

## Biodegradability of Diesel Using Specific Oxygen Uptake Rate

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### ABSTRACT

Pollution of the soil with petroleum products is a global challenge and this study measures the effect of surfactant addition on compost sample spiked with diesel oil to understand the degradability of diesel. The relationship between oxygen uptake and microbial activity on the biodegradation of diesel were studied. The compost was spiked with diesel oil at different concentrations of 0.05, 1.0 and 2.0g oil /8g of compost based on Nigerian intervention value of 5000mg oil/kg soil. Surfactant was added at a fixed volume of 0.2ml to the spiked compost samples. A respirometric technique known as specific oxygen uptake rate (SOUR) was employed to measure the changing oxygen concentration on aqueous suspension of spiked compost sample by the use of dissolved oxygen probes. The SOUR test showed that the increase in dissolved oxygen concentration reduced the oxygen consumption due to the presence of diesel. The amount of oxygen consumed during microbial activities was computed and the rate of dissolved oxygen concentration was highest (12.5mg O<sub>2</sub>/l) in 2g oil and lowest (8.06mg O<sub>2</sub>/l) in 0.05g oil.

**Keywords**-Biodegradation, diesel, surfactant, SOUR, dissolved oxygen, compost.

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### I. INTRODUCTION

The 21<sup>st</sup> century has witnessed transformation in soil bioremediation techniques. The discovery of oil, its exploration and exploitation have negatively affected the environment in diverse ways; for example the fertility of the soil is affected such that farmers grow crops with the addition of inorganic fertilizers to improve yield. Oil spillages have now become a common scenario in oil producing countries of the world including Nigeria. This phenomenon has greatly generated concern in developing and industrialized nations with a view to reclaiming back the lands for agricultural and other purposes. Bioremediation has been practiced for long in many countries because of its efficiency, cost effectiveness and its environmental friendliness [1, 2]. A lot of factors contribute to the increasing rate of oil spills. Firstly, equipment failure, takes the form of corroded and ruptured pipelines, valve and hose failure as well as lack of proper maintenance of the pipelines. Secondly, wilful sabotage of oil installations contributes to the increasing rate of oil spillage; this takes the form of illegal oil bunkering [3]. The consequences of oil spill included but not limited to ecological damages, economic and aesthetic loss. For example, oil spills cause damages to plants, it settles on beaches and kills marine animals like fishes, crabs and other crustaceans. Oil spills also poison algae, disrupts major food chains and decreases the yield of edible crustaceans. The effect of oil spill on the economy of the people living in the coastal areas cannot be overemphasized as they are mostly fishermen and farmers.

The use of diesel oil is becoming increasingly popular. It is used mostly in operating machines during production processes and in trucks for transportation of goods from one point to the other. The advancement in technology and interdependence of different economies has also given rise to the demand on goods and services and ultimately an increase in fuel (diesel) usage.

Bioremediation is fast becoming an attractive technology in treating lands contaminated by oil spills because of the numerous advantages it offers over the conventional technology, especially being a low cost technology [4]. Despite the advantages offered by bioremediation, it has its associated disadvantages such as lack of short term results. Specific oxygen uptake rate (SOUR) technique was initially adapted from the biological oxygen demand (BOD) bottle method of measuring oxygen uptake [5]. The technique involves a respirometric method based on oxygen consumption by microorganisms degrading a liquid suspension of the organic matter [6]. The SOUR test uses dissolved oxygen (DO) probe to measure changes in the oxygen

concentration of an aqueous compost suspension. The main advantage of this method is equipment availability and it allows the use of an aqueous sample suspension, thereby eliminating the potential for rate limitations due to lack of moisture [7]. According to Lasardi and Stentiford [6], the method offers a maximum reaction rates due to direct contact between microorganisms, substrate and oxygen and also the gas-liquid barrier for oxygen diffusion at the surface of the compost particles is omitted.

The aim of this study was to determine the biodegradability of diesel in the aqueous suspension of compost. The key objectives are to determine the relationship between oxygen uptake rate and microbial activity in degrading diesel oil and to determine the effect of the use of surfactants on the biodegradation of diesel.

## **II. MATERIALS AND METHODS**

The materials used for this research include diesel oil, horse manure and decomatic surfactant (biodegradable and non-foaming). The diesel was obtained from a petrol station near The University of Leeds and the surfactant collected from public health research laboratory, University of Leeds. The compost sample collected from a local farm in Leeds was two (2) year old matured composted horse manure. The horse manure was obtained from a farmyard in Northern England. The manure was passed through a 5mm sieve to remove stones and unwanted materials.

### **2.1 Preparation of Nutrient Solution**

The need for addition of various inorganic nutrient solutions was based on the assumption that the sample may have shortage of micro-nutrients essential for microbial activity and consequently limit respiration rate [8]. These nutrient solutions were used as source of inorganic nutrients to avoid nutrient limitation [9]. The following procedures were used to prepare the various inorganic nutrients.

#### **Magnesium sulphate**

22.5g of magnesium sulphate heptahydrate ( $MgSO_4 \cdot 7H_2O$ ) was dissolved in 150 ml of distilled water, and made up to 1000 ml in a standard flask.

#### **Ferric chloride**

0.257g of Iron (II) sulphate heptahydrate ( $FeSO_4 \cdot 7H_2O$ ), was dissolved in 150 ml of distilled water and made up to 1000 ml. This gradually becomes brown over some few minutes.

#### **Calcium chloride**

27.5g of calcium chloride anhydrous ( $CaCl_2$ ) or 36.43g of calcium chloride dehydrate ( $CaCl_2 \cdot 2H_2O$ ) was dissolved in 150 ml of distilled water and made up to 1000 ml.

#### **Phosphate buffer**

8.5g of potassium dihydrogen orthophosphate was dissolved in 150 ml distilled water. 21.75g of dipotassium hydrogen orthophosphate was dissolved in 150 ml distilled water and then added to the same flask. In addition, 33.4g disodium hydrogen orthophosphate dehydrate ( $Na_2HPO_4 \cdot 2H_2O$ ) was dissolved in 150 ml of distilled water and added to the same flask. The solution was made up to 1000 ml.

### **2.2 Treatments**

8.0g compost was mixed with varying weights of the diesel with the addition of surfactant at fixed volume (0.2ml) in some of the samples depending on the experimental conditions (Table 1). 750ml of distilled water was then measured out. The compost was put in the liquidiser with about two thirds of the water. 5ml each of  $CaCl_2$ ,  $FeCl_3$  and  $MgSO_4$  nutrient solution were added to ensure that nutrients are not rate limiting. This was then blended for 30 seconds, and the suspension was put into a one-litre Kilner jar. The remaining water was used to rinse the remaining solids left in the blender.

All the experimental conditions (Figure 1) were set up in two replicates and monitoring was undertaken for 72 hrs. Compost only solution was used as the control. Details of all the samples used are shown in Table 1.

Table 1. Experimental design

Treatment	Details of treatment
A	Compost + diesel oil (1.0g w/w)
B	Compost + diesel oil (2.0g w/w)
C	Compost + diesel oil (0.05g w/w)
D	Compost + diesel oil (1.0g w/w) + 0.2ml surfactant
E	Compost + diesel oil (2.0g w/w) + 0.2ml surfactant
F	Compost + diesel oil (0.05g w/w) + 0.2ml surfactant



Figure 1: The different compost samples used for SOUR test

### III. RESULTS AND DISCUSSION

#### 3.1 Dissolved oxygen (DO) concentration

The rate of dissolved oxygen concentrations in the various samples were quantitatively measured using dissolve oxygen probes and the plots of dissolved oxygen against time were shown in Figures 2 to 4. The highest dissolved oxygen concentration of 12.5 mg O<sub>2</sub>/l was observed in the sample with compost spiked at 2.0g weight of diesel oil (Figure 2), whereas a minimum of 8.06 mg O<sub>2</sub>/l was observed in the sample spiked with 0.05 g of diesel oil. The dissolved oxygen concentration of samples A and B (Figure 2) increased remarkably at the early stage within the first one hour reaching a maximum of 12.5 and 9.47 mg O<sub>2</sub>/l respectively. In sample C, no significant increase in dissolved oxygen concentration (8.06 mg O<sub>2</sub>/l) was observed. The dip observed in sample A could be due to aeration problem or mechanical fault from the stirrer because dissolved oxygen probes consumed dissolved oxygen even when the stirrer has stopped working [8].

The initial rapid increase observed could be as a result of microbial population trying to acclimatize to new environmental condition. This is in agreement with the findings of Lasardi and Stentiford [6] as low level of dissolved oxygen concentration indicates utilization of oxygen by microorganisms to oxidize the biodegradable material in the sample. The high concentration of diesel oil hindered microbial activity in degrading the readily bio-available fraction of the oil which led to high concentration of dissolved oxygen (low oxygen utilized). Similarly, Leahy and Colwell [10] reported that high concentration of hydrocarbons inhibited biodegradation due to the toxic effect exerted by volatile fraction of crude oil.

To determine the effect of diesel oil addition, a different experiment was carried out with compost only as control (Figure 3), to differentiate the quantity of oxygen consumed from compost spiked with and without diesel oil. After a sharp decrease was observed in the control sample at the onset of the experiment, dissolved oxygen concentration returned to almost its saturation level shortly afterwards. The initial rapid decrease might be as a result of increase in temperature, as the aqueous compost sample was incubated in a water bath at 30°C from the initial exposure of a typical soil temperature of about 15°C. Secondly, the addition of nutrient solutions could have resulted in rapid consumption of oxygen as the compost is likely to be deficient of nutrients due to the age of the compost.

The level of DO in compost only later decreased linearly with time from 6.85 to 6.40 mg O<sub>2</sub>/l due to oxygen demand in the compost in breaking down the available organic and lignin woody material. When compared with samples A, B and C which were spiked with diesel at a concentration of 1g, 2g and 0.05g respectively, an increase in dissolved oxygen concentration was observed. The increase in dissolved oxygen concentration indicated low oxygen consumption due to the presence of diesel oil, which perhaps hindered proper aeration and transfer of oxygen gas in the aqueous samples thereby affecting microbial activity in degrading the carbon content of the diesel oil.

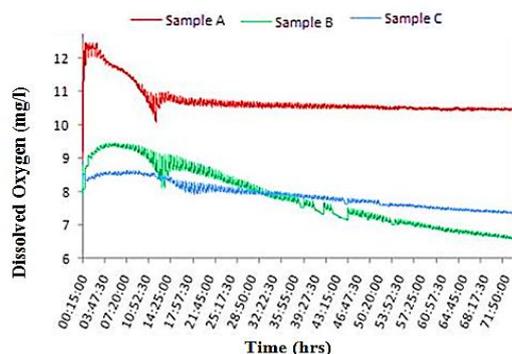


Figure 2: Dissolved oxygen concentration on spiked compost

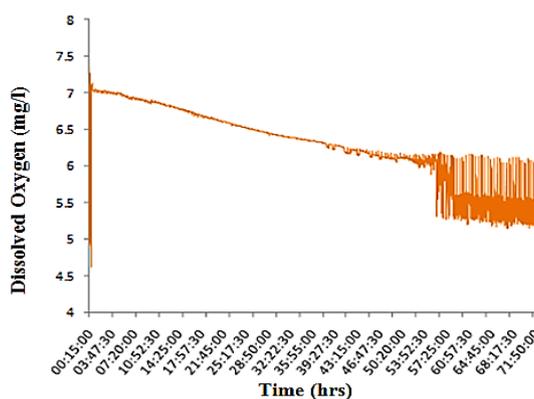


Figure 3: Dissolved oxygen concentration on compost (control).

### 3.2 Effects of surfactant

Surfactant addition was conducted to assess the effects on the biodegradation of the diesel oil. Figure 4 shows the dissolved oxygen rate on addition of surfactant to the spiked compost. The peak DO concentration in samples D, E and F were 12.05 mg O<sub>2</sub>/l, 13.50 mg O<sub>2</sub>/l and 8.06 mg O<sub>2</sub>/l respectively. The effect of surfactant addition was determined by comparing compost samples spiked with diesel at different concentrations (0.05g, 1.0g and 2.0g) without and with surfactant (Figures 2 and 4). The oxygen consumption rates in the samples without surfactant were similar when compared to the samples with surfactant at same diesel oil concentration except at 2g oil. This indicates that surfactant addition did not have much effect on the degradation of the diesel oil, which may be due to its toxicity to microorganisms and probably due to its high pH [11]. It is also possible that the surfactant added brought about substrate inhibition and oil was not used as carbon source by the microorganisms. The use of surfactants for bioremediation of contaminated sites could produce either an enhanced or inhibitory effects [2]. In the case of sample E, a significant DO rate was also observed and the trend was the same as that of sample D, but the rate of DO was slightly lower than that of sample D. The results indicate that sample D with 1g of oil has less toxic effects on the microorganism than sample E (2g oil). Both samples D and E show less biodegradability than sample F (0.05g oil) as it recorded the least DO (8.06 mg O<sub>2</sub>/l), an evidence of increased oxygen consumption signifying increased microbial activities. In general, high DO as observed in samples with high concentrations of diesel, suggests more toxicity on the microorganisms and is reflected in a reduced oxygen uptake by the microorganisms.

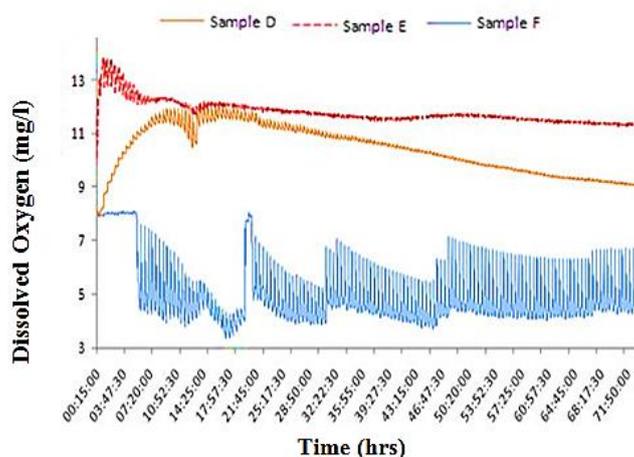


Figure 4: Dissolved oxygen concentration on spiked compost with addition of surfactant (0.2ml)

#### IV. CONCLUSION

Degradation of oil was highest in the samples spiked at different concentration of diesel oil without surfactant and lowest in the corresponding samples with surfactant. Thus, the addition of surfactant inhibited the rate of oxygen consumption by the microorganisms and limited the rate of biodegradation of the diesel oil. A longer experimental time will be beneficial to the determination of actual degradation of the oil.

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