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Short Communication

Anaerobic and aerobic growth of *Alcaligenes eutrophus* in gasoline contaminated soil of Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria

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ABSTRACT

Studies on the anaerobic and aerobic growth of *Alcaligenes eutrophus* bacteria show that the same growth factor suffice for both aerobic and anaerobic growth, thus supporting Mathava and Ligy formulation of photosynthesis and carbon assimilation in these forms, and his thesis that the previously observed need for peptone or yeast extract could be attributed to their content of essential growth factors. A bottle technique for anaerobic cultures and a flask technique for aerobic culture are described in detail. Photosynthesis in *A. eutrophus* bacteria is best observed when they are grown anaerobically. The experiments described here indicate that the culture media had to be modified to conform to certain special requirement imposed by anaerobic growth; the required growth factors remained the same. *A. eutrophus* bacteria display the same growth pattern in both aerobic and anaerobic condition in ethylene substrate while there were some slight changes of its growth pattern in both aerobic and anaerobic condition for xylene and benzene substrate which are aromatic compounds. This study shows that there was higher growth of *A. eutrophus* bacteria in aerobic condition than anaerobic condition. The findings suggest that *A. eutrophus* bacteria is most adapted to conditions present in soils contaminated with gasoline and hence can be exploited in bioremediation activities.

Keywords: Bacteria, photosynthesis, growth factors, growth pattern, hydrocarbons.

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INTRODUCTION

Contamination of soil environment by hydrocarbons (mostly petroleum hydrocarbons) is becoming prevalent across the globe. This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for the environmental health (Minai-Tehrani et al., 2009). The amount of natural crude oil seepage is estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year (Nlianjana and Preethy, 2011; Osuji and Adesiyan, 2005). Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution. These hydrocarbon pollutants usually cause disruptions of natural equilibrium between the living species and their natural environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Das and Chandran, 2011; Chukwuma, 2012).

Aromatic hydrocarbons are common environmental pollutants with toxic, genotoxic, mutagenic and carcinogenic properties (Minai-Tehrani and Herfatnanesh, 2007; Li et al., 2006). They mainly occur in petroleum industry activities. Oil spills because of pipeline breakages, tanks leakages or storage and transportation accidents can be considered as the most frequent causes of hydrocarbon release, included PAHs into soils. BTEX compounds are components of gasoline and aviation fuels that are carcinogenic and neurotoxic to most organisms (Minai-Tehrani et al., 2009).

Bacteria play a major role in hydrocarbon degradation. The reason for petroleum biodegradation is the ability of microorganisms to utilize hydrocarbons to satisfy their cell growth and energy needs. Low molecular weight alkanes are degraded most rapidly whereas mixed cultures carry out more extensive biodegradation of petroleum through pure cultures. Therefore, biodegradation using microorganisms is usually preferred. They play major role in PAHs removal from contaminated environments because of some advantages such as cost effectiveness and more complete cleanup (Abbas and Barack, 2005).

In this study, we determine the anaerobic and aerobic growth of *Alcaligenes eutrophus* bacteria indigenous to gasoline contaminated soil.

The objectives were:

(a) To isolate and identify bacteria associated with utilization of gasoline.
(b) To characterize the bacteria.
(c) To determine the *A. eutrophus* plate counts bacteria population in xylene, benzene and ethylene substrate.

MATERIALS AND METHODS

The sandy soil used in this experiment was obtained from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State, Nigeria. On collection, stones and refuse were separated from the soil sample. Further separation was carried out using 2 mm mesh size to remove the larger non-sandy fractions from the sandy.

The gasoline was purchased from Mobil Oil filling station in Oshodi, Lagos. The distilled water used for dissolving the soil, washing apparatus was obtained in Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.

Hydrogen peroxide (H_2O_2) 3% ($^{w}/_{v}$), a product of Sigma Aldrich, USA, Kovac's reagents, a product of Sigma Aldrich, USA, and gram staining reagents, a product of Sigma Aldrich, USA, were bought from Rochester Silicate Limited chemical store in Mushin, Lagos, Nigeria for biochemical characterization of bacterial isolates (Sumit, 2011; Mathava and Ligy, 2006).

The methanol solvent manufactured by Sigma Aldrich, USA, was used for extraction of gasoline from soil was bought from a chemical store in Lagos, Nigeria. The Nutrient Agar a product of Himedia laboratories pvt Ltd, used for supporting the growth of bacterial was purchased from Richmond Consultants store, Mushin, Lagos, Nigeria.

EXPERIMENTAL DESIGN

Anaerobic

The culture vessels were '250 ml.' Pyrex bottles with interchangeable stoppers. The 10 conical flasks of 250 ml (bioreactors) at room temperature. The air tightened bioreactors were designated N_1-N_{10} (anaerobic). 15 g of gasoline spiked soil were weighed into each of the bioreactor in a solution of Minimal Salt Medium (MSM) 150 ml and mixed properly at room temperature 30 ± 3°C and autoclave at 121°C for 15 min. The bioreactor N_2-N_{10} (anaerobic) were amended with 3 ml substrate, benzene N_2-N_4; xylene N_5-N_7 and ethylene N_8-N_{10} respectively after cooling and pure culture of *Alcaligenes eutrophus* bacteria. The bioreactor under treatment N_1 (control) was not amended with any biostimulating agent and microbes. In total, 10 microcosms were settled and incubated for 42 days. All bioreactors were mixed manually once per week to enhance oxygenation and kept moist during the 42 days experiment duration. The set-up was monitored under anaerobic condition. Samples were withdrawn at intervals of one week for microbial population analysis respectively (Agarry et al., 2012).

Aerobic

The culture vessels were '250 ml.' Pyrex bottles with interchangeable stoppers. The 10 conical flasks of 250 ml (bioreactors) at room temperature serve. The bioreactors slightly closed were designated D_1-D_{10} (Aerobic). 15 gram of gasoline spiked soil were weighed into each of the bioreactor in a solution of Minimal Salt Medium (MSM) 150 ml and mixed properly at room temperature 30 ± 3°C and Autoclave at 121°C for 15 min. The bioreactor D_2-D_{10} (Aerobic) were amended with 3 ml substrate, benzene D_2-D_4; xylene D_5-D_7 and ethylene D_8-D_{10} respectively after cooling and pure culture of *A. eutrophus* bacteria. The bioreactor under treatment D_1 (control) was not amended with any biostimulating agent and microbes. In total, 10 microcosms were settled and incubated for 42 days. All bioreactors were mixed manually once per week to enhance oxygenation and kept moist during the 42 days experiment duration. The set-up was monitored under aerobic condition. Samples were withdrawn at intervals of one week for microbial population analysis respectively (Agarry et al., 2012).

RESULTS AND DISCUSSION

The bacterial plate counts of *A. eutrophus* in the soil samples collected from FIIRO compound are presented in Figures 1 and 2.

Figure 1 shows that *A. eutrophus* plate counts of bacterial population in ethylene substrate show lag phase from week zero to one, exponential phase from week one to four, stationary phase from week four to five and decline phase from week five to six. The *A. eutrophus* plate counts of bacterial population in benzene substrate show lag phase from week zero to one, exponential phase from week one to four, stationary phase from week four to five and decline phase from week five to six. The *A. eutrophus* plate counts of bacterial population in xylene substrate show lag phase from week zero to one, exponential phase from week one to four, stationary phase from week four to five and decline phase from week five to six. The *A. eutrophus* bacteria have the highest bacterial population of 4.3×10^7 CFU/g in xylene substrate for week three.

Figure 2 shows that *A. eutrophus* plate counts of bacterial population in ethylene substrate show lag phase from week zero to one, exponential phase from week one to four, stationary phase from week four to five and decline phase from week five to six. The *A. eutrophus* plate counts of bacterial population in benzene substrate show lag phase from week zero to one, exponential phase from week one to three, decline phase from week three to four, stationary phase from week four to five and

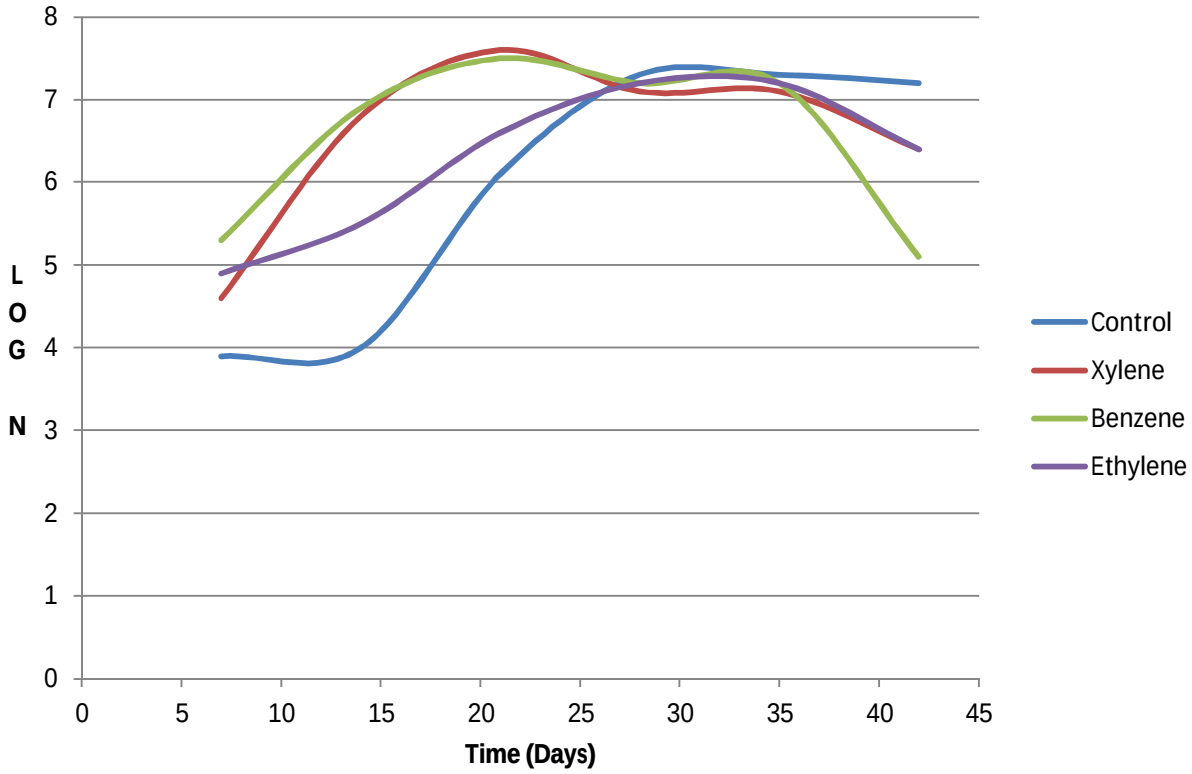

Figure 1. Variation of Log N with time, for *Alcaligenes eutrophus* (Aerobic).

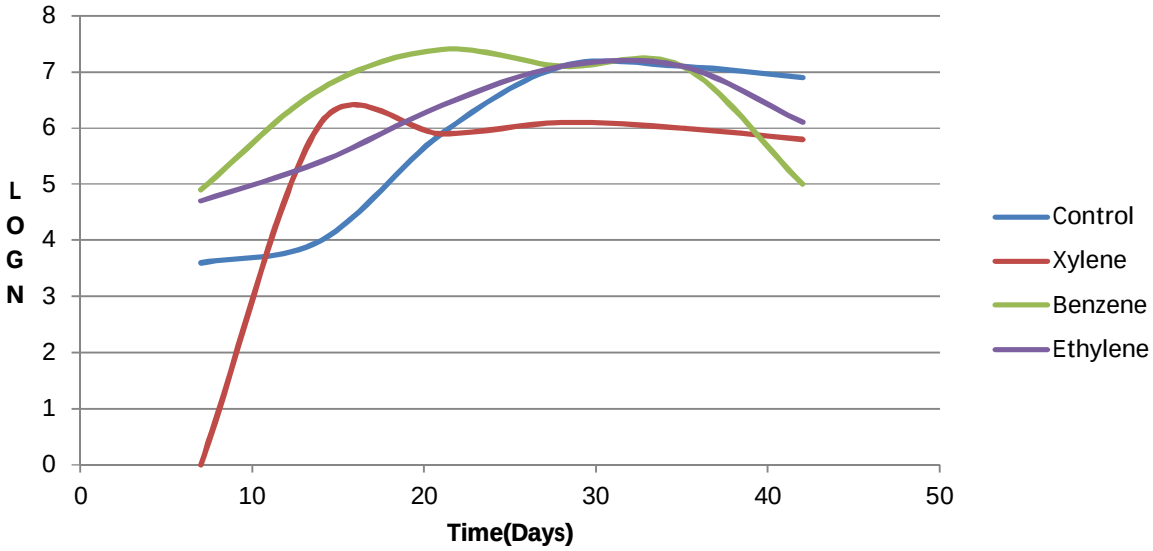

Figure 2. Variation of Log N with time for *Alcaligenes eutrophus* (Anaerobic).

decline phase from week five to six. The *A. eutrophus* plate counts of bacterial population in xylene substrate show lag phase from week zero to one, exponential phase from week one to two, decline phase from week two to three, stationary phase from week three to five and decline phase from week five to six. The *A. eutrophus* bacteria have the highest bacterial population of 1.76×10^7 CFU/g in ethylene substrate for week four.

Soils are considered viable with plate counts of 10^3 to 10^7 CFU/g (Cutright et al., 2015).

The plate counts of *A. eutrophus* bacteria in this study gave viable population in order of 3.6×10^4 to 4.3×10^7

CFU/g.

CONCLUSION

The study focused on anaerobic and aerobic growth of *A. eutrophus* bacteria indigenous to gasoline contaminated soil. This study shows that *A. eutrophus* bacteria indigenous to gasoline contaminated soil utilized xylene, benzene and ethylene substrate. This study also shows that *A. eutrophus* bacteria can be use for bioremediation of aromatic compounds contaminated soil such as benzene and xylene.

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