ISOLATION AND IDENTIFICATION OF DIESEL OIL-DEGRADING BACTERIA FROM ABATTOIR EFFLUENTS IN GWAGWALADA AREA COUNCIL, ABUJA, NIGERIA

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Received: December 22, 2019 **Accepted:** February 17, 2020

Abstract: The microbial population and hydrocarbon utilizing microorganisms from Gwagwalada abattoir effluents within Abuja metropolis was investigated. This was carried out by the isolation of bacteria from abattoir effluents using the pour plate method. The heterotrophic bacteria isolated include *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus*. Hydrocarbon utilizing bacteria identified belong to the genera *Pseudomonas, Bacillus, Escherichia,* and *Staphylococcus* but *Escherichia* and *Staphylococcus* were the most occurring bacteria while *Pseudomonas* and *Bacillus* species were the least occurring bacteria. The residual diesel oil recovered in the mineral salt medium containing *Pseudomonas aeruginosa, Bacillus subtilis*, *Escherichia coli,* and *Staphylococcus aureus* after 16 days of biodegradation was 0.03, 0.05, 0.08 and 0.08 ml per 10 ml of mineral salt medium, respectively from initial corresponding concentrations of 0.1 of diesel oil. The presence of bacteria which are indicators of faecal contamination as observed in this study are pointers to the dangers associated with the discharge of untreated abattoir wastes and effluent into the soil and water bodies. On the other hand, the abattoir effluent is rich in hydrocarbon utilizing microbes that can be harnessed for the clean-up of hydrocarbon contaminated soils.

Keywords: Diesel, oil-degrading bacteria, Abattoir effluents

Introduction

The abattoir is a specialized facility approved and registered by the regulatory authority for inspection of animals, hygienic slaughtering, processing and effective preservation and storage of meat products for human consumption (Atlas, 2011). Most abattoirs in Nigeria have no facilities for waste treatment; wastes are either disposed of in open dumps or are discharged into nearby streams, hence constituting an environmental menace (Das and Chandran, 2011). Waste water or effluent generated from the abattoir is characterized by the presence of a high concentration of whole blood of slaughtered food animals and suspended particles of semi-digested and undigested feeds within the stomach and intestine of slaughtered and dressed food animals (Atunaya, 2007).

In addition, there may also be the presence of pathogenic microorganisms, such as *Salmonella, Escherichia coli* (including serotype O157:H7), *Shigella*, parasite eggs and amoebic cysts which are of public health importance. Recent studies have shown that zoonoses from abattoir wastes are yet to be fully controlled in more than 80% of public abattoirs in Nigeria (Marquez-Rocha *et al.,* 2001). Also, several pathogenic bacteria and fungi species have been isolated from abattoir wastewater and surface water; including *Staphylococcus, Escherichia coli, Streptococcus, Salmonella, Aspergillus, Mucor, Saccharomyces spp and Penicillium spp* (Bull *et al*., 1982).

In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sights (Pitt and Skerman, 1992). The environment is a very important and necessary component for the existence of both man and other biotic organisms. The past two decades have witnessed a heightened concern over environmental degradation from pollution and depletion of natural resources. Organic and inorganic substances have been released into the environment as a result of domestic, agricultural and industrial activities (Mouchet, 1996; Lim *et al.,* 2010). The meat processing industry produces large volumes of slaughterhouse wastewater (SWW) due to the slaughtering of animals and cleaning of the slaughterhouse facilities and meat processing plants (MPPs) (Padilla-Gasca *et al*., 2011; Bustillo-Lecompte and Mehrvar, 2015).

In Nigeria, the abattoir industry is an important component of the livestock industry providing domestic meat supply to over 150 million people and employment opportunities for teaming population (Nafarnda *et al*., 2011). They are usually situated near aquatic environment where water from different untreated waste streams are discharged (Adelegan, 2002; Sangodoyin *et al*., 1992) and constitute public health concern to authorities. The impact of wastewater effluents on the quality of receiving water bodies are manifold and depend on volume of the discharge, chemical and microbiological concentration/composition of the effluents.

Slaughterhouse wastewaters (SWWs) contain high amounts of biodegradable organic matter, suspended and colloidal matter such as fats, proteins and cellulose (Nunez and Martinez, 1999; Caixeta *et al*., 2002). Biodegradable organic matter in receiving waters create high competition for oxygen within the ecosystem leading to high levels of biochemical oxygen demand (BOD) and a reduction in dissolved oxygen, which is detrimental to aquatic life. Nutrients (nitrogen and phosphorus) enrichment in receiving sensitive bodies of water can cause eutrophication by stimulating the growth of algae (called an algal bloom). Blooming and finally collapse of algae may lead to hypoxia/anoxia and hence mass mortality of benthic invertebrates and fish over large areas (Wu, 1999; Foroughi *et al*., 2010) due to aquatic dissolved oxygen depletion.

Among several clean-up techniques available to remove diesel oil pollutant from the soil, biodegradation by intrinsic populations of microorganisms is one of the primary mechanisms by which petroleum hydrocarbon pollutant can be removed from the environment since it is cheaper than other remediation technologies. These processes rely on the innate ability of microorganisms to mineralize hydrocarbons, leading to formation of CO_2, H_2O and cell biomass (Alexander, 1994; Das and Chandran, 2011).

Of all strategies to accelerate the biological breakdown of hydrocarbons in soil, biostimulating the intrinsic microorganisms by addition of nutrients is the most frequently used bioremediation technique as the introduction of

enormous amount of carbon sources as contaminant tends to result in quick reduction or depletion of the available nitrogen and phosphorus which are essential for microbial growth. The use of organic manures such as cow dung, poultry droppings, household refuse and effluents for crop production has been a usual practice amongst the subsistence farmers in West Africa since the prospect of obtaining adequate chemical fertilizers to meet the requirement of the large farming population is challenging.

Osemwota (2010) revealed that abattoir effluent increased the pH and available phosphorus of the soil. The increase in pH value coupled with that of the available phosphorus could help in stimulating bacterial growth since most aerobic bacteria involved in hydrocarbon degradation grow best at pH range of near neutral to slightly alkaline. This study therefore aims at assessing the diesel oil degradation potential of different bacteria isolated from abattoir effluent and how these bacteria can be used to reduce environmental pollution caused by oil spillage or its likes.

Materials and Methods

Sample collection

The abattoir effluent samples were collected from the abattoir located in Gwagwalada, (Gwagwalada Area Council) using sample bottles. The sample bottles were used to aseptically draw part of the wastewater running off the drainage system just as it was leaving the slaughter pavements and also at three other different spots; where the animal is being slaughtered, washed and dressed. Sample bottles were placed on ice during transport to the laboratory for analyses immediately after collection. The diesel oil used was purchased from a petrol filling station in Giri (Gwagwalada Area Council).

Culture media preparation

Culture media used were Nutrient agar (NA), Mannitol salt agar and Eosin methylene blue (EMB) agar and were all prepared according to the manufacturer's specification and sterilized by autoclaving for 15 min at 121°C.

Microbiological analysis

This was carried out according to the method described by Cheesbrough (2006). The samples collected at different spots were aseptically mixed together. Ten-fold serial dilutions of sample were aseptically carried out up to 10^9 dilution. 1 ml of the 10^{-3}, 10^{-5} and 10^{-7} dilutions of the samples were aseptically introduced into different plates in duplicates after which sterile prepared Nutrient agar was introduced using the pour plate technique. The plates were incubated at 37°C for 24 h after which they were examined for growth.

Representative colonies of bacteria were picked from different plates after the incubation period. Pure cultures of bacteria were obtained by streaking representative colonies of different morphological types on to freshly prepared Nutrient agar plates. The agar plates were further incubated at 37°C for 24 h. Discrete bacterial colonies, which developed on the plates, was observed and further cultured on differential and selective media (MacConkey agar, Mannitol salt agar, Cetrimide agar and Eosin methylene blue agar) used for subsequent characterization tests.

Identification of bacterial isolates

This was done on the basis of cultural appearance of colony, morphology, differential and selective media and by conventional biochemical tests (Cheesbrough, 2005). Gram staining and conventional biochemical test such as catalase, motility, indole, citrate utilization, coagulase, oxidase and starch hydrolysis tests were carried out. Bacterial isolates were identified in accordance with the schemes of the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Starch hydrolysis test

This test was used to identify bacteria that can hydrolyze starch using the enzyme α-amylase and oligo-1,6-glucosidase and was carried out according to the method of Akponah (2011). Nutrient agar was prepared according to the manufacturer directives; the agar was boiled to dissolve completely. Soluble starch was dissolved in a small amount of water and was heated slowly with constant stirring to form suspension. The agar was allowed to cool and then mixed with the nutrient agar solution and then sterilized at 121°C for 15 min. The prepared starch agar was allowed to cool and dispensed into petri dish and labeled. A single streak inoculation of test organism to be tested was made in the centre of the labeled plate. The plate was incubated for 48 h at 37^{0}C. The surface of the plate was flooded after 48 h of incubation with iodine solution using a dropper for 30 seconds. Excess iodine was decanted. Clear zones around the path of bacteria growth were observed.

Inoculation of diesel oil with bacterial isolates

A medium of Zajic and Suplisson (1972) consisting of diesel oil and various inorganic salts (KH_2PO_4 1.2 g/L, K_2HPO_4 1.8 g/L, NH_4Cl 4.0 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $FeSO_4.7H_2O$ 0.01 g/L, NaCl 0.1 g/L) were dissolved in 1000 mL of distilled water, sterilized and employed as the substrate for the bacterial isolates. 2 mL of 3 h broth culture (peptone broth) of the isolates were seeded into five test tubes containing the modified medium (mineral salt) and diesel oil while another five test tubes containing the medium (mineral salt and diesel oil) without the bacteria served as the control and then incubated at 37°C for 16 days and records of optical density was taken on four days interval starting with day zero. All the experiments were carried out in duplicates.

Determination of turbidity during biodegradation

This was carried out using a spectrophotometer (JENWAY 6400). The sample cell was rinsed with sample, and then filled with the sample, carefully placed in the spectrophotometer. Readings of the absorbance were taken at 520 nm.

Determination of temperature during biodegradation

Temperature of the degrading sample was determined with the bulb of mercury-in-glass laboratory thermometer. The initial temperature was taking on day zero and at interval of four days. This was done by dipping the bulb of mercury-in-glass laboratory thermometer into the degrading sample and the reading taken.

Determination of total viable count

Total viable count of the inoculated organism was carried out using Nutrient agar. At four days interval, the diesel oil + organism sample + medium were serially diluted in 10 folds (plated that number of organisms). Two dilution factors of each organism were plated on the Nutrient agar, each in duplicate and incubated at 37°C for 24 h. After incubation, the total viable count was determined.

Statistical analysis

Descriptive statistics and analysis of variance (ANOVA) was performed using procedure of SPSS version 16. Experimental precision achieved was reported at 5% probability level.

Results and Discussion

The organisms capable of diesel degradation isolated from abattoir effluent in this study were *Pseudomonas aeuruginosa* and *Bacillus subtilis* (Table 1). This is in line with the previous report by Osemwota (2010). Previous work reported the presence of these bacteria in palm oil mill effluent, Kitchen effluent, animal dung and poultry droppings and the degrading potential of these bacteria (Okwute *et al.,* 2016). Other isolates include *Escherichia coli and Staphylococcus aureus* which have little degrading potential.

Table 1: Biochemical characteristics of bacteria isolated from abattoir wastes

Organism	Catalase test	Coagulase test	Citrate test	Oxidase test	Indole test	Methyl red	V-P test	Starch hydrolysis
Pseudomonas aeruginosa	+	-	+	+	-	-	-	-
Bacillus subtilis	+	-	+	+	-	+	+	+
Escherichia coli	+	-	-	-	+	+	-	-
Staphylococcus aureus	+	+	-	-	-	+	+	+

Figure 1 showed the results of the total viable count of the bacterial isolates during the biodegradation of diesel. It revealed the highest counts for *Pseudomonas aeruginosa* ($8.33 \times 10^8 \pm 0.9$ cfu/ml) and *Bacillus subtilis* ($6.60 \times 10^8 \pm 0.7$ cfu/ml) followed by *Staphylococcus aureus* (3.50×10^8 cfu/ml) and *Escherichia coli* (3.00×10^8 cfu/ml) colonies occurred on the 12th day. The initial decrease in bacterial count observed at day 8th in this study may be due to the toxic effect of diesel oil. This agrees with the report of Umanu and Owoseni (2013). There were no significant differences in the viable counts at 0.05 probability level.

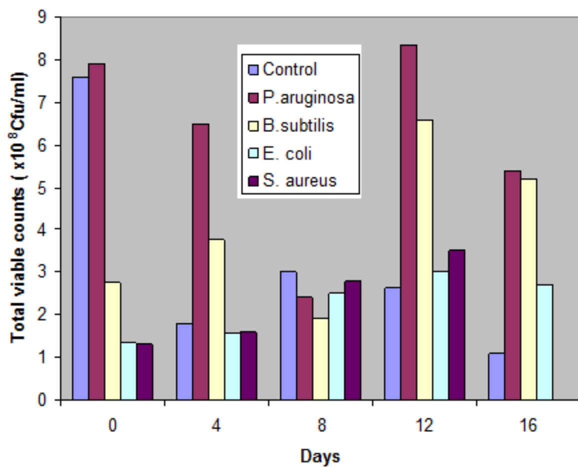

Fig. 1: Total viable counts of bacteria degrading diesel

Figure 2 shows the optical density/turbidity of the suspended bacteria in mineral salt medium containing diesel. There was a continuous increase in optical density from day 0. *Pseudomonas aeruginosa* was more turbid compared to the other bacteria followed by *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. The degree of turbidity during biodegradation of diesel corresponded with increase in hydrocarbon utilization by *Pseudomonas aeruginosa* and *Bacillus subtilis*. This is in contrast with the findings of Popoola (2006) who reported higher degradation of crude oil by *Bacillus subtilis* compared to *Pseudomonas aeruginosa.*

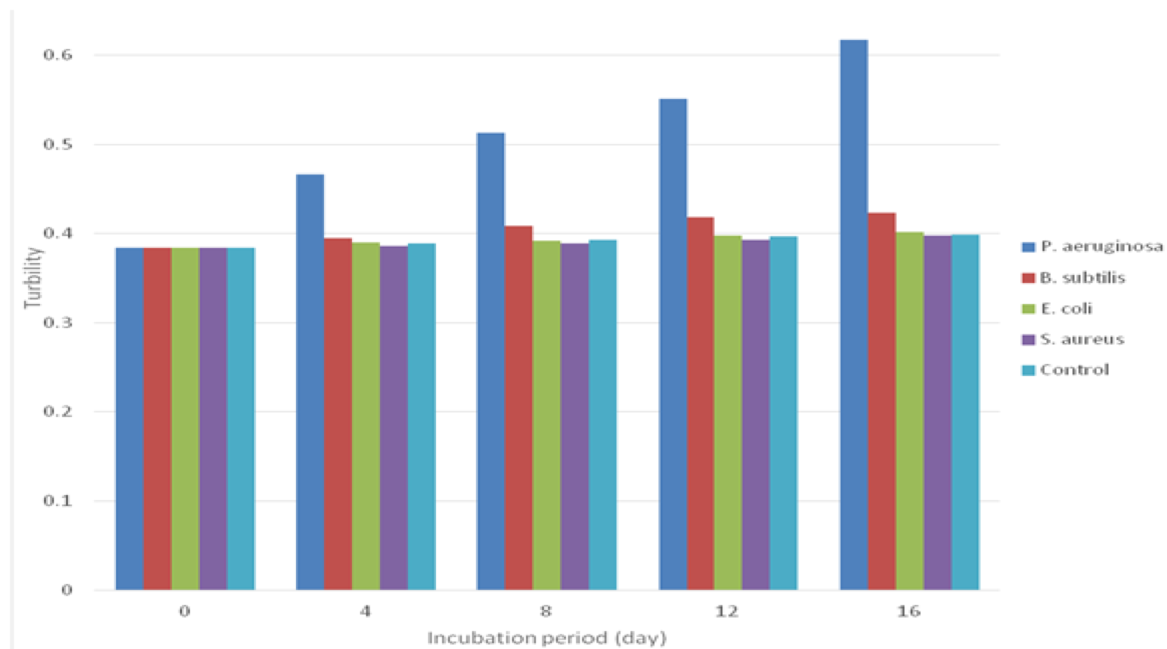

Fig. 2: Turbidity of bacteria isolates during biodegradation of diesel

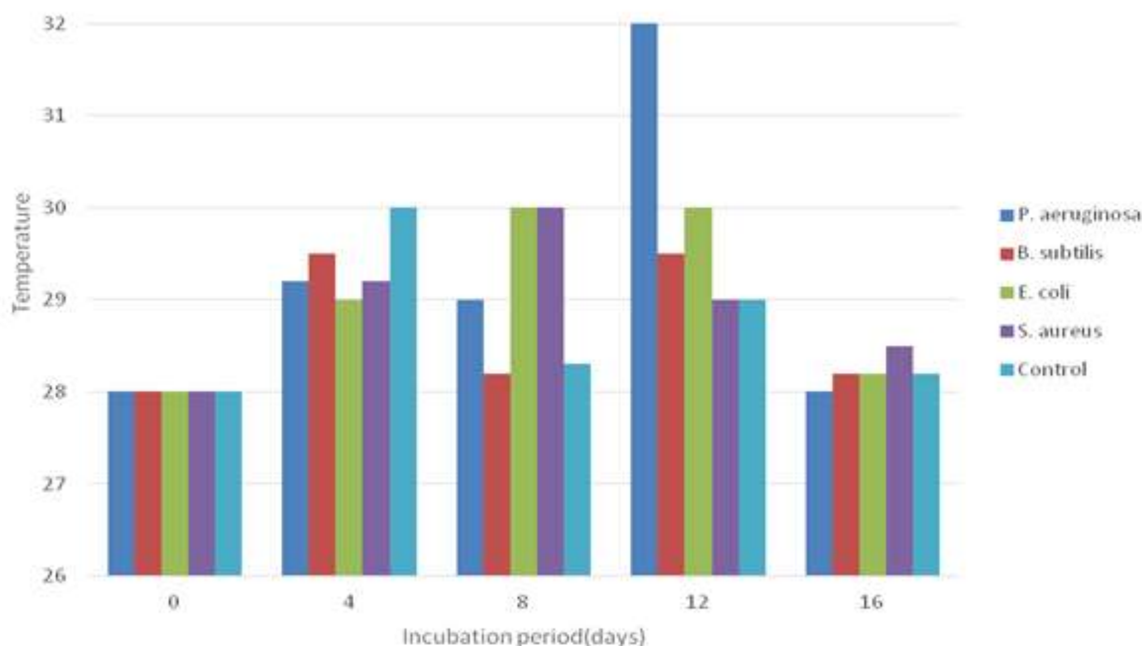

Fig. 3: Temperature of the medium during biodegradation of diesel

Figure 3 shows the effect of temperature on the bacteria isolates during biodegradation of diesel oil. The temperature fluctuations show the varying degrees at which the various bacteria isolates thrived. Temperature increase favours degradation of hydrocarbon (Margesin and Schinner, 1997) and this was observed on day 12. *Pseudomonas aeruginosa* showed more degrading capability when the temperature rose from 29 to 32°C, then *Escherichia coli, Bacillus subtilis*, *and Staphylococcus aureus.* Margesin and Schinner (1997) are of the opinion that hydrocarbon loss increased with incubation time and temperature. However, in this study, increase in temperature had more effect on diesel loss than incubation time. This may be due to the fact that majority of microorganisms thrive better at mesophilic temperatures than at low or very high temperatures.

Conclusion

In conclusion, abattoir effluents contain a measurable amount of hydrocarbon degraders which include *Pseudomonas aeuruginosa, Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus*. Therefore, bioremediation of diesel oil contaminated soil can be influenced using abattoir effluent or through biostimulation of hydrocarbon degraders found in abattoir effluents. In addition, abattoir wastes could serves as source of nutrients (nitrate and phosphate) for hydrocarbon degraders.

Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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