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CRUDE OIL UTILIZATION AND DEGRADATION POTENTIAL OF MICROBES ISOLATED FROM AGED CRUDE OIL POLLUTED SOIL IN NIGER DELTA, NIGERIA

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Abstract

Naturally occurring attenuation process for restoration of crude oil impacted soil is dependent on the crude oil transformation capabilities of indigenous microorganisms that are ubiquitous in the soil. This potential of the microbes can be explored in the design of a remediation strategy to manage crude oil pollution in the soil.

In this study, microbes including bacteria and fungi were isolated from an aged crude oil polluted soil and characterized using morphological and biochemical methods. The potential of the microbial isolates to utilize and degrade crude oil was evaluated in liquid medium using turbidity assay and estimation of total petroleum hydrocarbon (TPH).

Microbial screening revealed the presence of microbial genera including bacteria and fungi in the aged crude oil polluted soil with ability to utilize and degrade crude oil in liquid medium. The probable identity of the indigenous microorganisms ranked in their increasing capacity to utilize and degrade crude oil was: Enterobacter < E. coli < Micrococcus < Aeromonas < Corynebacterium < Bacillus < Pseudomonas (bacteria) and Fusarium < Penicillium < Aspergillus (Fungi).

These microbial isolates especially Pseudomonas, Bacillus, Aspergillus and Penicillium with high oleophilic abilities are effective candidates that can be used as microbial consortium in bioremediation plan for the restoration of the crude oil impacted soil.

Key words: bioremediation, crude oil pollution, indigenous microorganisms, microbial potential, soil attenuation

INTRODUCTION

The crude oil producing area of Nigeria is located in the southern part of the country covering a total area of about 112,000 square kilometers (Sam et al., 2017; Ite et al., 2018). Oil exploration and production activities in this Niger Delta region sometimes involve undesirable discharge of crude oil and associated waste to the surroundings (Zabbey et al., 2017; Sam and Zabbey, 2018). Available reports placed this region to be one of the highest oil - impacted region globally (Sam and Zabbey, 2018) with serious degradation of the environment and negative effect on the ecosystem including impact on human health (Ite et al, 2018;). Although a number of approaches are available to remedy this environmental challenge, the environmentally friendly microbial remediation driven by soil microbes is the focus of this work.

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Crude oil pollution disturbs the dynamics of soil microbial community and stimulates activity of oleophilic microbes with capacity to utilize petroleum hydrocarbons as source of carbon while degrading the pollutant to less toxic forms or intermediates (Chikere et al., 2017). Naturally occurring attenuation process for crude oil impacted soil proceeds slowly with low rate of detoxification (Azubuike et al., 2016; Berchez and Stanciu, 2019; Safdari et al., 2018). The slow removal of the pollution increases the exposure time of the pollutants to the ecosystem with its attendant negative effects and therefore requires intervention.

One of such bioremediation enhancement approach termed bioaugumentation relies on increasing bioremediation efficiency through the introduction of specific crude oil degrading microbes to the system (Varjani, 2017). It is applied in both in situ and ex situ techniques when the indigenous crude oil degrading microbial population is not sufficient (Sabău and Șandor, 2018; Safdari et al., 2018; Siles and Margesin, 2018). The use of microbial consortium in this technology is preferred as it will provide metabolic diversity to achieve degradation of a wider range of crude oil components (Macaulay and Rees, 2014). A major limitation of this intervention is the concern for the survival of the introduced inoculum due to competition with the indigenous microbes (Simarro et al., 2013; Macaulay and Rees, 2014). This limitation can be enhanced by adapting the crude oil degraders in the laboratory before introduction to the site or system (Macaulay and Rees, 2014; Dzionek et al., 2016).

The primary crude oil degrading microbes found in the soil are mainly bacteria and fungi (Safdari et al., 2018).

Bacteria are considered to be very versatile microorganisms with capability to transform oil in any impacted system (Chikere et al., 2011). A major contributory factor to the enhanced crude oil degrading capability of bacteria is their possession of plasmids (Chikere et al., 2011). This increases bacterial ability to adapt and respond to soil pollutant with shorter turnover and higher metabolic actions compared to fungi (Fabian et al., 2017). Some reported important crude oil degrading bacteria genera isolated from soil include: *Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Burkholderia, Collimonas, Corynebacterium, Dietzia, Flavobacterium, Gordonia, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Rhodococcus and Sphingomonas* (Olukunle, 2013; Varjani, 2017).

Fungi have lower metabolic demand for nutrient and wider enzymatic capabilities compared to bacteria (Danger et al., 2016; Fabian et al., 2017). Filamentous fungi in the soil survive extreme conditions better than bacteria through their extensive mycelia network and action of extracellular enzymes to make contact with the contaminant (Godoy et al., 2016). They are more

active in initiating degradation of oil to yield activated metabolites that are further metabolized by both fungal and bacterial action (Harms et al., 2011). Some bacteria however are also known to be mobile and able to move towards contaminant by chemotactic process (Furuno et al., 2009). *Aspergillus, Candida, Cunninghamella, Fusarium, Penicillium, Trichoderma* are some oleophilic fungi genera isolated from impacted-soil in Niger Delta region (Ugochukwu et al., 2008; Chikere et al., 2011; Olukunle, 2013; Ejechi and Ozochi, 2015).

An innovative approach to bioremediation is the site-specific consideration of factors including the identities and capacities of microbial population that can be manipulated to enhance the process of soil restoration (Tibbett et al., 2011; Safdari et al., 2018). Hence, as a preliminary step in the design of an enhanced bioremediation plan, this study is aimed at evaluating the crude oil utilization and degradation potential of microbes isolated from aged crude oil polluted soil in Niger Delta of Nigeria. This will provide a basis for ranking and selection of isolates to be used as a consortium in bioaugumentation process for the impacted site remediation.

MATERIAL AND METHOD

Collection of soil samples

Crude oil contaminated soil sample was collected from an industrial area in Koko situated in Warri North Local Government Area of Delta State, Nigeria on coordinate N: 5^{o}59'53.7036", and E: 5^{o}27'53.8992". A number of oil related companies operate in this community. Uncontaminated soil sample was also collected here from a area without any history of crude oil pollution.

The composite soil samples collected from 0 – 50 cm depth were aggregated in sterile polythene bags and taken to the laboratory. Preliminary field screening of the sampling site was done prior to sample collection by visual screening and oil sheen test following EPA 589/05 guideline (Environmental Protection Authority of South Australia – EPA SA, 2005). The physical appearance of the site was observed while some quantity of the soil was taken in a silver plate and water was added to cover it; shaken and observed for oil sheen formation.

Isolation, characterization and identification of microorganisms

Isolation of bacteria and fungi from the contaminated soil was done according to method of Olukunle, 2013. One (1) g contaminated soil sample was mixed with 9 mL sterile distilled water to obtain a soil suspension that was subjected to ten-fold serial dilution. For bacterial isolates, an aliquot (1 mL) of 10^{-4} and 10^{-5} dilutions were inoculated into nutrient agar (NA) in sterile Petri dishes using pour plate technique. Inoculated plates were

incubated at 30 ^{o}C for 24 hours. The streak method was then used to subculture individual isolates from the mixed culture grown on NA. Individual (pure) bacterial colonies were characterized using the taxonomy scheme of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). For fungal isolates, the aliquot (1 mL) was inoculated into potato dextrose agar (PDA) and the plates were incubated at 28 ^{o}C for 72 hours. The plates were supplemented with streptomycin to prevent bacterial growth. Pure cultures of the fungal isolates obtained from subcultures were identified by morphological and microscopic techniques (Barnet and Hunter, 1972).

Evaluation of crude oil utilization potential of the microbial isolates

Mineral salt medium (MSM) of Zajic and Supplisson, 1972, composed of K_2HPO_4 (1.8 g), KH_2PO_4 (1.2 g), NH_4Cl (4.0 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1 g), $FeSO_4.7H_2O$ (0.01 g) in 1 litre of distilled water was used to evaluate the crude oil utilization and degradation potential of all the microbial isolates (Hamzah et al., 2014). Ten (10) mL of MSM supplemented with 2 % crude oil (v/v) was transferred to separate test tubes, sterilized by autoclaving at 121 ^{o}C for 15 minutes and cooled.

Two drops of cell suspension of each microbial isolates (bacteria and fungi) was inoculated into separate test tubes, respectively and incubated at room temperature (28 ^{o}C) for 12 days (Ajuzie et al., 2015). The microbial cell suspension was prepared by adding a loop full of each isolate to 2 mL of sterile MSM. An uninoculated test tube with the crude oil supplemented medium served as control. Turbidity measurement at 600 nm was done every two days (Kumari et al., 2012; Aunsberg et al., 2015)

Evaluation of crude oil degradation potential of the microbial isolates

Fifty (50) mL of MSM supplemented with 2 % crude oil (v/v) was transferred to separate conical flasks, sterilized by autoclaving at 121 ^{o}C for 15 minutes and cooled. One (1) mL of cell suspension of each microbial isolate (bacteria and fungi) was inoculated into separate conical flasks respectively and incubated at 28 ^{o}C on a rotatory shaker at 150 rpm for 12 days (Ajuzie et al., 2015). The microbial cell suspension was prepared by adding a loop full of each isolate to 2 mL of sterile MSM. An uninoculated conical flask with the crude oil supplemented medium served as control. Total petroleum hydrocarbon (TPH) was estimated every two days (Kumari et al., 2012; Aunsbjerg et al., 2015). The content of each conical flask was loaded on silica gel column and the crude oil component was eluted with n-hexane for spectrophotometric estimation at 400 nm (Ogbeh et al., 2019). The concentration of crude oil in the eluate was estimated from n-hexane/crude oil standard curve using the absorbance obtained.

Data analysis

All experiments were performed in triplicates. The data were presented as means ± standard error of mean (SEM).

RESULTS AND DISCUSSION

The direct visual examination of the sampling site indicated visible dark patches on the soil surface and positive crude oil sheen test with visible oil sheen seen on the surface of the polluted soil suspension. (Figure 1). This is indicative of crude oil pollution in the soil.

Fig. 1. Oil sheen test result for unpolluted soil (left) and crude oil polluted soil (right)

Microorganisms are ubiquitous in the soil where they abound in vast diversity (Braga et al., 2016). They interact with themselves and the environment enabling them to be involved in several soil functions such as nutrient cycling, mineralization and filtering of contaminants (Braga et al., 2016; Safdari et al., 2018). The probable identities of the characterized bacterial and fungal isolates that were subjected to screening in liquid medium in this study were *Enterobacter*, *E. coli*, *Micrococcus*, *Aeromonas*, *Corynebacterium*, *Bacillus*, and *Pseudomonas* (bacteria) as well as *Fusarium*, *Penicillium* and *Aspergillus* (Fungi). This microbial population structure is reflective of microbial population dynamics in a polluted soil. Crude oil pollution is known to disturb microbial dynamics in the soil leading to selective increase in only oleophilic microbes (Azubuike et al., 2016; Siles and Margesin, 2018). This changing community structure is responsible for the lower microbial diversity with time of the pollutant in the soil (Braga et al., 2016; Siles and Margesin, 2018).

The positive interaction of the crude oil degrading microbial population at the onset of the degradation process later becomes antagonistic owing to competition for limiting nutrients (Zhang et al., 2014). This leads to the predominance of bacteria over fungi (Zhang et al., 2014).

Furthermore, the limited microbial diversity and limited number of indigenous fungal isolates obtained in this study is characteristic of aged crude oil pollution (Yao et al., 2015; Godoy et al., 2016). Crude oil spilled on land is subjected to change in composition and toxicity with time as a result of weathering and other processes (Jiang et al., 2016). Evaporation of volatile components and leaching of soluble fractions cause accumulation of polycyclic aromatic hydrocarbons (PAHs) that are of great environmental concern; they are toxic, mutagenic and carcinogenic with low biodegradability (Jain et al., 2011; Macaulay and Rees, 2014).

From the results obtained in the turbidity assay (Figure 2), *Pseudomonas* exhibited the highest growth potential while *E. coli* had the lowest capacity to utilize crude oil for growth among the bacterial isolates.

The result of the crude oil utilization potential of the fungal isolates is presented in Figure 3.

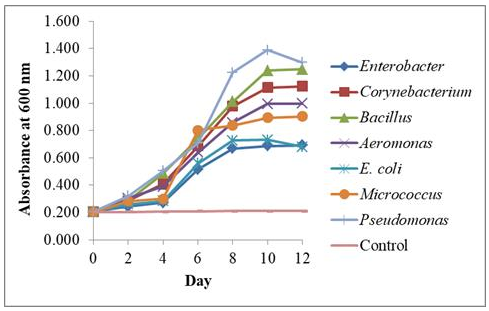

Fig. 2. Growth potential of bacterial isolates in liquid medium

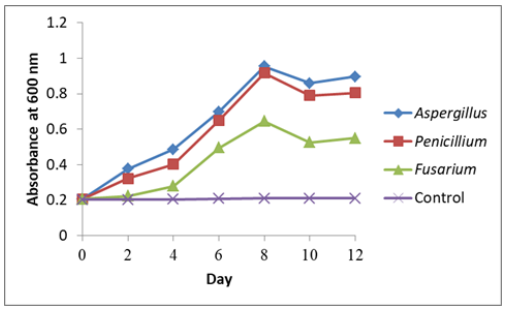

Fig. 3. Growth potential of fungal isolates in liquid medium

The highest growth ability in crude oil was exhibited by *Aspergillus* while *Fusarium* had the lowest potential. *Pseudomonas* also had the highest crude oil degrading potential with *Enterobacter* exhibiting the lowest ability in liquid medium for the bacterial isolates (Figure 4). The crude oil degrading potential of the fungal isolates in liquid medium revealed that *Aspergillus* and *Fusarium* returned the highest and lowest potentials to degrade crude oil respectively (Figure. 5).

Turbidity measurement is an indirect determination of microbial cell mass (Abbondazi et al., 2003). The crude oil degrading microorganisms are able to transform and mineralise the pollutant for use as an energy source as well as other cellular functions while reducing the pollutant concentration in the environment in the process (Polyak et al., 2018).

The bacterial isolates including *Pseudomonas*, *Bacillus*, *Micrococcus* and *Corynebacterium* isolated and characterized in this study are known oleophilic bacteria in contaminated soil (Boboye et al., 2010; Olukunle, 2013, Xu et al., 2018).

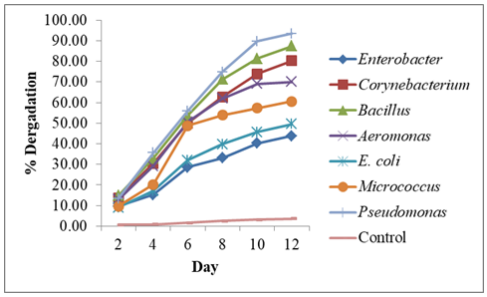

Fig. 4. Crude oil degrading potential of bacterial isolates in liquid medium

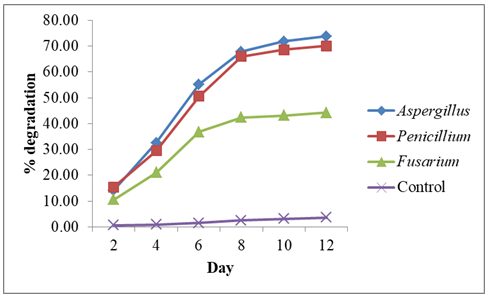

Fig. 5. Crude oil degrading potential of fungal isolates in liquid medium

Similarly, the crude oil degrading fungi isolated in this work namely: *Aspergillus*, *Penicillium* and *Fusarium* sp have also been previously identified in impacted soil (Olukunle, 2013; Yao et al., 2015). These microbial isolates especially *Pseudomonas, Bacillus, Aspergillus* and *Penicillium* (Jain et al., 2011; Godoy et al., 2016) with high oleophilic abilities are effective candidates that can be used as microbial consortia in bioremediation studies.

CONCLUSIONS

Microbes are ubiquitous in soil where they perform a variety of functions including pollutant transformation and mineralization. Crude oil pollution in the soil initially disturbs the dynamics of soil microbial population but gradually leads to increased presence of primary oleophilic microbes with abilities to utilize crude oil as the sole source of carbon while degrading the pollutant in the process.

In this study, nine bacteria genera and three fungi species have been isolated from an aged crude oil polluted soil, characterized and screened for their crude oil utilization and degradation capabilities. The reported crude oil utilization and degradation potentials of the highly ranked isolates will benefit a bioremediation plan for the restoration of crude oil impacted-soil that is widespread in the Niger Delta region of Nigeria.

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