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Assessment of Indigenous Bacteria from Biodiesel Effluents Contaminated Site

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ABSTRACT: This study was conducted in order to identify indigenous microorganisms which have the capability to degrade biodiesel contaminated sites. Bacterial isolates were identified on the basis of morphological and biochemical characterization in which nine bacteria were isolated from the site, *Staphylococcus aureus* and *micrococcus letus* were found to be hydrocarbon degraders during the degradation test. The efficiency of biodegradation capability of isolates was measured by UV spectroscopy for 14 days wavelength of 600nm. The optimal temperatures at which the biodegradation occurred at 30 – 37^{0}C. The result obtained demonstrated the potentials of these isolates in situ and/or ex situ bioremediation.

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Indigenous soil Microorganisms are tiny colonies of life that are found and propagated from the immediate vicinity of the growing location supplies readily available nutrients directly to the soil-rhizosphere system. Microorganisms play an important role in making soil good for growing plants. These microorganisms can also be collected and cultured. Natural Farming promotes the use of Indigenous soil Microorganisms. The microorganisms that have been living in the local area for a long time are best for farming because they are very powerful and effective. They have survived and can survive the extreme climatic conditions of the local environment much better than artificially produced microorganisms (Soma and Sai, 2013).

There are several scientific studies that address biological treatment of soils contaminated by petroleum products (Vieira *et al*., 2009a,b). The search for alternative sources of energy and sustainable processes in order to reduce environmental pollution and global warming has spurred the global market for clean fuels such as biodiesel, which is a renewable and environmentally safe alternative to fossil fuels. Many studies have been planned to either prevent the contamination or clean up the polluted sites after the contamination (Head and Swannell, 1999; Jones, 1998; Margesin and Schinner, 1997; Sayler and Ripp, 2000). Physical, chemical and biological methods can be used for cleaning up the polluted sites. It also shows that microorganisms have broad range of enzymes that enable them to degrade many chemicals (Chen *et al*., 1999; Kanaly and Harayama, 2000; Watanabe, 2001). The application of microorganisms in oil biodegradation has been shown at different environmental conditions (Margesin and Schinner, 2001; Whyte *et al*., 1997). This study is aimed at assessing indigenous microorganisms (bacteria) from soil capable of degrading biodiesel effluent.

MATERIALS AND METHODS

Materials: The Gram's iodine stain, Gram's crystal violet stain, Gram's safranin solution, nutrient broth, ethanol, Methyl Red – Voges Proskauer (MRVP), Nutrient Agar, Nutrient Broth, Potato Dextrose Agar, Potato Dextrose Broth, Hydrogen peroxide, Petri Dishes, Conical flask, Measuring cylinder, wire loop, spirit lamp, test tubes, Autoclave, auger, weighing balance, biodiesel effluents, etc.

Sample collection: Soil samples were collected from a site opposite the Botanic Garden, Faculty of Life Sciences, University of Benin with an auger from the biodiesel effluent contaminated site measuring 5m × 5m at three different depth of 0 – 10cm, 10 – 20cm and 20 – 30cm respectively.

Isolation of bacteria and fungi from soil sample: Bacterial species were isolated from the collected soil samples by serial dilution and agar plating method wherein the soil sample was diluted from 10^{-1} to 10^{-3} dilutions and the diluted soil samples was inoculated into a sterile Petri dish before pouring Nutrient agar plates and Potato Dextrose agar respectively. The

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inoculated plates were incubated at 37°C for 24 hours (bacteria) and at room temperature for 72hours (fungi) and yeast. Mixed cultures obtained after incubation (bacteria) were labelled accordingly and were purified by streaking on sterile nutrient agar plates. The purity of cultures was cross checked by gram staining procedure.

Staining and biochemical activities of purified cultures: In order to identify the purified cultures tentatively on the basis of Bergey's manual various staining and biochemical tests were performed namely Gram staining, Catalase test, Indole test, citrate utilization test, Urease test, Motility test, oxidase test, coagulase test, Glucose fermentation, fructose fermentation, and Lactose fermentation (Aneja, 2003).

Biodegradation and growth studies: Growth and degradation studies over a time course were carried out using biodiesel effluent. In this study, the bacteria, yeast and fungi were inoculated into 10ml of Mineral Salt Medium (MSM) containing 10ml biodiesel effluent and 5ml of the inoculum. While, for control preparation, 10 ml of biodiesel effluent (biodiesel effluent was added into 10ml MSM without inoculum. After that, the culture was incubated at 30°C for 14 days. At 24 hours interval during the incubation, microbial growth in culture tubes was determined spectrophotometrically by measuring absorbance at wavelength 600nm with UV-visible spectrophotometer.

RESULT AND DISCUSSION

The indigenous bacteria isolated from the biodiesel effluent polluted site on the basis of biochemical and morphological characterization (Table 1) were *Staphylococcus aureus*, *Proteus* spp., *Streptococcus* spp., *Escherichia coli*, *Micrococcus letus*, *Clostridium* spp., *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus epididymis*. Akpomie (2013) and Mohammed (2011) isolated similar microorganisms with those isolated in the study from tannery effluent but reported that *Pseudomonas* spp. and *Bacillus* spp. were the major isolates.

All the bacterial isolates grew well within the temperature range (28 – 37°C) indicating they were mesophilic however *Staphylococcus aureus* and *micrococcus letus* were still able to thrive well at 45°C. *Staphylococcus aureus* and *micrococcus letus* had the optimum growth at 28°C.

All the other organisms had the optimum growth at 37°C. The growth may be attributed to the enzymes being stable and optimally metabolically active at 37°C.

Table 1: Total Bacteria count

Sampling period (Month)	Depth of soil (cm)	Total Bacteria count ($\times 10^3$) 37°C for 24hours
1	0 – 10	2.5×10^4
	10 – 20	2.4×10^4
	20 – 30	1.2×10^4
2	0 – 10	6.0×10^4
	10 – 20	6.9×10^4
	20 – 30	2.6×10^4
3	0 – 10	1.25×10^5
	10 – 20	4.0×10^4
	20 – 30	7.0×10^4
4	0 – 10	5.5×10^4
	10 – 20	4.0×10^4
	20 – 30	-

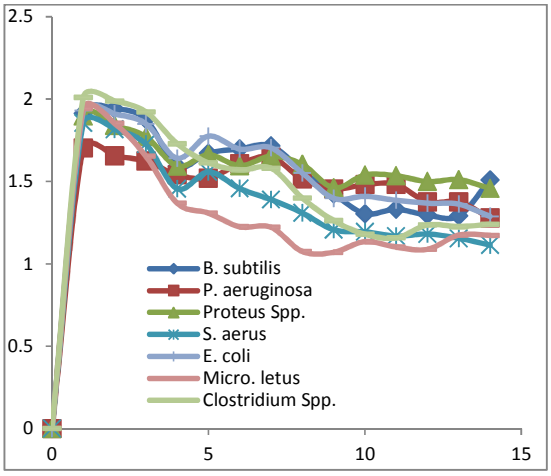

Fig 1: Optical Density @ 600nm of bacteria isolates during biodegradation of biodiesel effluents

The results in Figure 1 showed, the cell density was relatively low on the first, second and third day then increased remarkably from the fourth to the seventh day. In the following three days cell density remains relatively constant and decreased in the ninth day. The degradation rate was only a few on the first day, and increased linearly in the next days. The growth curve indicated that the bacteria were in the adaptation phase on the first day when bacteria could not grow and reproduce immediately. The bacteria would take a period of time to grow in the new culture medium. The second day and the third day was the logarithmic growth phase, when bacterial metabolism was dynamic and synthesis of new cellular material was fast. Bacteria entered into the stationary phase from the fourth day and entered into the death phase from the ninth day. Nine indigenous bacterial were capable of utilizing biodiesel effluents as source of carbon from the contaminated site.

Although *Pseudomonas* and *Acinetobacter* species are the most common bacteria hydrocarbon – degraders reported by Barathi (2001), Bhettacharya *et al*. (2002), Pokethitiyook *et al.* (2003) and Van Hamme *et al*. (2003). *Acinetobacter* spp. are widespread in nature and can remove or degrade a wide range of organic such as phenol, toluene (Zilli *et al*., 2001) and inorganic compounds such as phosphates and metal (Boswell *et al*., 2001). In this study, it was discovered that *Staphylococcus aureus* had the potentials to biodegrade the pollutant (biodiesel effluent) in the environment.

Bioremediation has been widely received by the public. However, a number of factors must be taken into consideration before in situ bioremediation can be applied. These include (i) type and concentration of biodiesel effluent concentration; (ii) prevalent climatic conditions; (iii) type of environment that has been contaminated; and (iv) nutrient content as well as pH of the contaminated site (Rosenberg, 1992).

Conclusion: The hydrocarbon degradation experiment demonstrated in this study showed that *Staphylococcus aureus* is useful to assess the potential for natural attenuation of hydrocarbon contaminated environment. Furthermore, the hydrocarbon degrading microorganism which was isolated from hydrocarbon polluted area was found to be the highest performance microorganisms isolated. This suggested that, these microorganisms have great potentials to be used as hydrocarbon degrading organism in bioremediation of oil contaminated areas.

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