International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 5 Issue 5, May 2018

ISSN: 2394-4404

The Effect Of Microbes On Contaminated Soil, A Basis For Environmental Waste Control

Ugah Chukwuemeka Stanley

Abstract: ***The effect of time on biodegradation of petrol-contaminated soil was investigated after intervals of 14 days. The investigation was carried at the back of faculty of Applied and Natural Science new Laboratory complex of Ebonyi State University. Thereafter, the isolated microbes were added to the polluted soil sample for microbial reaction after which the mixture was heated for 15mins to stop further degradation. n-hexane was used to extract the remaining petrol in the reacting mixture. Xylene, was used to determine the absorbance of the recovered petrol spectrophotometrically. Result show that biodegradation reduced the level of petrol in the soil after 2, 4, 6, 7, 8, 10, 12, and 14 days in the order of 75%, 71%, 52%, 36%, 26%, 11%, and 0.01% respectively . The petrol level in the soil sample dropped from 0.956 to 0.013 after fourteen days of microbial action, achieving a maximum degradation. The order of reaction is rate order with constant of 0.1335 per day. The optimum percentage biodegradation of petrol occurred on the sixth day of reaction. The recovered microbes showed excellent ability of petrol (99.99%) degradation of soil contaminated.***

Keywords: Microbes, Petrol (PMS), Soil, Xylene, N-hexane, U.V spectrophotometer

I. INTRODUCTION

Pollution of soil is a worldwide problem that can result in uptake and accumulation of toxic chemicals in food chains and harms the flora and fauna of affected habitats. The contamination of resources by organic chemical is a significant environmental problem with an estimate of 300,000 to 400,000 contaminated sites in the USA alone (Doust, H.G et al. 1992; and USEPA 2000). Contaminated site often contain a numerous pollutants, which can constitute a risk to a human health, animals and or the environment. Although substantial progress has been made in reducing industrial releases over recent years, a considerable number of known polluted sites are existing and new ones are continually being discovered. Many of these sites threaten to become sources of contamination of drinking water supplies and thereby constitute a substantial health hazard for current and future generation. To remedy this situation, numerous remediation techniques have been developed (USEPA 1992).

Primarily, due to cost and time consideration physical and chemical treatment processes are currently the most widely used remediation methods. Nevertheless, biological remediation alone or in combination with other methods has gain an established place as a soil restoration technology. In these bioremediation the microorganism such as (enzymes, bacteria or fungi) metabolize contaminants either through oxidative or reductive processes under favorable conditions. The microorganism can oxidatively degrade the organic contaminants completely into non-toxic by-product such as carbon dioxide and water or organic acids and methane (USEPA 1991).

II. BIOREMEDIATION PROCESSES

Bioremediation is a common technology for the treatment of organic compound; the use of this technology for the treatment of heavy metals is still new (USEPA 1985). Therefore, the fundamental process involved in biodegradation of organic contaminant will be the focus of this work. Bioremediation process may be directed towards accomplishing by:

- ✓ Complete oxidation of organic contaminants (termed mineralization).
- ✓ Biotransformation of organic chemical into smaller (hopefully less toxic) constituent, or

Reduction of highly electrophilic halo-and nitro groups by transferring electron from an electron donor (typically a sugar

or fatty acid) to the contaminant resulting in less toxic compound (Means, J.L et al. 1994). Microbes are known for their metabolic diversity. One consequence of this diversity is the fact that many toxic or persistent anthropogenic organic compounds are degraded by microbial activities. In sample terms, microorganism must gain energy from the transformation of contaminants in order to survive. In addition, they must also have a source of carbon to build new cell materials, in absent of these, biodegradation will not proceed. The case of biodegradation of organic pollutants, the carbon typically comes from the pollutant being degraded. Although a multitude of reaction are used by microbes to degrade and transform pollutants. In oxidation attacks, microbes oxidize contaminant by transferring electron from the contaminant (termed the electron donor) to an electron acceptor to gain energy (Thomas, J.M et. al. 1989). Typically electron acceptor are oxygen, nitrate, fe(iii), sulfate and carbon dioxide.

In reductive attacks, microbes utilize some easily metabolized organic electron donor (such as sugar or short chain fatty acids) and transfer the electron to the pollutant to gain energy. This process is only possible with electrophilic pollutant such as halogenated aliphatic and explosive which contain nitro group.

III. BIOCHEMISTRY OF BIODEGRADATION

All reactions in cell are controlled by enzymes. Enzymes catalyzes both the oxidation and reduction of organic compound for energy (called catabolic reactions) as well the production of new cell component during growth (called anabolic reactions). The degradation of any organic molecules thus, requires the production and efficient utilization of enzymes. This enzyme needs transfer of electron from electron donor to the electron acceptor which requires electro carrying molecules such as NADH. These carries transport electron from an electron donor to the terminal electron acceptor through an electron transport chain. Microorganism need appropriate environmental conditions ti survive and grow. These conditions include, appropriate PH, temperature, oxygen, nutrient and lack of inhibiting or toxic compound (Thomas, J.M et. al.1989 and Cookson, J.T 1995). Typically, bioremediation is most sufficient at a PH near 7. However, bioremediation can be achieved between PH valves of 5.5 and 8.5. Most bioremediation system operates over a temperature range of 150c to 450c. Aerobic microorganism needs a certain amount of oxygen not only to survive but also to mediate their reactions. Generally, oxygen concentration greater than 2mg/L is required for aerobic microorganism to efficiently degrade organic contaminant. Microorganism need nutrient for their growth, the major need are identified with the generalized biomass formular (C60 H82 O25 N12 P) and include carbon, hydrogen, oxygen demand (BOD) of the contaminated soil. The carbon to nitrogen to phosphorus ratio (by weight) requires 120:10:1. Other nutrient such as sodium, potassium, ammonium, calcium, magnesium, iron etc need in minor quantities in the concentration range of one to hundred (i.e. 1 - 100) mg/L. In addition, traces less than 1mg/L of nutrient such as manganese, cobalt, nickel, boron copper, zinc various organic (vitamins) and molybdenum are need. High concentration of any contaminant can frequently be toxic to microbes. Some contaminant even at low concentration may be toxic microbes.

It is also desirable to maintain the soil moisture level between 40 to 80% of field capacity. Different classes of organic contaminant have different microbial bioremediation pathways and thus different consideration for bioremediation strategies (Means, J.L et al 1994).

OBJECTIVE OF THE STUDY

The objective of this research is to create a natural means by which a contaminated soil can be free through the process of bioremediation using microorganism such as bacteria, fungi etc. Nevertheless, biological remediation alone or in combination with other methods has gained an established place as a soil restoration technology. These microorganism metabolizes this contaminants either through oxidation or reduction process under favorable condition to give out non-toxic by-product such as carbon dioxide and water.

IV. MATERIAL AND METHODS

100g of soil sample control was analyzed using U.V spectrophotometer, the analyzed sample was to determine the initial concentration of petroleum hydrocarbon (PMS) present in the soil sample. The soil sample was soaked with 500mls of n-hexane for twent-four hours with constant shaking in the electric shaker. It was allowed to settle and was decanted. The decanted liquid was then filtered using filter paper to remove particles size or impurities. Distillation was carried out with a sample distillation system, after which a total of 1.3mls of oil extraction of petroleum hydrocarbon (PMS) was recovered using measuring cylinder. 10mls of xylene was used to dilute the extracted oil for the determination of initial concentration using U.V spectrophotometer at wavelength of 350nm. The absorbance of the diluted extract was 0.956.

The microbial contaminated soil sample was analyzed via-viza as the same as sample control, after an interval of fourteen days. The soil sample was then heated to stop further degradation ability based on it time interval. Cooling water was cold to the temperature of 150°c using ice block to bring down the temperature. The distillation temperature was 50°c using water bath with regulatory temperature setting, The distillation was done for one and half hour. The petroleum hydrocarbon (PMS) recovered after distillation process was 1.5, 2.2, 1.2, 1.8, 2.5, 3 and1.3mls for soil sample of 1, 2, 3, 4, 5, 6, and 7 respectively. Each total extract for the soil sample was diluted with 10mls of xylene to test for the concentration or absorbance of the petroleum hydrocarbon present in each of the soil sample by using U.V spectrophotometer.

V. RESULTS AND DISCUSSION

After fourteen days of inoculation of microbes to the contaminated soil sample, the greatest percentage degradation

of total petroleum hydrocarbon (PMS) was observed on the last day. The result is shown below;

S/N	Time (days)	Wavelength (nm)	Absorbance (mg/L)	percentage of petrol remaining (%)	Percentage of degradation (%)
0	0	350	0.956	100	0.00
1	2	350	0.723	75	0.003615
2	4	350	0.680	71	0.0272
3	6	350	0.495	52	0.0297
4	8	350	0.343	36	0.02744
5	10	350	0.251	26	0.0251
6	12	350	0.102	11	0.01224
7	14	350	0.013	0.01	0.00182

Table 1: Effect of time on biodegradation of petrol contaminated soil

Percentage degradation is given by:

$$\frac{\text{Absorbance} \times \text{time interval}}{100}$$

And percentage of petrol remaining is given by:

$$\frac{\text{Absorbance of time interval}}{\text{Initial absorbance at time zero}} \times \frac{100}{1}$$

In the table one above, the sample control shows the highest absorbance of 0.956 in petroleum hydrocarbon (PMS). But as the pre-selected microbes were inoculated, the lowest rate of degradation of the petrol was observed on the last day. During that period, more than 2% of the petroleum was degraded with a small and continual decrease until the end of the experiment. The effect of time on biodegradation of PMS contaminated soil was investigated by monitoring the concentration of the PMS remaining after a given interval for the soil sample.

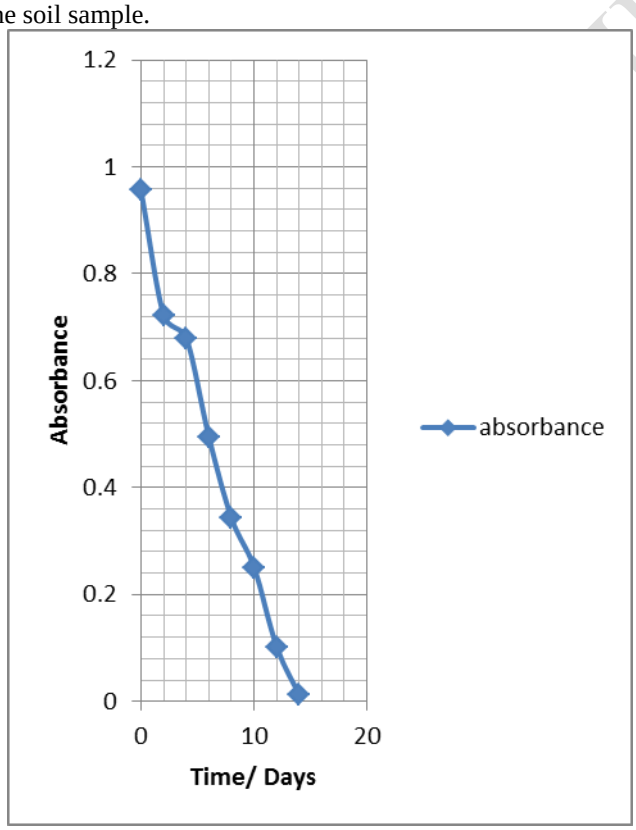

Figure 1: The effect of time on biodegradation of PMS

Also from table one above, the optimum percentage degradation of PMS was observed on the sixth day of the experiment. This means that the optimum percentage degradation was found to be 0.0297%

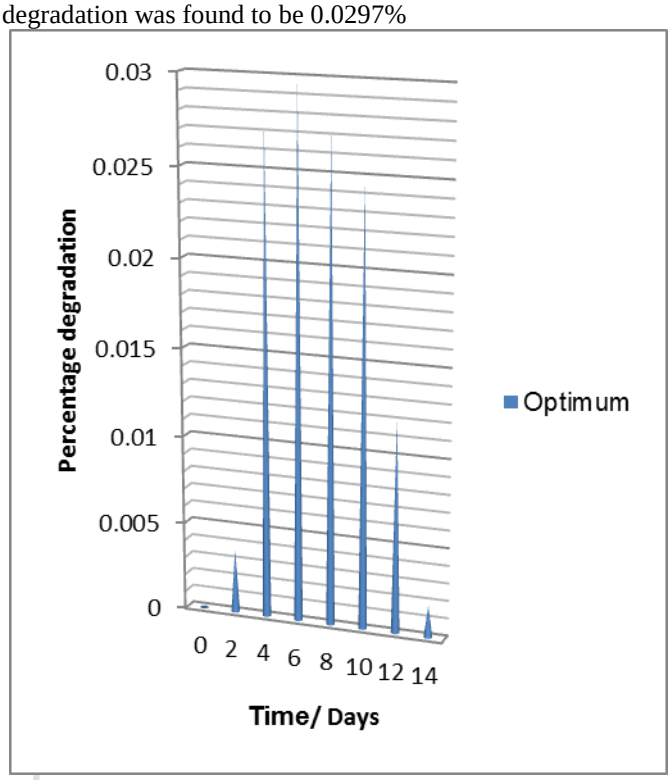

Figure 2: The optimum percentage degradation of PMS

S/N	Days	A_t	A_0	$A^{\propto}$	$A_0 - A^{\propto}$	$A_t - A^{\propto}$	$\frac{A_0 - A^{\propto}}{A_t - A^{\propto}}$	$K_1 = \frac{2.303}{t} \text{Log} \frac{A_0 - A^{\propto}}{A_t - A^{\propto}}$
0	0	0.956	0.956	0.013	0.943	0.943	1.000	- - -
1	2	0.732	0.956	0.013	0.943	0.710	1.328	0.123
2	4	0.680	0.956	0.013	0.943	0.667	1.413	0.150
3	6	0.495	0.956	0.013	0.943	0.482	1.956	0.291
4	8	0.343	0.956	0.013	0.943	0.330	2.857	0.455
5	10	0.251	0.956	0.013	0.943	0.238	3.962	0.597
6	12	0.102	0.956	0.013	0.943	0.089	10.595	1.025
7	14	0.013	0.956	0.013	0.943	- - -	---	- - -

Table 2: Data On Kinetics Of Biodegradation

From table 2 above, it indicates that the rate of microbial reaction on contaminated soil sample follows first order reaction by using first order reaction equation $K_1 = \frac{2.303}{t} \log \frac{A_0 - A^{\propto}}{A_t - A^{\propto}}$

Where, t is the day's interval

A_0 is the initial concentration or absorbance

A_t is the total absorbance after inoculating the microbes at day's interval

$A^{\propto}$ is the absorbance of the soil sample.

Since the above values of rate constant for first order reaction at different day's intervals K_2, K_4, K_6, K_8, and K_{10} are fairly constant hence it is a first order reaction.

The rate constant $K = \frac{0.141 + 0.086 + 0.111 + 0.130 + 0.137 + 0.196}{6}$

So therefore, K = 0.1335.

ISSN: 2394-4404

VI. CONCLUSION

The effect of time on biodegradation of PMS contaminated soil shows that the microbes actually attack the petroleum hydrocarbon present in the soil sample, Because the sample control have the highest absorbance of 0.956. But as the pre-selected microbes were inoculated, the lowest absorbance was observed to be 0.013 on the last day of the experiment. It also indicate that the rate of microbial reaction on contaminated soil sample follows first order reaction and also the optimum percentage degradation of PMS was observed on the sixth day of the experiment.

REFERENCES

[1] Doust, H.G and Huang, J.C (1992); the fate and transport of hazardous chemicals in the subsurface environment. Water Sci. 25: 169-176
[2] United State Environment Protection Agency. (2000); Engineered approaches to insitu bioremediation of chlorinated solvent: fundamentals and applications. EPA-540-R/00-998, Cincinnati. OH. Pp 8-12.
[3] United State Environmental Protection Agency (1991); Understanding bioremediation: a guide book for citizens. EPA/540/2-91/002, office of research and development, Washington, D.C. Pp 167-178.
[4] United State Environmental Protection Agency (1992); Bioremediation of hazardous waste EPA600/R/-92, office of research and development. p3.
[5] United State Environmental Protection Agency (1985); EPA guide for identifying clean up alternative at hazardous waste site and spits, biological treatment. EPA/600/3-83/063.p11.
[6] Means, J.L., and Hinchee, R.E (1994); Emerging technology for bioremediation of metals. Lewis publishers, Boca ration, fl. Pp 136-140
[7] Thomas, J.M., and Ward, C.H (1989); in-situ bioremediation of organic contaminants in the surface. Environment science. Technology, vol. 23 No. 7. Pp 760-766
[8] Cookson, J.T (1995); Bioremediation Engineering design and application, mc Graw-Hill, New York. Pp 19-22.