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# Bioremediation of gasoline contaminated agricultural soil by bioaugmentation

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## HIGHLIGHTS

- Bioaugmentation results in optimum dehydrogenase activity.
- 75.70% degradation can be attained by bioaugmentation with *M.luteus*.
- Microbial consortium results in accelerated degradation rates within two weeks.
- First order kinetic fitted into the present study adequately.

## ARTICLE INFO

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## ABSTRACT

Bioaugmentation can occur either by the use of a single strain microorganism or between different microorganisms (fungi and bacteria). In the present study, we carefully evaluated these two bioaugmentation forms on the degradation of total petroleum hydrocarbon (TPH) in gasoline polluted soil. Microbial activity indexed by dehydrogenase (DHA) assay and effect of pH on biodegradation were also measured. After 8 week incubation, all the studied systems showed varying degree of TPH removal from the gasoline polluted soil. Highest percentage of degradation (75.70%) was observed in *Micrococcus luteus*, 71.10% in *Rhizopus arrhizus* and 66.40% in the consortium though removal efficiencies were not statistically different among the bioaugmentation options but differed significantly with the control at $p < 0.05$. The natural attenuation (control) system had the least TPH removal efficiency (54.04%). The study reported that bioaugmentation involving microbial consortium resulted in initial accelerated biodegradation rates. Fungi bioaugmented systems had low pH condition throughout the experiment. Results of microbial dynamics showed that population density of total heterotrophic microorganisms were higher than actual gasoline degraders and studies using kinetic parameters supported that significant gasoline hydrocarbon removal occurred in the bioaugmentation systems.



## 1. Introduction

Gasoline, popularly referred to as petrol is a transparent, petroleum-derived liquid. It is used primarily as fuel in internal combustion engines. It consists mostly of organic compounds obtained by the fractional distillation of petroleum, enhanced with a variety of additives (Kao and Wang, 2000; Souza et al., 2009; Pinedo et al., 2013). These correspond to small chain

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alkane's carbon six (6) to carbon ten (10) with low boiling points of 60–70 °C. They include; iso-pentane, 2, 3-dimethyl butane, n-butane, n-pentane and volatile aromatic compounds such as mono-aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene. These are collectively referred to as gasoline range organics (GRO). Gasoline utilized in vast number of internal combustion engines used in contemporary transport and industry, has a significant impact on the environment, both in local effects (e.g., smog) and in global effects (e.g., effect on the climate) (Tejada et al., 2008; Almansoory et al., 2015). Just like other hydrocarbon fractions, gasoline enters the environment uncombusted in the following ways; as liquid, as vapors, leakage/accidental discharges during production, transport and delivery as well as from storage tanks (Nwankwegu et al., 2016a; Okonkwo et al., 2016; Yano et al., 2016). Apart from the polynuclear aromatic hydrocarbon (PAHs), gasoline also has been found to be carcinogenic (Swick et al., 2014; Wang et al., 2016a). It is capable of eliciting human diseases such as skin irritations and burns through exposure (Okonkwo et al., 2016). Sequel to these negative effects, there is however, a need for a suitable method of gasoline removal from the environment.

Soil pollution by hydrocarbon and its derivatives has become an unavoidable phenomenon on the environment and efficacy of some traditional clean up/remediation methods like incineration, land farming has been seriously questioned (Lu et al., 2015; Cozzarelli et al., 2014; Campesi et al., 2015; Peng et al., 2016; Nwankwegu et al., 2016a). This is because traditional clean-up methods are usually cost ineffective and eco-hazardous. Apart from this, gasoline is highly inflammable and clean up technologies involving incineration or other traditional approaches often not recommended in polluted environments near residential areas.

Consequently, an eco-friendly and cost effective remediation strategy known as bioremediation has been considered a more reliable alternative in oil hydrocarbon spill response situations. It has been defined by many researchers as the use of living things (microorganisms and plants) to clean up contaminated environments (Nipokoloulou et al., 2013; Chen et al., 2015; Zhang et al., 2015; Kang et al., 2016; Salgado et al., 2016). It utilizes the metabolic potentials of some microorganisms to deal with hydrocarbon contaminants. Bioremediation has been so far practiced using three broad methods notably;
Bioaugmentation (allochthonous microbial strains or genetically engineered microbes)
Biostimulation (adding nutrients or electron acceptor),
Finally remediation by enhanced natural attenuation (i.e. natural ability of the soil's microorganisms).

Furthermore, of these three bioremediation approaches, bioaugmentation has been shown by many researchers to be the most effective (Bento et al., 2005; Hassanshahian et al., 2014; Andreolli et al., 2015). On the other hand, bioremediation has been practiced in various forms; use of microbial consortium (Goux et al., 2013; Ghazali et al., 2004; Yi and Li, 2008; Jian et al., 2012), use of single strain microorganisms (Liming et al., 2014), and use of genetically engineered microorganisms (Rodrigues et al., 2006; Wei et al., 2008; Mrozik and Piotrowska–Seget, 2010; Okafor and Nwankwegu, 2016). Although, appreciable successes have been reported among these, the rationale that no known microorganism has the exceptional ability to completely degrade hydrocarbon pollutants and its intermediates has made bioaugmentation a promising approach. This is because a particular hydrocarbon fraction toxic to one organism and capable of impairing its biodegradative potential might be an utilizable substrate for another organism during the biotransformation phenomena that often accompanied bioremediation.

Presently, there is paucity of scientific information on the bioremediation performance from combined bacterial and fungal consortium. Therefore, the aims of the present study were to evaluate the bioremediation effectiveness of gasoline polluted soil using combined consortium of *Micrococcus luteus* (AJ536198) and *Rhizopus arrhizus* (JN942919), study the degradation performances of individual microbial species as well as model the TPH biodegradation using linear kinetic modeling.

## 2. Materials and methods

### 2.1. Sample collection and microcosm design

Hydrocarbon contaminated soil was collected from a mangrove swamp at Elibrada, Emuoha Local Government of River State, one of the major oil producing areas in Nigeria. Soil was collected into a sterilized plastic container using a hand trowel (Eziuzor and Okpokwasili, 2009; Nwankwegu et al., 2016c). It was transported to the microbiology laboratory of Nnamdi Azikiwe University, Awka, Nigeria. Experiments were set up in four different microcosms after sieving each containing 750 g of dry weight soil placed in set of opened aluminum pans of surface area 625 $cm^2$ and a volume of 2528 $cm^3$ at temperature $30 \pm 1.0$ °C and moisture $15.20 \pm 1.34\%$. The experimental design of the study is provided in Table 1. The properties of the polluted mangrove soil were evaluated (Table 2). To stimulate a condition of major spill, each of the four sample divisions as set up in four different aluminum pans were further polluted with 150 ml of gasoline purchased from a local petrol station in Awka metropolis and then tilled periodically with four different hand trowels to ensure homogeneous pollution (Orji et al., 2012).

### 2.2. Bioaugmentation treatment

The soil sample was bioaugmented with 50 ml of $3.2 \times 10^8$ cells $ml^{-1}$ *Micrococcus luteus* (AJ536198) and this was designated as "Mb system". The second was bioaugmented with *Rhizopus arrhizus* (JN942919) and designated as "Rb