# Biodegradation of diesel-polluted soil using Penicillium sp and Bacillus subtilis

**Emurotu, Marvelous Olubunmi**
Microbiology Department, Kogi State University, Anyigba Nigeria
bunmi_emurotu@yahoo.com

**Abstract**
Study was carried out on soil sample gotten from Kogi State University. This soil sample was sterilized in the laboratory and polluted with diesel. The diesel-polluted soil was biodegraded using Penicillium sp., and Bacillus subtilis. This was carried out for a period of 21 days in microbiology laboratory of Kogi State University Anyigba. Some physicochemical parameters of the soil were also analyzed. The study revealed that Penicillium sp., and Bacillus subtilis made the soil sample to become alkaline compared to the control. The soil inoculated with Penicilliun sp was more alkaline than soil inoculated with Bacillus subtilis. There were higher rates of turbidity in both inoculums (Penicillium sp., and Bacillus subtilis) than the control. The study also revealed that Penicillium sp had higher percentage of biodegradation at days 19 and 21 which were 9.41 and 12.90 and rates of biodegradation were 1.75X $10^{-4}$ and 2.18 $X10^{-4}$ respectively compared to Bacillus subtilis whose highest percentage of biodegradation was also at 19 and 21 which were 7.06 and 10.59 and rates of biodegradation were 1.31 $X10^{-4}$ and 1.78 $X10^{-4}$ respectively. It can therefore be deduced from this study that Penicillium sp degraded diesel at a higher rate than Bacillus subtilis.

**Key words**: biodegradation, diesel, polluted, Penicillium sp., Bacillus subtilis

## 1. INTRODUCTION

Nigeria is a major producer of crude oil in the world and pollution of the environment due to oil spillage has steadily increased. In the Niger Delta area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment (Kori-Siakpere, 1998; Odiete, 1999). Crude oil originating from different parts of the world will differ considerably in their physical and chemical properties. These differences become important in relation to the behavior of spilled oil in environment and subsequent clean up techniques (Awabanjo, 1981).

Diesel oil, which is one of the major products of crude oil, constitutes a major source of pollution in our environment. With the combined dependence on diesel oil by some vehicles and generators, greater quantities are being transported over long distances, therefore diesel oil can enter into the environment through wrecks of oil tankers carrying diesel oil, cleaning of diesel tanks by merchants, war ships carrying diesel oil and motor mechanics (Hill and Moxey, 1980). Diesel oil spills on agricultural land generally reduce plant growth. Suggested reasons for the reduced plant growth in diesel oil contaminated soils range from direct toxic effect on plants (Baker, 1982) and reduced germination (Udo and Fayemi, 1975) to unsatisfactory soil condition due to insufficient aeration of the soil because of the displacement of air from the space between the soil particles by diesel oil.

Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods (Ijah and Okang, 1993). This is possible be- cause microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy (Ijah and Antai, 1988; Ezeji *et al*., 2005; Antai and Mgbomo, 1993). In this study, an investigation was made on the biodegrading capability of diesel by *Penicillium sp*.and *Pseudomonas aeruginosa* under laboratory condition.

## 2. MATERIALS AND METHODS

### Sample collection and preparation

Top soil sample was collected from the premises of the Kogi State University, Anyigba in sterilized plastic container and taken to the laboratory. Soil sample meant for degradation studies was sterilized using oven at 100$^{o}$C for 15 min, after which it was allowed to cool to room temperature for further treatments.

**Isolation of Test Microorganisms**

*Penicillium sp*.and *Bacillus subtilis* were collected from stock cultures from the laboratory of Microbiology Department, Kogi State University Anyigba**.** These microorganisms were streaked on nutrient agar plates (for *Bacillus subtilis*) and potato dextrose agar plate (for *Penicillium sp*). The plates were incubated at 37$^{o}$C for 24h and 27 $^{o}$C for 72h the two organisms respectively. After incubation, the plates that contained between 30 to 200 colonies were used. The isolates were characterized based on cultural characteristics such as, cell morphology and other biochemical tests to re-affirm them being *Penicillium sp*.and *Bacillus subtilis*. This was done according to the method of Okpokwasili and Ananchukwu (1988).

**Samples treatment.**

The soil samples in each group were treated as follows:

**Group A**: sample of 20 g sterilized soil mixed with 1 ml (0.85g) of diesel oil plus 0.1 ml of *Bacillus subtilis*

**Group B:** sample of 20 g sterilized soil mixed with 1 ml( 0.85g) of diesel oil plus 0.1 ml of *Penicillium sp*

**GroupC**: sample of 20g of sterilized soil mixed with 1ml of distilled water and 1ml of diesel. Group C serve as control. Udeme and Antai 1988 method.

**Determination of diesel oil degradation**

The ability of *Bacillus subtilis and Penicillium sp* to degrade diesel oil was demonstrated in terms of reduction in the quantity of diesel oil introduced to pollute the soil samples. The rate of utilization was monitored on the first day (day zero) of the study and subsequently at 3-days interval for 21 days. Carbon tetrachloride was employed as the extractant. On each day, one sample per single treatment was analyzed for the quantity of residual diesel oil using the methods of Udeme and Antai (1988).

Each of the 20 g soil treatment samples was mixed with 10 ml of carbon tetrachloride, placed in a separating flask, shaken vigorously for 3 min and allowed to settle for 5 min. The liquid phase was separated by allowing the mousse (diesel oil – carbon tetrachloride) to pass gradually through a funnel fitted with filter paper (Whatman No 1). Anhydrous sodium sulphate spread on the filter paper was employed to remove any moisture in the mixture. The liquid phase was collected in a beaker. The beaker containing the extract was placed on the hot plate and the extractant, allowed to evaporate at 50$^{o}$C. The beaker with the residual diesel oil was allowed to cool to room temperature and weighed to determine the quantity of residual diesel oil by difference, according to Udeme and Antai (1988).

The percentage of diesel oil degraded at three days interval was determined from the equation:

$$\%\ diesel\ of\ \deg raded = \frac{Weight\ of\ diesel\ oil\ \deg raded}{Original\ weight\ of\ deisel\ \text{int}roduced} \times 100$$

Where the weight of diesel oil degraded = Original weight of diesel oil - weight of residual diesel oil obtained after evaporating the extractant.

$$Rate\ of\ \deg radation = \frac{Weight\ of\ diesel\ oil\ \deg raded\ (g)}{Time\ taken\ (h)}$$

**pH:** This was determined using the pH meter. It was standardized using a 5ml Buffer of pH6.86, 5ml of the sample was poured into a beaker. The protective cap of the electrode was removed, rinsed and immersed in the beaker for 3-5 minutes. The reading displayed was recorded as the pH of the sample, Udeme and Antai (1988).

**Turbidity**: This was determined using digital turbidity meter (WGZ-1B Shanghai Xinrui, China). A 5ml of the solution was poured into a beaker. the electrode of the turbidity meter was inserted into the beaker. The reading displayed on the screen was recorded as the turbidity of the sample, Udeme and Antai (1988).

## 3. Results and Discusion

The observed pH range (day 0 to 21) is given in Table 1. *Bacillus subtilis* ranged from 5.8 to 7.3. Corresponding values in *Penicillium sp* ranged from 5.8 to 7.5. The observed result shows

Table 1**:** pH of diesel polluted soil undergoing biodegradation

| Days | pH of soil innoculated with *Penicillium sp* | pH of soil innoculated with *Bacillus subtilis* | Control |
|---|---|---|---|
| 0 | 5.8 | 5.8 | 5.8 |
| 3 | 7.2 | 7.0 | 5.8 |
| 6 | 7.3 | 7.2 | 5.8 |
| 9 | 7.5 | 7.3 | 5.8 |
| 12 | 7.3 | 7.3 | 5.8 |
| 16 | 7.2 | 6.0 | 5.8 |
| 19 | 7.2 | 6.2 | 5.8 |
| 21 | 7.0 | 6.3 | 5.8 |

that the addition of diesel increased the pH to alkalinity in both *Bacillus subtilis and Penicillium sp*. Highest observed pH was 7.5 and 7.3 for *Penicillium sp* and *Bacillus subtilis* respectively on day 9. From day 12 it started decreasing and on day 21 the pH became neutral. Similar trend was observed in *Bacillus subtilis*. However, in *Bacillus subtilis* it decreases into acidity from day 16. The pH of the control soil was acidic all through 5.8. The results obtained compared to the control showed that microorganisms addition increased the pH of the soil samples which enhanced

biodegration rate of diesel in soil. Similar pH range has also been reported (Stephen *et al*., 2015). The highest pH observed in *Penicillium sp* medium indicates higher metabolic activity by *Penicillium sp* compared to *Bacillus subtilis* (Stephen *et al*., 2015).

Table 2 shows the results for turbidity of diesel polluted soil undergoing biodegradation. The observed differences in turbidity of diesel polluted soil undergoing biodegradation when *Penicillium sp* and *Bacillus subtilis* was added is not significant. However, when compared with control soil result showed decrease in turbidity in soil polluted with *Penicillium sp* and *Bacillus subtilis*. Decrease in turbidity was obseved in both inoculum after day 3 and 6. However, as from day 9 to 21 there was a slight increase but not much differences for *Penicillium sp* (13.5 to 13.6) and corresponding values for *Bacillus subtilis* ranged from 12.9 to 13.1. This shows that there was a higher growth rate in *Penicillium sp* than *Bacillus subtilis*. Turbidity increase has been reported to be due to the ability of the added organisms to make use of diesel as substrate to carry out metabolism in addition to the presence of mineral salt in the medium which is a necessary requirement for biodegrative activities (Ezeji *et.al*. 2005).

*Pseudomonas aeruginosa* performed best in the biodegradation of diesel polluted soil *Penicillium sp*, this is because *Bacillus subtilis* utilize the diesel better thereby reducing the dissolved nutrient particles in the medium (Brusseau, 1998).

*Penicillium sp* (mold) has a higher potential to degrade diesel at 1.75 x $10^5$ and 2.182 x$10^5$ at day 19 and 21 compared to *Bacillus subtilis* (bacteria) with 1.31 x$10^6$ and 1.78 x$10^6$ at day 19 and 21. As earlier reported by Rahaman *et al*., 2003 and Broojiman *et al*., 2009 that molds are the most agents in hydrocarbon degradation and they work as primary degraders of spill oil in the evnviroment.

Table 2: Turbidity of diesel polluted soil undergoing biodegradation

| Days | Turbidity of soil innoculated with *Penicillium sp* | Turbidity of soil innoculated with *Bacillus subtilis* | Control |
|---|---|---|---|
| 0 | 19.0 | 19.0 | 0 |
| 3 | 11.4 | 13.3 | 0 |
| 6 | 11.3 | 13.1 | 0 |
| 9 | 13.5 | 12.9 | 0 |
| 12 | 13.5 | 13.0 | 0 |
| 16 | 13.5 | 13.0 | 0 |
| 19 | 13.6 | 13.1 | 0 |
| 21 | 13.6 | 13.1 | 0 |

| Days extracted | Sample description | Weight of diesel after being biodegraded with *Penicilllium sp* | Weight of diesel after being biodegraded with *Bacillus subtilis* | Weight of diesel (control) |
|---|---|---|---|---|
| 0 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum. | 0.85 | 0.85 | 0.85 |
| 3 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum | 0.84 | 0.85 | 0.85 |
| 6 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum | 0.78 | 0.84 | 0.85 |
| 9 | 20 g of Sterilized soil mixed with 1 ml of diesel + 0.1ml of innoculum | 0.78 | 0.80 | 0.85 |
| 12 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum | 0.78 | 0.79 | 0.85 |
| 16 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum | 0.77 | 0.79 | 0.85 |
| 19 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum | 0.77 | 0.74 | 0.85 |
| 21 | 20g of Sterilized soil mixed with 1ml of diesel + 0.1ml of innoculum | 0.74 | 0.74 | 0.85 |

**Table 3:** The level of diesel extracted after the addition of *Penicilllium sp* and *Bacillus subtilis* compared with control
Note:1ml of diesel=0.85g

Table 4: The rate of degradation of *Penicillium sp*., percentage of diesel oil degraded and weight of diesel oil degraded.

| Days | Weight of diesel extracted (g) | Weight of diesel degraded | % of degradation | Rate of degradation(g/h) |
|---|---|---|---|---|
| 0 | 0.85 | 0.00 | 0.00 | 0.00 |
| 3 | 0.84 | 0.01 | 1.18 | $1.38x10^{-4}$ |
| 6 | 0.78 | 0.07 | 8.24 | $4.86x10^{-4}$ |
| 9 | 0.78 | 0.07 | 8.24 | $3.32x10^{-4}$ |
| 12 | 0.78 | 0.07 | 8.24 | $2.43x10^{-4}$ |
| 16 | 0.77 | 0.08 | 9.41 | $2.08x10^{-4}$ |
| 19 | 0.77 | 0.08 | 9.41 | $1.75x10^{-4}$ |
| 21 | 0.74 | 0.11 | 12.90 | $2.18x10^{-4}$ |

Table 5: The rate of degradation of *Bacillus subtilis*, percentage of diesel oil degraded and weight of diesel oil degraded.

| Days | Weight of diesel extracted (g) | Weight of diesel degraded | % of degradation | Rate of degradation (g/h) |
|---|---|---|---|---|
| 0 | 0.85 | 0.00 | 0.00 | 0.00 |
| 3 | 0.85 | 0.00 | 0.00 | 0.00 |
| 6 | 0.84 | 0.01 | 1.18 | $6.0 x10^{-5}$ |
| 9 | 0.84 | 0.01 | 1.18 | $4.62 x10^{-5}$ |
| 12 | 0.80 | 0.05 | 5.88 | $1.74 x10^{-4}$ |
| 16 | 0.79 | 0.06 | 7.06 | $1.56 x10^{-4}$ |
| 19 | 0.79 | 0.06 | 7.06 | $1.31 x10^{-4}$ |
| 21 | 0.76 | 0.09 | 10.59 | $1.78 x10^{-4}$ |

*Penicillium sp* (mold) has a higher percentage of degradability compare to *Bacillus subtilis* (bacteria) which is in line with some earlier report that hydrocarbon in the evnviroment are biodegraded primarly by yeast, fungi, and bacteria, of which the efficiency of biodegradation ranges from 6% to 87% for soil fungi, 0.13% to 85% (Jones *et al*., 1970; Pinholt *et al*., 1979) for soil bacteria and 0.003% to 100% (Hill and Moxy *et al* 1980; Mulkin *et al* 1974) for marine bacteria.

## 4. CONCLUSION

This study revealed that *Penicillium sp*. degrade diesel polluted soil than *Bacillus subtilis*. The rate of degradation activities is more in neutral soil compared to acidic soil. This study has revealed that both *Penicillium sp* and *Bacillus subtilis* (bacterium) should be encouraged for proper cleanup of diesel polluted soil so as to reduce the effect associated with diesel polluted soil.

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