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EFFECT OF TEMPERATURE, pH AND NUTRIENT SUPPLEMENTATION ON THE GROWTH AND CRUDE OIL BIODEGRADATION OF PSEUDOMONAS AERUGINOSA

Nwosu, D.C[1]., Ekumankama, E.O[2]., Aneke, N.A.G[1].

[1]Chemical Engineering Department, Enugu State University of Science and Technology, Enugu State Nigeria.,

s[2]Department of Food Science and Technology, Ebonyi State University, Abakaliki Ebonyi State Nigeria.

Abstract

The effect of temperature, pH and nutrient supplementation on the growth and crude oil utilisation of Pseudomonas aeruginosa isolated from crude oil contaminated soil from Ndokwa East Local Govt area of Delta State was studied. The bacterium was isolated and identified based on the cultural, morphological and biochemical characteristics. This was incubated with known values of crude oil to investigate the growth and metabolic capability of this organism at five different values of temperature, pH and nutrient supplementation. The result showed that growth and degradation were highest at 37^{o}C, for pH, growth was highest at pH 5.32 while degradation was highest at pH 8.68 and for nutrient supplementation, growth was highest at nutrient concentration of 0.434g/ml of oil while degradation was highest at nutrient concentration of 1.085g/ml.

Keywords: Pseudomonas aeruginosa, Growth, Crude oil, Biodegradation.

1. Introduction

Pollution and environmental hazards have been on increasing trend due to industrial activities and technological development. The increasing global demand for crude oil and its products brought about the increase in production and the associated environmental pollution. Unfortunately, the efforts of the oil industry operators have not stopped the discharge of the oil into the environment. Oil spills both on land and sea is on the increase as a result of increase in demand and use. In the course of prospecting, drilling, production, refining, storage and distribution, some quantities of the oil are released into the environment. [1] reported that between 1976 and 1980, Nigeria recorded 784 incidents of oil spillage which resulted in the loss of 1,336,870 barrels of oil. [2] reported that apart from the physical loss to Nigeria nation, the impacts of oil spillage are noticeable in the flora and fauna of the oil producing communities.

Crops grow on soils that have the right amount and proportion of nutrients, water, oxygen and organisms. Petroleum hydrocarbons alter the fertility status of soil and hence reduce their ability to support proper crop growth and development [3]. The oil makes most of the essential nutrients unavailable for plants and crops utilisation and thus reduces the fertility of the soil. [4] observed that oil spills are destructive to both vegetation and animal in the soil not only because of their contact toxicity, but also because hydrocarbons in the soil reduces oxygen tension and increases anaerobiosis which is harmful to plant roots. The crude oil polluted land is barren and if left untreated will take more than ten years before it can be useful for agricultural purposes again. Biodegradation is the natural process of recovering crude oil polluted soil. It is a very slow process. To reclaim such agricultural lands and to clean up the polluted environment, different methods maybe necessary. Of the different methods available for remediation of contaminated environment, biological method of remediation is more acceptable because it is cost effective and environmental friendly. Biological method involves the use of biological agents such as bacteria, fungi or green plants to combat the contaminant. Indigenous bacteria to polluted soil biodegrade the oil and use them for growth and energy. Biodegradation is the process by which microorganisms transform or mineralize organic contaminants through metabolic or enzymatic processes into useful and non-hazardous substances which are then integrated into natural biogeochemical cycles [5]. This metabolic ability (biodegradation) of these bacteria is harnessed and used to breakdown the contaminant in a short time. The successful application of the metabolic property of bacteria to contaminated systems require knowledge of the characteristics of the site and the parameters that affects the microbial degradation of the pollutants. Petroleum hydrocarbon biodegradability in the soil is influenced by the complex array of factors such as temperature, nutrients, pH value, concentration and bioavailability. As these parameters constitute limiting factors for

the growth of the microorganism and the rate of degradation of crude oil, this work is aimed at determining the effect of temperature, pH and nutrient concentration on the growth and biodegradation of crude oil in the contaminated soil by *Pseudomonas aeruginosa*.

2. Materials and Methods

Crude oil was obtained from a farmland that have no history of oil spill in Ndokwa East local Goernment Area in Delta State, the polluted soil sample was obtained from a contaminated farmland also in Ndokwa East. Nitrogen fertilizer was purchased from Ogige market, Nsukka, while the Microbiological Media and Biochemicals were obtained from Joechem Laboratory Chemicals Shop, Enugu Road, Nsukka.

2.1 ISOLATION AND IDENTIFICATION OF THE ORGANISM.

Two mineral salts media were formulated and designated A and B for the isolation of the hydrocarbon degrading bacterium. Medium A was also used in screening the isolate for its ability to degrade hydrocarbons as well as for biodegradation studies. Medium B is mineral salt agar. Medium A was obtained by mixing $(NH_4)2SO_4$ (1.25g); $(NH_4)_2HPO_4$,(1.95g); KH_2PO_4,(0.85g); $MgSO_4.7H_2O$,(0.09g); $CaCl_2$,(0.001g) and distilled water, 1,000ml. medium B was obtained by mixing K_2HPO_4,(0.05g); NH_4Cl,(1.0g); Na_2SO_4,(2.0g); KNO_3,(2.0g); $MgSO_4$,(1.0g); agar, (15.0g) distilled water, (1,000ml); $CaCl_2$, (0.001g). The pH of the media were adjusted to 7.0.

Medium A was dispensed in 50ml volumes in duplicate 250ml Erlenmeyer flasks and sterilized at 121^{o}C for 15 minutes. Another 250ml flask containing 200ml of medium B was also sterilized at 121^{o}C for 15 minutes and dispensed into Petri-dishes. These were allowed to solidify, dried and kept for 24 hours to test for sterility.

PROCEDURE

The oil polluted soil sample was thoroughly stirred and 2g was used to inoculate each flask of medium A. Thereafter 1.0ml of kerosene filtered through a 5mm Whatmann syringe filter was introduced into each flask and the flask incubated on an orbital shaker (Gallenkamp, England) at 30^{o}C for 24 hours. After incubation a loopful of the content of each flask was used to streak triplicate petri-dishes of medium B using a sterile wire loop and incubated at room temperature (25-30^{o}C) for 48 hours. Kerosene was added as carbon source to each petri-dish by the vapour phase transfer method. This was done by placing sterile filter papers impregnated with sterile kerosene on each petri-dish cover and incubating in an inverted position. Resulting colonies were repeatedly sub cultured in this manner twice and pure isolates were obtained and stored in nutrient agar slants.

2.2 IDENTIFICATION OF THE ISOLATES.

The isolates were identified on the basis of microscopic examination and biochemical tests according to Bergey's manual of Determinative Bacteriology. The isolate was identified according to cultural morphology, shape size pigmentation on nutrient agar medium. The microscopic examination included spore staining and gram staining. The biochemical tests performed included catalase, oxidase, and starch hydrolysis, and nitrate reduction, growth on 7% NaCl, Arginine hydrolysis and Voges Proskauer test. Tests for the fermentation of sucrose, dextrose, mannitol, lactose, maltose, mannose and xylose were carried out according to standard procedures. The biochemical characterisation of the isolate was confirmed using API (50 CH BIOMETRIEUX, France) kit.

2.3 BIODEGRADATION STUDIES

The effects of temperature, pH and nutrient supplementation on crude oil utilisation by the chosen isolated bacterium (*Pseudomonas aeruginosa*) were investigated. The degree of utilisation was evaluated using bacterial number (measured in colony forming units (cfu) per gram of soil) and quantity of residual oil.

Each of the factors tested (i.e. temperature, pH and nutrient supplementation) was applied at five different levels to the organism (*Pseudomonas aeruginosa*). The nutrient supplementation was applied at a carbon to nitrogen ratio (C:N ratio) of 10:1, 10:2, 10:3, 10:4, and 10:5. The control for nutrient supplementation was 10:0 (i.e. no fertilizer).

Pristine sandy loamy soil obtained from Ndokwa East Local Govt. area of Delta State was air-dried, sieved and dispensed in 200g weights into thirty-two (32) 500ml Erlenmeyer flasks. The soil samples were sterilized by autoclaving thrice at 121^{o}C for 15 minutes. The 32 flasks were placed into three cells, each cell containing ten flasks which were duplicates of the variables (temperature, pH or nutrient) being tested for. Two flasks served as controls for the nutrient supplement. All the flasks were contaminated with filter-sterilised Bonny light crude oil at 20% (v/w) level of pollution. Each of the 32 flasks were inoculated with 5ml of inoculum of the bacterial isolate (*Pseudomonas aeruginosa*) suspended in normal saline to a level of 0.5 McFarland standard. The nutrient supplementation flasks also received sterilised solution of urea fertilizer. The study lasted for four weeks (one month) and samples were collected

weekly for analysis to determine the bacterial number and the residual oil. N-hexane was used as extractant for the determination of residual oil.

2.4 ESTIMATION OF THE BACTERIAL NUMBER (CFU/ML).

Ten-fold serial dilution was done. One gram (1g) of the soil sample was measured out from each of the sample flasks and dispensed into the first test tube containing 9ml of sterile distilled water for serial dilution. Each tube was shaken vigorously for thorough mixing of the mixture. 1ml was then pipetted from this and transferred into another test tube containing 9ml sterile distilled water to give a 10^{-1} dilution. The sample was diluted serially up to 10^{-10}. This was done for each of the 32 flasks. The enumeration proper was done by inoculating 0.1ml aliquot from an appropriate serially diluted sample onto mineral salts agar medium (medium B). Kerosene supplied carbon by the vapour phase transfer method mentioned earlier. Inoculated plates were incubated for 48 hours at room temperature (25-30^{o}C) after which the resulting colonies were counted and the bacterial number estimated as colony forming units (cfu) using the formula below;

$$(\text{cfu}) = \frac{\text{average number of colonies x original dilution}}{\text{volume of inoculum x dilution factor}}$$

$$= \frac{Y \times 10^{o}}{V \times 10^{-x}}$$

Where Y = Average number of colonies

X = volume of inoculum

10^{-x} = dilution factor

10^{o}=1= original dilution

2.5 EXTRACTION OF RESIDUAL OIL

Twenty milli- litre of n-hexane was poured into each bottle containing 5g crude oil polluted soil with the microorganism and centrifuged at 1000rpm for 5 minutes and decanted into a sterile test-tube. The extraction was performed twice with 10ml n-hexane each time. The total extract which contains the residual crude oil was left over night for the n-hexane to evaporate while leaving the residual oil in the test tube. This was weighed and the volume calculated based on the fractional recovery rate (FRR). For each, two extractions were done and the average value recorded.

2.6 FRACTIONAL RECOVERY RATE (FRR)

Ten milli-litre of crude oil was introduced into 20g of soil. Using the method of Toogood and McGill (1977) [6], the crude oil was extracted from the soil. It was discovered that the percentage recovery rate was consistently 80% (i.e. from the 10ml introduced into the soil, 8.0ml was consistently recovered with the remaining adsorbed to the soil particles). There was a consistent percentage loss of about 20% which amounts to 2ml of the 10ml used from the above, it was possible then to calculate the fractional lost.

Amount of oil used = 10ml
Amount of oil recovered = 8.0ml
Recovery per ml of oil = 8/10 = 0.8ml
The fractional recovery rate = 0.8ml
Percentage recovery rate = 80%
The fractional lost per ml of oil = 0.2ml per ml of oil

The computed fractional lost was added to the residual oil obtained from the extraction of residual oil. The actual quantity of oil degraded will now be the quantity used minus the total recovery residue and the fractional loss computed.

3. RESULTS AND DISCUSSION

3.1 Characterisation of isolate

The isolate used in the study was isolated from the crude oil polluted soil and identified based on its morphological and biochemical properties. The isolate exhibited positive results for motility, oxidase, catalase, Voges Proskauer test and negative results for gram staining, sporulation, starch hydrolysis, nitrate reduction, growth on 7% NaCl, Arginine

hydrolysis tests, sucrose, mannitol, lactose, maltose, mannose and xylose fermentation tests (Table 1). The strain implicated is *Pseudomonas aeruginosa*.The growth of this bacterial strain and its ability to degrade crude oil and use it as source of energy and carbon under different conditions of temperature, pH and nutrient supplementation were investigated.

Table 1 Biochemical tests carried out for the identification of the isolate *Pseudomonas aeruginosa*

Tests	**Results**
Colour of colonies	Greenish
Shape	Rod
Motility	+
Gram stain	_
Spore stain	_
Catalase	+
Oxidase	+
Starch hydrolysis	_
Nitrate reduction	_
Growth on 7% Nad	_
Arginine hydrolysis	_
Voges Proskaner	+
Sucrose fermentation	_
Dextrose fermentation	+
Mannitol fermentation	_
Lactose fermentation	_
Maltose fermentation	_
Mannose fermentation	_
Xylose fermentation	_
Implicated Organism	*Pseudomonas aeruginosa*

\+ = Positive reaction to the test
_ = Negative reaction to the test

The growth of *Pseudomonas aeruginosa* inoculated into the crude oil polluted soil varied with temperature and is shown in the plots of bacterial population against time and population against temperature in Figures 1 and 2. .The viable microbial cells also varied with pH (Figures 3 and 4) and with nutrient concentrations as shown in Figures 5 and 6. The breakdown and utilisation of crude oil monitored gravimetrically by the weight loss of crude oil introduced into the soil showed *Pseudomonas aeruginosa* degraded highest crude oil at temperature of 37^{o}C, Figure 7. Figure 8 showed the pattern of biodegradation at the five different pH values the study was conducted. The biodegradation of the crude oil at the different nutrient concentration values is shown in Figure 9.

3.2 EFFECT OF TIME AND TEMPERATURE ON GROWTH

The growth of *Pseudomonas aeruginosa*. Temperature affects the chemistry of the contaminants as well as the physiology and diversity of the microbial flora [7]. This study was done under five different temperatures of 30.27^{O}C, 33^{O}C, 37^{O}C, 41^{O}C and 43.7^{O}C.

Figure 1 is the contour plot of bacterial number against time showing the effect of time and temperature on the growth of *Pseudomonas aeruginosa.* The results obtained showed a steady

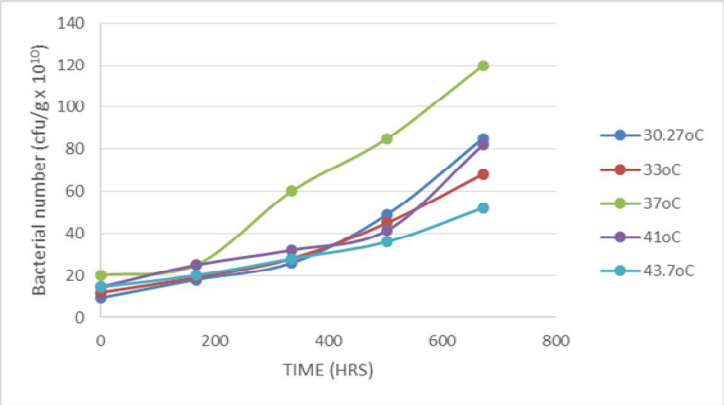

Figure 1: Effect of Temperature on the Growth of *Pseudomonas aeruginosa* at pH of 7.0 and 10.85g of nutrient

The result shows that for a growth period up to 672 hours, the microbial count increased from 9.5 x 10^{10} to 85 x 10^{10} cfu/g soil at incubation temperature of 30.27^{O}C, from 12 x 10^{10} to 68 x 10^{10} cfu/g soil at incubation temperature of 33^{O}C, 20 x 10^{10} to 120 x 10^{10} cfu/g soil at 37^{O}C, from 10 x10^{10} to 82 x 10^{10} cfu/g soil at 41^{O}C and from 14.5 x 10^{10} to 52 x 10^{10} cfu/g soil at 43.7^{O}C. The method of plating technique was used in the enumeration of the cell number. This was done every seven days for a total of 28 days. The bacterial growth increased with increase in the incubation period (weeks). This can be attributed to multiplication of cells of the microorganism as it fed on the crude oil. The viable cells increased with increase in temperature up to 37^{O}C and later decreased at temperatures higher than 37^{O}C. This is shown in the plot of variation of bacterial number against temperature Figure 2.

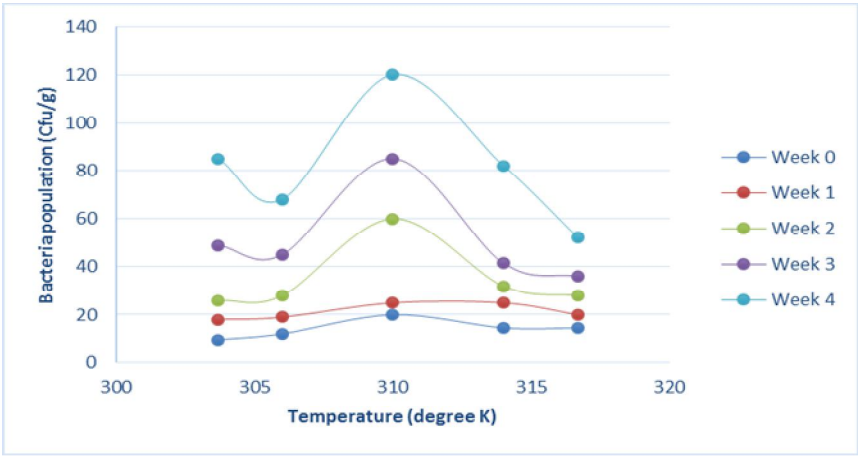

Figure 2: Variation of bacterial population with Temperature and Time at pH 7.0 and 10.85g nutrient

From this Figure 2 it can be seen that at low temperatures the microbial number from the initial time to the 2nd week remained constant. This is because at lower temperatures the viscosity of the crude oil is low and the bacteria cannot access it. Temperature plays an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as physiology of the bacteria. Availability of the hydrocarbons for the microorganisms to access depends on the temperature of the crude oil in the soil. At lower temperatures the bacteria could not access the hydrocarbons and with time some of them started dying off and this became more pronounced in the 3rd and 4th weeks. The viable cells increased with increase in temperature up to 37^{O}C and later decreased at temperatures higher than 37^{O}C. This suggests that the optimal temperature of the organism in the medium is 37^{O}C. At temperature higher than 37^{O}C the death of cell became higher than growth rate of cells. Another possible reason for the decrease in the bacteria population observed in Figure 2 could be what [8] reported in their work , that decrease in population could occur as a result of physical damage or shock in transferring the organism. In their work in biodegradation of Bonny light and Bonny medium crude oil, they noted that the initial outcome of natural microbial population in contact with petroleum hydrocarbon is most often a reduction in the microbial biomass followed by an increase in biodegraders. Also [9], reported that crude oil introduced into the soil causes initial damage to the soil biota. The increase in population after the initial decrease starts when most of the biodegraders recovered from the initial shock, they produce enzymes that were able to degrade the crude oil which they use as food and energy source. [9] added that the growth is exponential

3.3 EFFECT OF TIME AND pH ON GROWTH

The effect of different pH values on the growth of *Pseudomonas aeruginosa* inoculated into crude oil polluted soil was studied. The pH values used are between 5.32 and 8.68. Each organism has an optimal pH at which it grows and acts best. [10] observed that optimal pH of any organism is the measure of the pH of their extracellular environment but the intracellular pH of the organism remains close to neutrality because DNA is acid –labile and RNA is alkaline- labile. The organism with optimum pH that is not neutral has adaptations to be able to grow in that type of environment. It means that extremes in pH of soils would have negative effect on the growth of bacteria and also on the ability of the organisms to degrade hydrocarbons[10]. The viable microbial cells varied with time and pH. This is shown in Figures 3 and 4 with the highest values occurring at pH of 5.32.

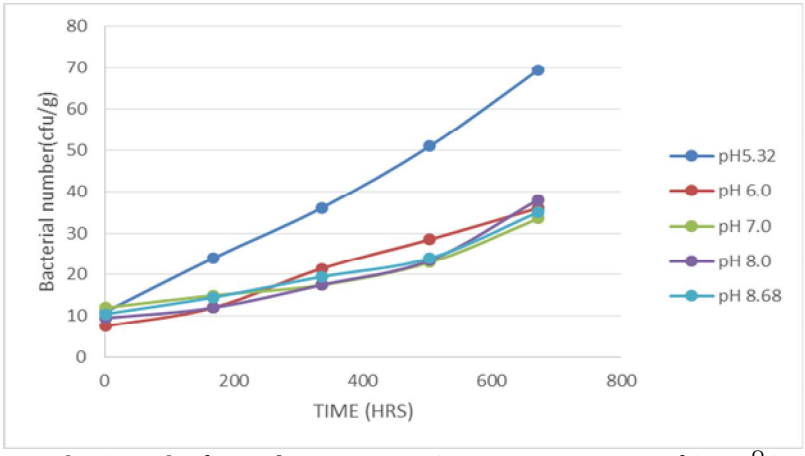

Figure 3: Effect of pH on the Growth of *Pseudomonas aeruginosa* at temperature of 30.27^{O}C and nutrient of 10.85g

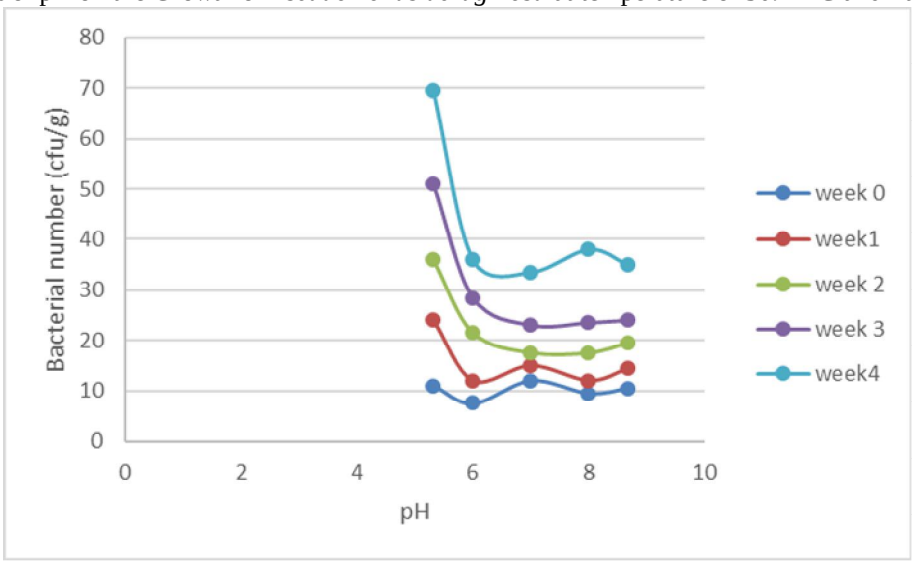

Figure 4: Variation of *Pseudomonas aeruginosa* population with pH and Time at pH of 7.0 and 10.85g nutrient

From the results obtained, it can be seen that working at pH of 5.32, the microbial count varied from 11 x 10^{10} to 69 x 10^{10} cfu/g soil for incubation period up to 672 hours. For the same incubation period, the microbial count varied from 7.5 x 10^{10} to 36 x 10^{10} cfu/g soil, 12 x 10^{10} to 33 x 10^{10} cfu/g soil, 9.5 x 10^{10} to 38 x 10^{10} cfu/g soil, and from 10.5 x 10^{10} to 35 x 10^{10} cfu/g soil for operation at pH of 6.0, 7.0, 8.0 and 8.68 respectively. From Figures 3 and 4, the highest bacterial number was obtained at pH 5.32 with a viable bacterial population of 69 x 10^{10} cfu/g soil in the 4th week followed by that in 3th week with bacterial population of 51 x 10 cfu/ g soil also at pH of 5.32. Apart from the viable microbial cells recorded at this pH of 5.32, it can be seen that after 4 weeks that the bacterial number did not significantly vary with pH as shown in Figures 3 and 4. Figure 3 showed that cell number generally increased with time. This is expected since the cells were feeding on the substrate (oil) growing and multiplying by cell division.

3.4 EFFECT OF TIME AND NUTRIENT ON GROWTH

Different concentrations of the nutrient (nitrogen) were used and the effect of these on the growth of *Pseudomonas aeruginosa* measured. The bacterial population (growth) plotted against time is shown in Figure 5.

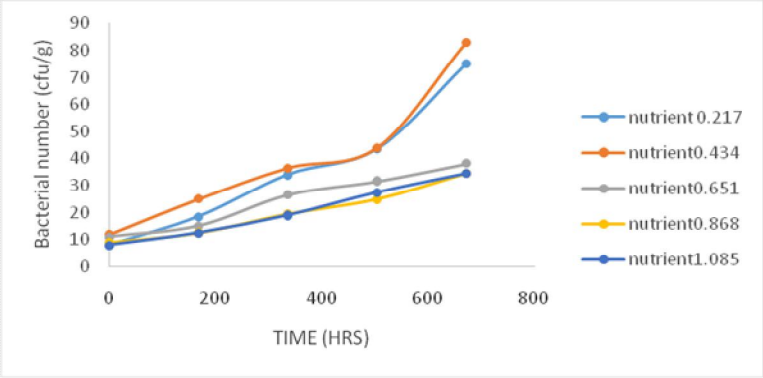

Figure 5: Effect of nutrient on the growth of *Pseudomonas aeruginosa* at temperature of 30.27^{O}C and pH 7.0

The highest growth of the organism was at nutrient level of 0.434g/ml of oil which is equivalent to carbon to nitrogen ratio of (C : N) 10 : 2 followed by that of nutrient concentration level of 0.217g/ml of oil which has the C : N ratio of 10 : 1. However, the general trend of cell number increasing with time was observed. The variation of *Pseudomonas aeruginosa* cell number with nutrient concentration and time is shown in Figure 6. At the initial time, it was observed that the nutrient concentration effect was noticed only at a concentration of 0.885g/ml of oil. With time the increase in the viable number became evident, increasing with time as seen in Figure 6 showing maximum at 0.434g/ml of oil.

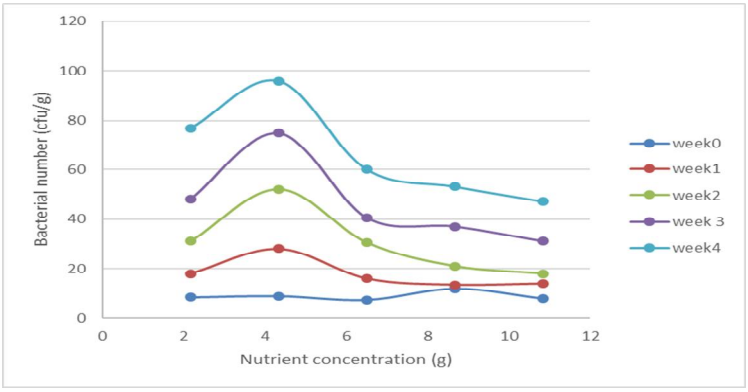

Figure 6: Variation of *Pseudomonas aeruginosa* number with nutrient concentration and time at temperature of 30.27^{O}C and pH 7.0

3.5 EFFECT OF TIME AND TEMPERATURE ON THE UTILIZATION OF CRUDE OIL

The effect of temperature on the break down and utilization of the crude oil by *Pseudomonas aeruginosa* followed the growth shown in Figures 1 and 2. The residual crude oil decreased with time as shown in Figure 7.This is expected since the organism was utilizing it as food for energy and growth.

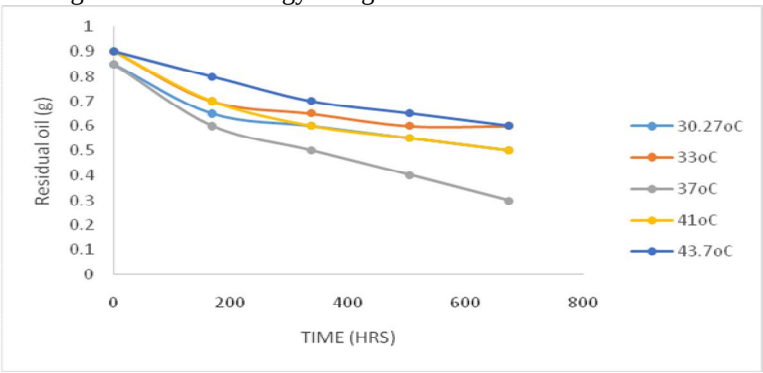

Figure 7: Effect of temperature on *Pseudomonas aeruginosa* degradation of crude oil

This suggests that the organism degraded the oil highest at the temperature of 37^{O}C which is the temperature with the highest population. From initial time to fourth week, the oil concentration progressively decreased from the initial of 0.85g to 0.3g in twenty-eight days. This steady decrease of crude oil from the initial value of 0.8g to 0.3g is attributed

to utilization by the cells leading to the increase in the microbial biomass at the optimum temperature of 37^{O}C. This observation is in agreement with the report of [11]- [12] that microbial degradation of petroleum hydrocarbon in the environment is influenced by factors such as population of the hydrocarbon degraders, temperature and nutrient availability. The growth was highest at this temperature and biodegradation was also found to be highest at this temperature of 37^{O}C and time resulting to the least residual oil recorded as shown in Figure 7. The extent of degradation of the crude oil by *P. aeruginosa* is about 65%. This is consistent with the report of [13], that *P. aeruginosa spp*. strain degraded Escravos crude oil between 60 to 66% after 20 days of incubation. [14] noted that the optimum temperature for growth and crude oil degradation is 37^{O}C for *Pseudomonas aeruginosa* (www.textbookof bacteriology.net)

3.6 EFFECT OF TIME AND pH ON CRUDE OIL DEGFADATION (OR DEPLETION)

Figure 8 shows the effect of pH on the depletion of the crude oil by Pseudomonas aeruginosa.

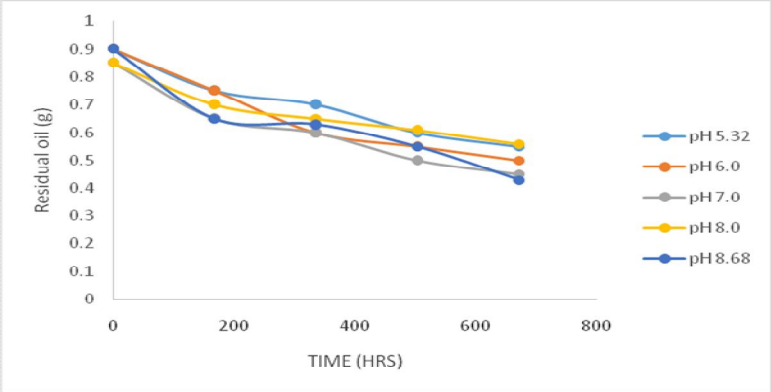

Figure 8: Effect of pH on crude oil degradation by *Pseudomonas aeruginosa*

From the results it can be seen that the optimum pH for biodegradation of crude oil is between pH 6.0 to 8.0, [15]. In this study, the highest biodegradation of oil by *Pseudomonas aeruginosa* was at pH 7.0 and 8.68 having 0.45g/ml and 0.43g/ml residual oil respectively. This corresponds to about 47% and 52% degradation respectively. This result is in agreement with the observation of [16], that biodegradation rate increased as the microbial population increased as a result of favoured pH between 6.0 to 9.6. [17], gave soil pH 5.5 to 8.8 as favourable condition for microbial activity and pH 6.0 to 8.8 as optimum value for an oil degradation. Therefore the result obtained in this work is consistent with those of [15], [16] and [17].

3.7 EFFECT OF TIME AND NUTRIENT ON CRUDE OIL DEGRADATION (OR DEPLETION)

The effect of nutrient concentration on the depletion of crude oil is presented in Figure 9.Nutrient are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen [18].

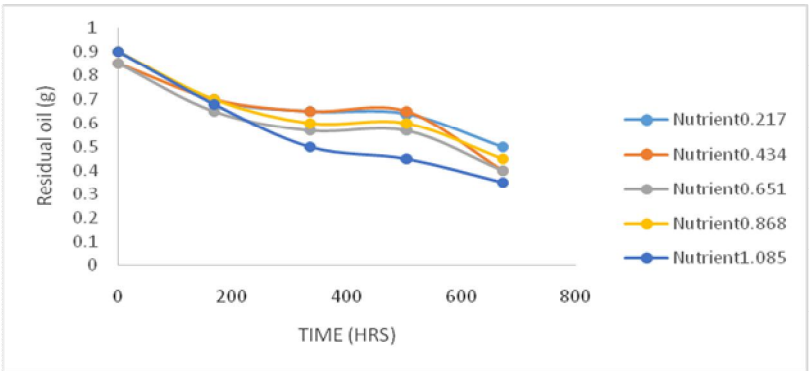

Figure 9: Effect of Nutrient on the biodegradation of Crude oil by *Pseudomonas aeruginosa*

[16] observed the rate of microbial degradation was dependent on availability of nutrient source. Shortage or absence of these nutrients could become limiting factor affecting the biodegradation processes. Oil spills on land increases the supply of carbon significantly and the availability of nitrogen becomes the limiting factor for oil degradation. The ratio of nitrogen to carbon in the soil which should be around 10:1 and 10:2 produces excessively high carbon to nitrogen ratio which are unfavourable for microbial activity and biodegradation when there is oil spill. This work was done using five different nutrient concentrations 0.217g/ml of oil, 0.434g/ml, 0.651g/ml, 0.868g/ml and 1.085g/ml of oil. The highest biodegradation was at nutrient concentration of 1.085g/ml followed by those at 0.434g/ml and 0.651g/ml.

The oil decreased from the initial value of 0.9g/ml to 0.35g/ml in 4 weeks, Figure 9. This is about 61% degradation of the hydrocarbon by *Pseudomonas aeruginosa*. This rate of biodegradation observed is as a result of the increase in microbial biomass and correct nutrient availability.

4.CONCLUSION

Pseudomonas aeruginosa has the capacity to degrade petroleum hydrocarbon when the favourable conditions are met. The result of this work showed that the rate of biodegradation is related to the microbial biomass. Under the three conditions studied, temperature, pH and nutrient supplementation, the highest growth was at the temperature of $37^{O}C$ with microbial population of $120x10^{10}$cfu/g soil. The percentage oil removal is 65%. The highest growth in the case of pH was that at pH 5.32 with a microbial population of $69.5x10^{10}$cfu/g soil and percentage degradation of 52%. For the growth and degradation recorded with the nutrient supplementation, the highest microbial population was $83.0x10^{10}$cfu/g soil. The percentage oil removal is 61%.

REFERENCES

[1]. 1. Awobajo, S.A. (1981). An analysis of spill incidents in Nigeria (1976—1980). IN: Proc. Seminar on Petroleum Industry and Nigerian Environment, NNPC/FMOW and Housing PT; Warri.
[2]. Ikporukpo, C.O. (1983). Perceptible aspects of the environmental effect of oil exploration on Nigerian rural Community. In Nigeria Environment: Status and Trends Nigeria (Geographical Association).
[3]. Abii, T.A, and Nwosu, P.C. (2009). The effect of oil spillage on the soil of Eleme in Rivers State of the Niger Delta Area of Nigeria. Research Journal of Environmental Sciences, 3:316—320.
[4]. Bossert, I. and Bartha, R.A. (1984). The fate of petroleum in the soil ecosystem. In Atlas, R.M. (Ed), Petroleum Microbiology. Macmillan, New York, 435—473.
[5]. Snezana, M.; Bozo, D.; & Srdan, R. (2013). Petroleum hydrocarbon biodegradability in soil – Implications for bioremediation. http://dx.doi.org/10.5772/50108.
[6]. Toogood, J.A. and McGill, W.B. (1977). The reclamation of Agricultural soils after oil spills: 11 extension. The University of Alberta, A.I.P. Publishers, Edmonton.
[7]. Nilanjana, D. and Preethy, C. (2010). Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology Research International, 13. Doi: 10.4061/2011/941810
[8]. Ekpo, M.A. and Ekpo, E.I. (2006). Utilization of Bonny light and Bonny medium crude oils by microorganisms isolated from Qua Iboe River Estuary. Nig. J. Microbial. 20: 832—839.
[9]. Odu, C.T.I (1972). Microbiology of soils contaminated with petroleum hydrocarbons III. National rehabilitation and reclamation of soils affected. Institute of Petroleum Technology Publication. Pp 77-102.
[10]. Margesin, R. (2000). Potential of cold-adapted microorganisms for bioremediation of oil polluted alpine soils. International biodeg. 46, 3-10.
[11]. Atlas, R.M. and Bartha, R. (1972). Degradation and mineralization of petroleum in seawater: limitation by nitrogen and phosphorus. Biotechnol. Bioeng. 14: 309 – 817.
[12]. Atlas, R.M. and Bartha, R. (1973). Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environ. Sci. Technol. 7: 538 – 541.
[13]. Adebusoye, S.A, Ilori, M.D, Amund, D.D, Tesiola, O.D & Olatope, S.O (2007). Microbial degradation of petroleum in a polluted tropical stream. World J. Microbiol. 23: 1149-1159.
[14]. Kenneth Todar. (www.textbookofbacteriology.net)
[15]. Leahy, J.G and Colwell, R.R. (1990). Microbial Degradation of Hydrocarbons in the Environment. Microbial Reviews, 53(3), 305-315.
[16]. Akpoveta, O.V; Eghareavba, F.; Medjor, O.W; Osario, K.I & Enyemike, E.D (2011). Microbial degradation and its kinetics on crude oil polluted soil. Research Journal of Chemical Sciences, 1(6), pp 8-14.
[17]. Bijay, T; Ajay, K.K.C; & Anish, G. (2012). A review on biodegradation of petroleum hydrocarbon contaminants in soil. Kathmandu University Journal of Science, Engineering and Technology. Vol. 8, No 1, pp 164-170.
[18]. Atlas, R.M (1975). Effects of temperature and crude oil composition on petroleum biodegradation. Appl. Microbial. 30; 396-403.