used for this study were cultured in the Microbiology Department of Covenant University using Nutrient Agar (NA) and Potato Dextrose Agar (PDA) respectively as feed.

3. SAMPLE PREPARATION

The raw (untreated) crude oil polluted water (RUCOW) was made by mixing volumes of Escravoes light (crude oil) and water. This RUCOW in the ratio 1: 10 was then stored in three different black plastic containers with carefully drilled holes to provide the required air for aerobic activity, until required. Before the experiment was started the RUCOW was allowed to stand for 1 week allowing the indigenous microbes to grow and accustom to the medium. 0.2M sodium nitrate was prepared by dissolving 56.1g of sodium nitrate in 3300ml (3.3litres) of crude oil polluted water. Then *Aspergillus niger*, *Pseudomonas aeruginosa* and combination of the two microbes were inoculated into three of the containers of crude oil polluted water and the fourth was left as control. The above procedure was repeated but this time Treated crude oil polluted water (TCOW) sample was used in place of the RUCOW sample.

4. ANALYSES DESCRIPTION

4.1 Biological Oxygen Demand

Reagents and Apparatus used: Winkler's solution A, Winkler's solution B and Starch solution, Sodium thiosulphate, Conc. Sulphuric acid, black cellophane bags, Burette, pipettes and measuring cylinder.

4.2 Procedure

Four 250 ml reagent bottles were filled up with the four polluted water samples (RUCOW) and stoppered tightly. To each 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 18M 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 with 0.2M Sodium thiosulphate ($Na_2S_2O_3$) solution. The initial blue black coloration turned colorless at the endpoint, as the volume of 0.2 M ($Na_2S_2O_3$) solution used was recorded. The remaining samples were covered with black cellophane bags to prevent the penetration of light and then left at room temperature (29^0C – 30^0C) for days. At intervals of 5 days, the above procedure was repeated for the contents of these four samples and for each, the volume of 0.2M $Na_2S_2O_3$ used was recorded. The entire procedures were similarly repeated for the other sample (TCOW).

The BODs of the samples were calculated as follows:

$$C_A = \left[\frac{C_B V_B}{V_A}\right] X \frac{n_A}{n_B} X \frac{32gO_2}{mol}\ X\ 1000mg/g$$

Where C_A = concentration of dissolved oxygen (DO) in the polluted sample, (mg/l)

V_A = volume of polluted oil sample for titration (50ml)

C_B = concentration of sodium thiosulphate ($Na_2S_2O_3$) solution. (0.2M)

V_B = Titre value or volume of $Na_2S_2O_3$ used for titration.

From the stoichiometric equations of the Winkler's test for dissolved oxygen

n_A= number of moles of O_2 = 1
n_B= number of moles of $Na_2S_2O_3$ = 4

$$BOD_5 = DO_0 - DO_5$$

Where DO_0 = Dissolved oxygen concentration at the beginning (zero time)
DO_5 = Dissolved oxygen concentration after 5 days incubation period

The above procedures were repeated for other samples.

4.3 Total Hydrocarbon Content

Reagents and Apparatus used: Toluene**,** Jenway 6405 UV/VIS Spectrophotometer and Separating funnel.
The oil content of the polluted water samples were determined by shaking 5 g each of RUCOW or TCOW with 10 ml of toluene. The two phases formed (water phase and crude oil-toluene phase) were separated by using a 250ml separating funnel. The hydrocarbon content of the toluene phase was calculated by measuring the absorbance of the sample at 420nm using a spectrophotometer. The hydrocarbon content was read off a calibrated curve of hydrocarbon in toluene chart.

5. RESULTS AND DISCUSSION

From both Figs.1 and 2 The Pseudomonas aeruginosa (bacteria sample) was seen to have undergone the highest bio-remediation of the hydrocarbon in both the raw and treated crudes than the rest microbes (Pseudomonas aeruginosa combined with Aspergillus niger, Aspergillus niger (fungi sample) alone and the control sample) used. Aerobic bioremediation provides the oxygen which is a major requirement for effective biodegradation of crudes. The results show that a minimum of 45 days was required for the microorganisms to biodegrade the hydrocarbons in the water to an acceptable level and turn it to less toxic substances (CO_2 and H_2O). The BOD of the bacteria sample fell from 1839.2mg/l to 38.3mg/l (97.9%) in the raw crude and 1746.6mg/l to 2.4mg/l (99.8%) in the treated crude. The sample inoculated with combined bacteria and fungi fell from 1839.2mg/l to 113.8mg/l or 93.8% and 1746.6mg/l to 36.3mg/l or 97.9%, then the sample inoculated with fungi from 1839.2mg/l to 196.1mg/l or 89.3% and 1746.6mg/l to 79.8mg/l or 95.4%, and the control sample which fell from 1839.2mg/l to 289.5mg/l (or 84.5%) and 1746.6mg/l to 116.5mg/l (or 93.3%) in the raw and treated crude oils polluted water samples respectively.

From figs. 1 to 4 both the BOD and THC values reveal that all the microbes generally find it easier to biodegrade the treated crude oil polluted water than the raw crude oil polluted water. This is because the raw crude oil sample contains not only hydrocarbons which serve as energy and food for the microorganisms to thrive on but also other elements like nickel, copper, vanadium and iron which are not favorable for the growth of the microorganism. The presence of these other elements slow down the activity of the microorganism hence the growth rate is reduced and their demand for oxygen is reduced. This leads to the slow fall in the BOD and THC values of the raw crude oil polluted water. The treated crude oil polluted water which contains basically hydrogen and carbon has a higher demand for oxygen and thus their BODs fall faster. Figs. 5 to 7 illustrate the plot of the rate of BOD depletion against time for TCOW sample. They fitted to a varying degree of accuracy with exponential decay curves. This is shown by the high coefficients of correlation (R^2 = 0.9808, R^2 = 0.9744 & R^2 = 0.9662) obtained from the rate plots for the three microbes - Pseudomonas aeruginosa (bacteria), Pseudomonas aeruginosa combined with Aspergillus niger (bacteria-fungi) and Aspergillus niger (fungi) respectively. It is suspected that R^2 gives an indication of the degree of bio-remediation of treated crude oil polluted water sample using microbes. The higher the R^2 value, the higher the bio-remediation, as evident with the three microbes used in this work. The exponential factor could also be used to predict or know the degree of bioremediation. The more negative the exponents the higher and faster the rate of bioremediation. For the three microbes, their exponents are given as e^{-108t} (bacteria), $e^{-0.085t}$ (bacteria-fungi) and $e^{-0.0071t}$ (fungi). The TCOW sample bio-remediated, went through the highest bio-remediation with bacteria, followed by the combined bacteria - fungi and the fungi alone.

6. CONCLUSION

The study of the effect of three microbes on aerobic bioremediation of both raw and treated crude oil polluted water samples indicate that:

Pseudomonas aeruginosa is a better organism for bioremediation than Aspergillus nigger.

It is easier to biodegrade treated crude oil polluted water sample than the raw crude oil polluted water sample.

It is better to use Pseudomonas aeruginosa alone than its combination with Aspergillus nigger. Their combination does not biodegrade crude oil polluted water as fast as Pseudomonas aeruginosa alone. This is because they are both of different strains and either may hinder the growth of the other thus slowing down the biodegradable activity and increasing bioremediation time.

The bio-remediation activity of fungi-bacteria combination (Aspergillus nigger - Pseudomonas aeruginosa) is more effective than that of fungi (Aspergillus nigger) alone.

The exponents of the decay curves give an indication of the rate and extent of bio-remediation.

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Table 1a: Effect of Bacteria (Pseudomonas aeruginosa) on the Rate of Depletion of Biological Oxygen Demand of Treated Crude Oil Polluted Water with Time.

Time (days)	2	5	10	20	35
Rate (mg/l-day)	225	104	57.8	26.7	5

Table 1b: Effect of Combined Bacteria and Fungi (Pseudomonas aeruginosa & Aspergillus Niger) on the Rate of Depletion of Biological Oxygen Demand of Treated Crude Oil Polluted Water with Time.

Time (days)	7	10	17	25	35
Rate (mg/l-day)	93.33	70	46.7	28.9	5

Table 1c: Effect of Fungi (Aspergillus Niger) on the Rate of Depletion of BOD of Treated Crude Oil Polluted Water with Time

Time (days)	9	16	20	30	35
Rate (mg/l.day)	120	62.5	45	26.7	9.23

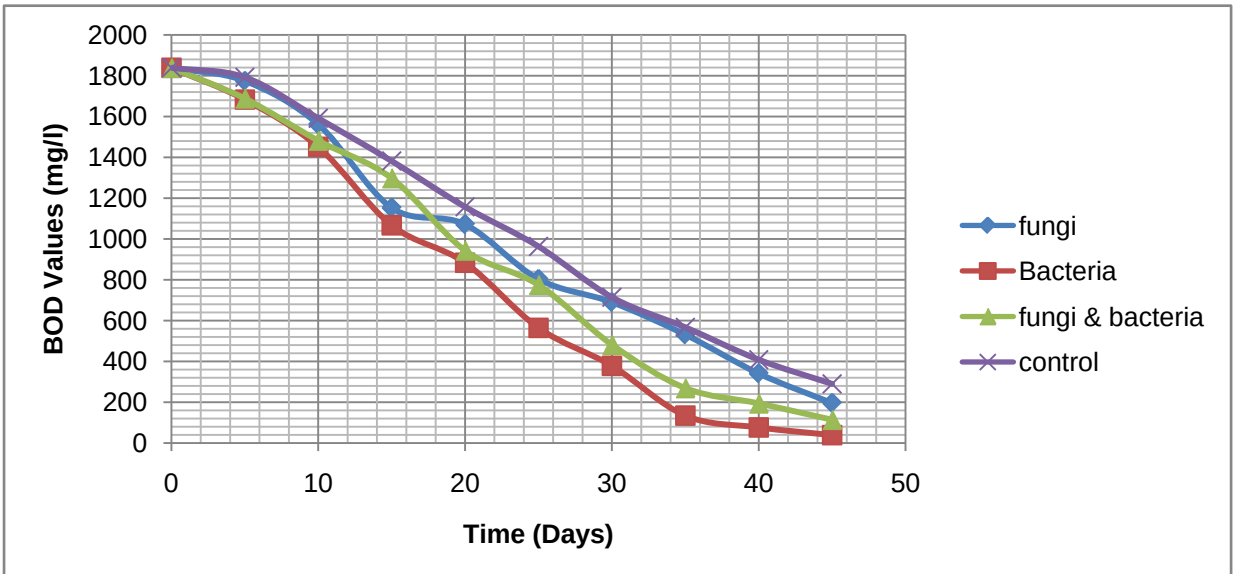

Fig.1: BOD Values for Aerobic Bioremediation of Raw Crude Oil Polluted Water

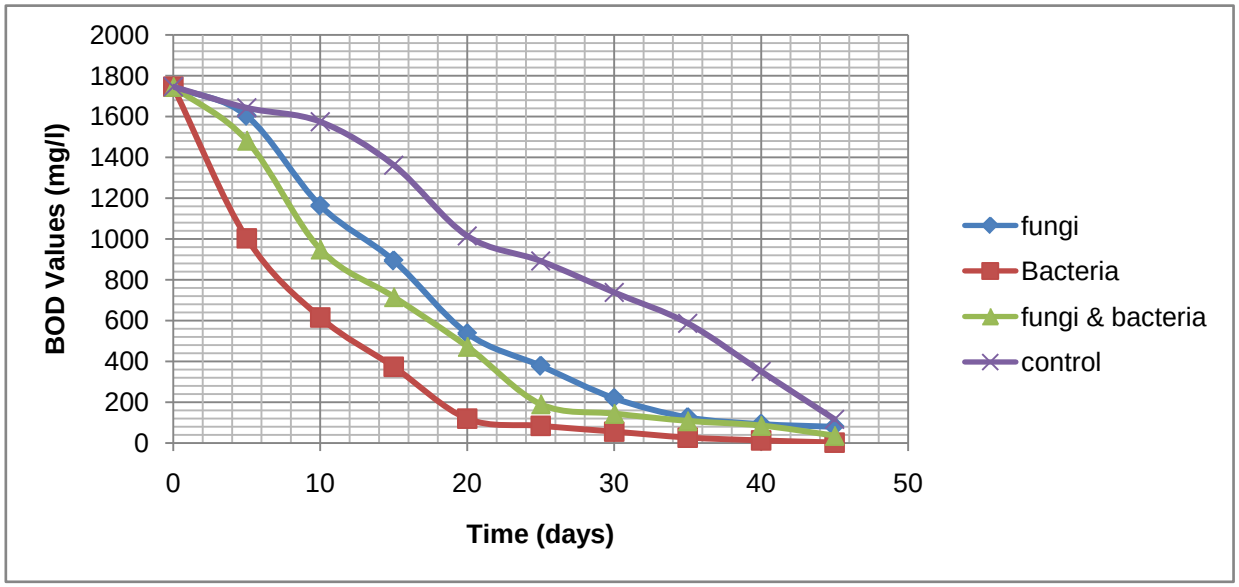

Fig.2: BOD Values for Aerobic Bioremediation of Treated Crude Oil Polluted Water

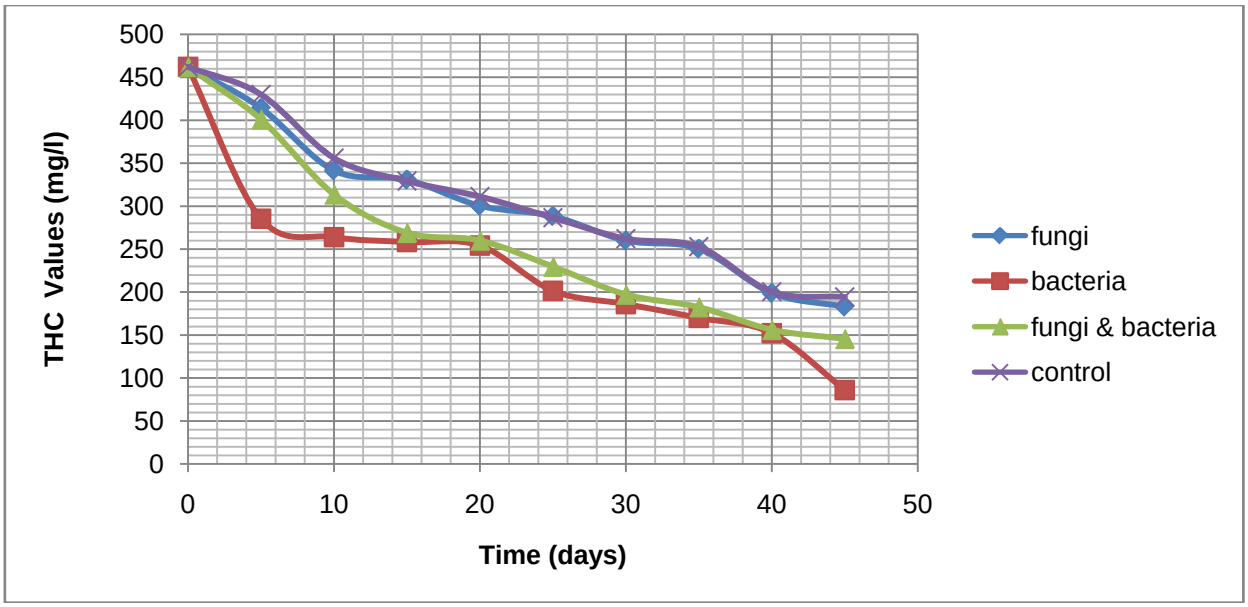

Fig.3: THC Values for Aerobic Bioremediation of Raw Crude Oil Polluted Water

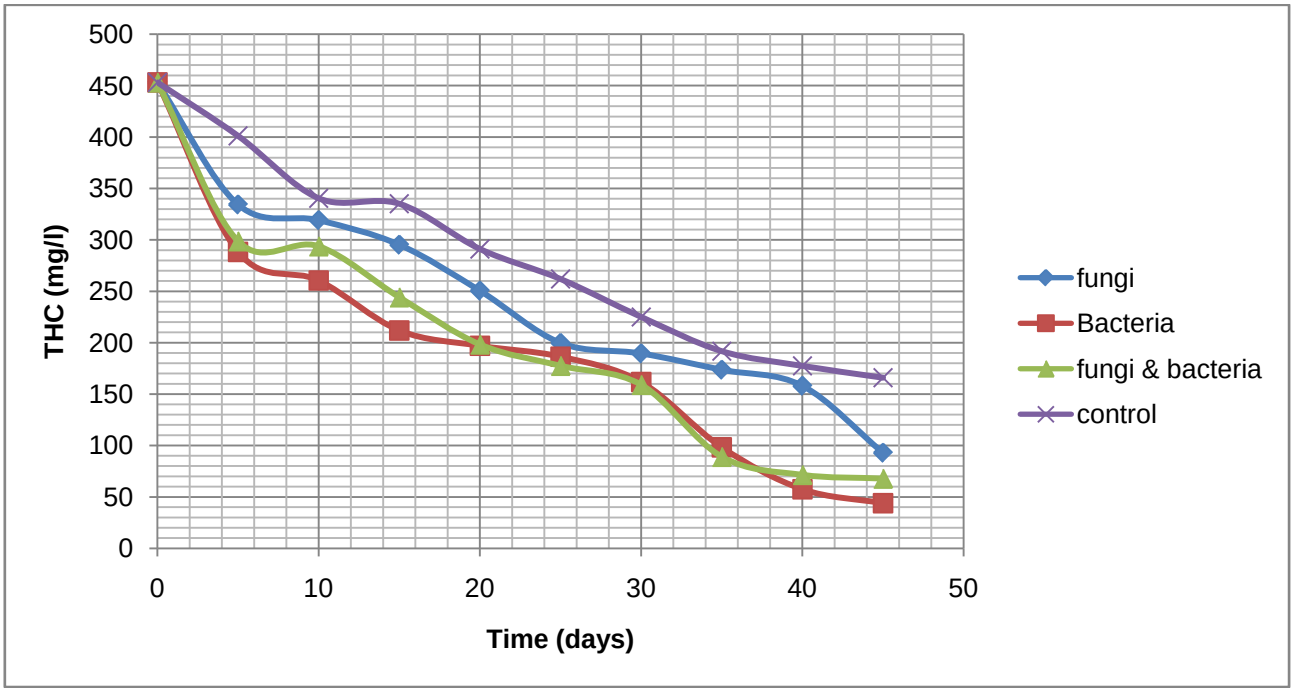

Fig.4: THC Values for Aerobic Bioremediation of Treated Crude Oil Polluted Water

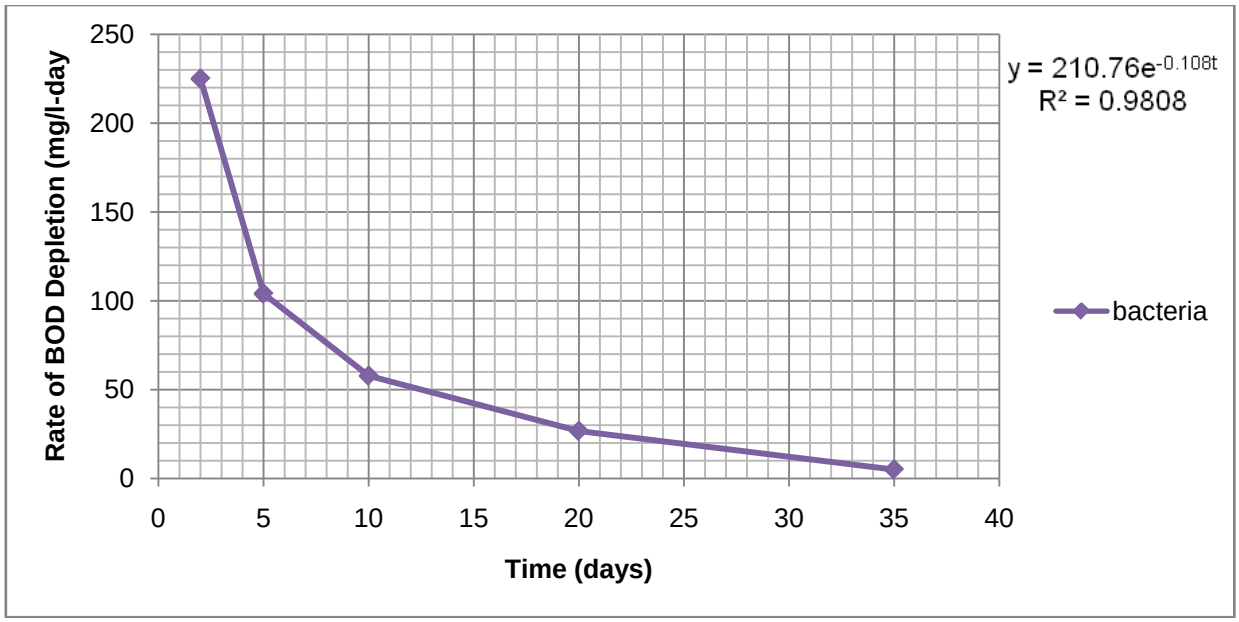

Fig.5 Effect of Bacteria on the Rate of Depletion of BOD of Treated Crude Oil Polluted Water with Time

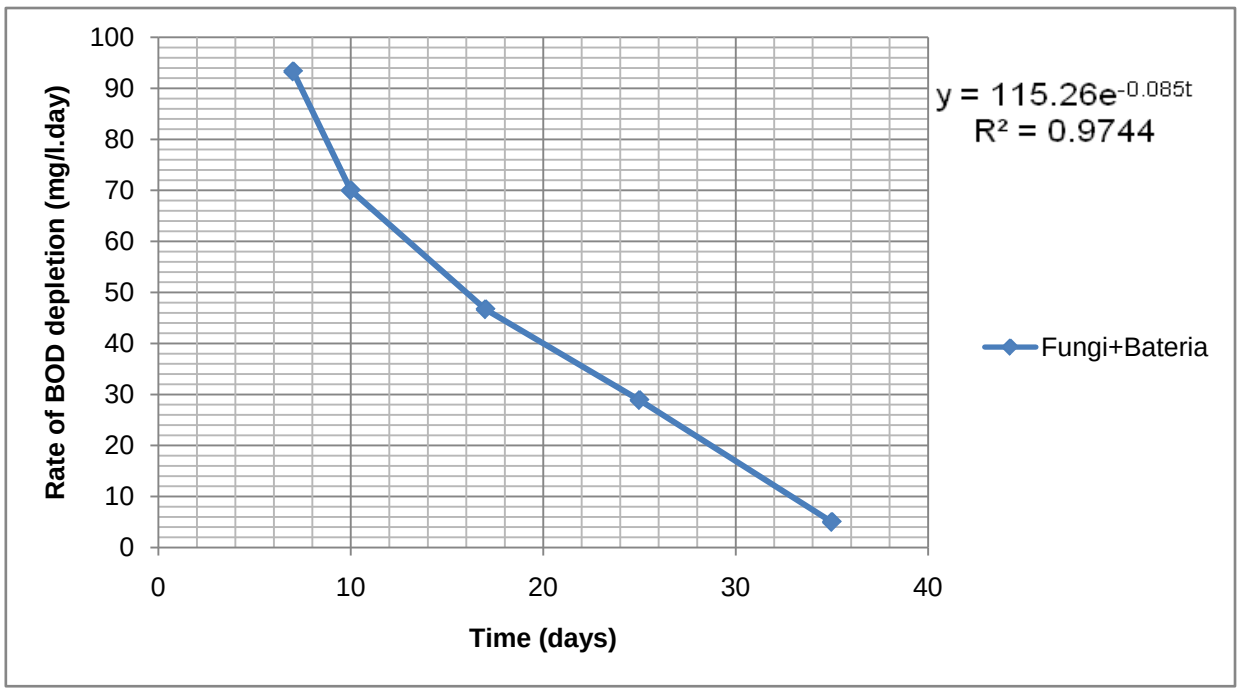

Fig.6 Effect of Combined Bacteria and Fungi on the Rate of Depletion of BOD of Treated Crude Oil Polluted Water with Time

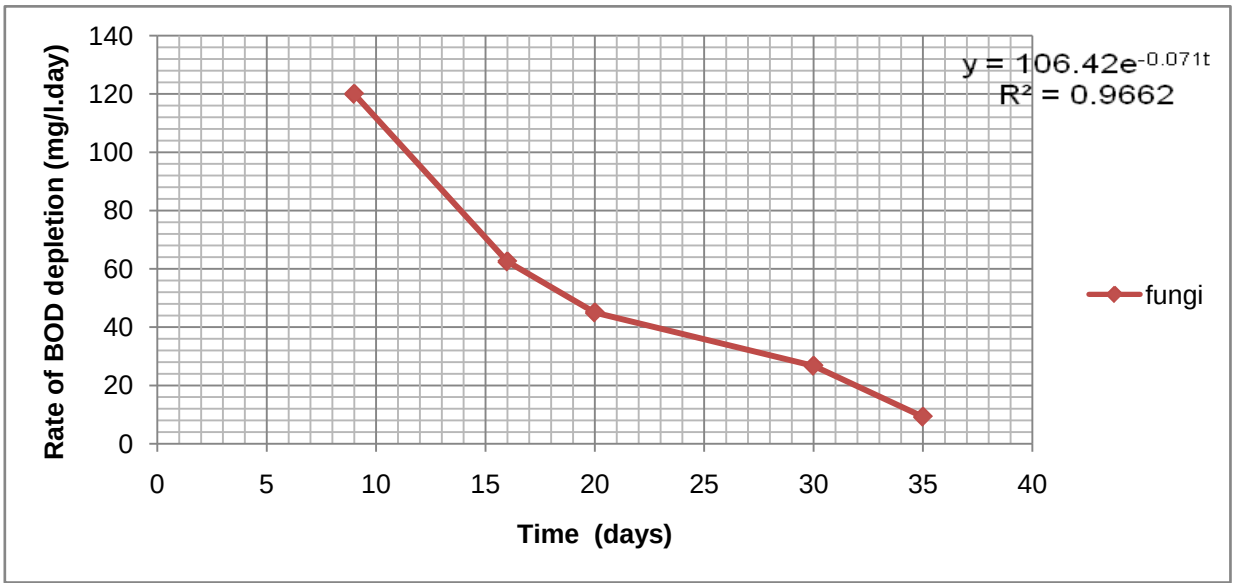

Fig 7: Effect of Fungi (Aspergillus Niger) on the Rate of Depletion of BOD of Treated Crude Oil Polluted Water with Time