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Degradative Ability of Silver Particles Synthesized by Gram-negative Bacteria of Some Crops Rhizosphere on Crude Oil Polluted Soil

F. O. Ekundayo[1], D. B. Orisadipe[1] and I. A. Onifade[1*]

[1]Department of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author FOE conceived, designed and supervised the study. Authors IAO and DBO performed the research work under close supervision of author FOE. Authors IAO and DBO performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To degrade the crude oil polluted soil with silver particles synthesized by some Gram negative rhizosphere bacteria of cocoa, orange and timber obtained from Idanre, Ondo State, Nigeria.
Place and Duration of Study: Soil samples were obtained from Idanre, Ondo State while crude oil polluted soil samples were obtained from Zion town in Ese- Odo at Ilaje, Ondo State, between June and September 2018. Also, Bonnylight Crude oil was collected from Warri in Nigeria. This research work was carried out at the Department of Microbiology Laboratory, Federal University of Technology, Akure.
Methodology: Soil samples (rhizosphere) were collected in sterile polythene bags and tightly packed and transported for analysis. The types and loads of bacteria present in the soil samples were determined. Identification and characterization of various bacterial isolates were based on Gram staining techniques and different biochemical tests. The physicochemical properties of the crude oil polluted soil were determined. The microbial biomass and the synthesis of silver particles were carried out while the bioremediation of the crude oil polluted soil with the silver particles which were performed in triplicate. Also, bioremediation of crude oil with the bacterial isolates were

**Corresponding author: E-mail: isrealonifade@gmail.com;*

performed in triplicate. Five millilitres (5 ml) of silver particles produced was inoculated into 50 g of sterilized crude oil polluted soil in a plastic container with 5ml of basal medium and 3 ml of the harvested cells of the isolates was inoculated into 10 ml of the basal medium with 30ml of crude oil in a conical flask. The unsterilized soil without inoculum and silver particles were served as control, also crude oil without inoculum and silver nanoparticles were served as a control.

Results: *Citrobacter freundii, Yersinia pestis, Edwardsiella tarda*, were isolated from rhizosphere of cocoa. *Serratia marcescens*, *Providencia stuatii* were isolated from rhizosphere of orange while *Enterobacter agglutinate, Moellerella wisconsis* were isolated from rhizosphere of timber. The silver particles bioremediation on polluted soil ranges were recorded within 5 days interval for 30 days. It was deduced that *Enterobacter sp* appeared to be the fastest on polluted soil followed by *Citrobacter sp, Providencia sp, Serratia sp* and *Yersinia sp.*

Conclusion: Findings from this study revealed the effects of rhizosphere Gram negative bacteria in cleaning up crude oil polluted soil environment and the usefulness of silver particles in remediating hydrocarbon polluted soil.

Keywords: Rhizosphere; gram-negative bacteria; crude oil polluted soil; silver particles.

1. INTRODUCTION

Presence of Petroleum hydrocarbons influence the biodiversity, distribution and pollution of microorganisms in an environment. Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compound [1].

Biodegradation of petroleum hydrocarbons in the environment may be limited by a large number of factors. An important limiting factor in the biodegradation polluted soils is often the low bioavailability and solubility of the hydrocarbon [2]. Crude oil is one of the most significant pollutants in the environment as it is capable of causing serious damages to humans and the ecosystem. Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer. The microorganisms in the degradation of petroleum and its products has been established as an efficient, economic, versatile and environmentally sound treatment [3].

The search for effective and efficient methods of oil removal from contaminated sites has intensified in recent years, because microbial degradation for cleaning untreated oil spills is slow. Microbial remediation of a hydrocarbon–contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil [4]. These microorganisms can degrade a wide range of target constituents present in oily sludge. A large number of *Pseudomonas* strains capable of degrading petroleum have been isolated from soil and aquifers. Other petroleum hydrocarbon-degraders include *Yokenella* sp, *Alcaligenes* sp, *Roseomonas* sp., *Stenotrophomonas* sp, *Acinetobacter* sp, *Flavobacter* sp, *Corynebacterium* sp, *Streptococcus* sp, *Providencia* sp, *Sphingobacterium* sp, *Capnocytophaga* sp, *Moraxella* sp, and *Bacillus* sp. Microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy. The growth and proliferation of oil utilizing microorganisms in polluted soil is greatly influenced by the availability of nutrients and their hydrocarbonoclastic property [5-9].

Rhizosphere bacterial communities have become a major focal point of research in recent years, especially regarding how they affect plants and vice versa. Changes in microbial density and diversity within the rhizosphere occur in a spatial temporal manner. The soil zone closest to the plant roots has the most density and diversity of microbes (18). The lack of methods to consistently isolate rhizosphere samples in a spatially defined manner is a major bottleneck in rhizosphere microbiology. Microbes with increasing affinities to and distance from the plant root can be isolated using increasing strengths of physical disruption. Sonication is an excellent choice due to the ability to gently remove rhizosphere soil and bacterial biofilms without damaging plant root.

2. MATERIALS AND METHODS

2.1 Study Area Description

Ondo is a State in South-western Nigeria, created on February 3, 1976. Akure which is the state capital is situated in the tropical rainforest zone in Nigeria. The state had a total population

of 3,460,877 as at the 2006 population census, with the total land area of 15,500 km^2. Ondo lies about 7°25' North of the equator and 5°05' East of meridian. The rural settlements under study for this research are: Idanre, Ese-Odo, Ilaje, Ondo State.

2.2 Collection of Samples

Soil samples were collected at 5cm from the rhizosphere of cocoa, orange and timber from Idanre, Ondo State. Crude oil polluted soil samples were collected from Zion town in Ese-Odo at Ilaje, Ondo State. Soil samples were collected in sterile polythene bags and tightly packed. They were then carefully transported to the laboratory for the analysis. Bonnylight Crude oil was collected from Warri in Nigeria.

2.3 Physicochemical Characterization of the Crude Oil Contaminated Polluted Soil

For Crude oil contaminated soil characterization, selected physical and chemical properties were determined using the well-established laboratory procedures. Samples were analysed for the following physicochemical parameters: Clay, Silt, sand, Coarse sand, Gravel, pH, Nitrogen, Phosphorus available in the polluted soil, and the total hydrocarbon content (THC) (Table 1).

2.4 Isolation, Identification and Characterization of Isolates

Three-fold serial dilution was performed on the above soil samples. An aliquot (0.2 mL) of each diluent were plated on sterile Nutrient agar (NA) using the pour plate method. Incubation was done at 37±2°C for 24hrs. Developed bacteria colonies were aseptically sub-cultured a number of times into fresh NA plates and incubated 37 ± 2°C for 24hrs until pure cultures of bacteria were obtained. Pure cultures were afterwards transferred onto McCartney bottles of doubled strength slant incubated at 37±2°C for 24hrs and stored at 4°C in the refrigerator.

The bacteria isolates were identified according to colonial morphology, surface, shape, size, margin and pigmentation on nutrient agar medium. The microscopic examination included Gram-staining, The biochemical tests including citrate utilization, starch hydrolysis, methyl red, Voges-Proskauer test (MR-VP), triple sugar iron test (TSI) for lactose, dextrose, sucrose, glucose and mannitol fermentation, carbohydrate fermentation, H_2S production, indole production test, urease test, catalase test, citrate test, indole test and sugar fermentation test were carried out according to standard procedures and identified using Bergey's Manual of Systematic Bacteriology [10].

2.5 Preparation of Biomass and Silver Particles

An inoculating loop was used to pick bacteria colony and inoculated on sterile nutrient broth and incubated at 37±2°C for 24hrs. For the harvested cells, the broth cultures were centrifuged at 5000 rpm for five min and suspended in 2 mL sterile distilled water. The content was centrifuged again at 5000 rpm for five min to wash the cells. Harvested cells were obtained after decanting the supernatant [11].

2.6 Synthesis of Silver Particles

Equal volume of the Silver nitrate solution and the supernatant was added and then monitored for three days for the formation of nanoparticles [11].

2.7 Degradation of Crude Oil and Polluted Soil Using Bacteria Cells and Silver Particles

The enrichment procedure as described by [12] was used in the estimation of hydrocarbon utilizers. Liquid basal medium contained Na_2HPO_4 (2.0 g), K_2SO_4 (0.17 g), NH_4NO_3 (4.0 g), KH_2PO_4 (0.53 g), $MgSO_4 \cdot 7H_2O$ (0.2 g) and 1000ml of distilled water. Sterilization was done at 121°C for 15 min. 3 ml of harvested cells was inoculated into 10 mL of the basal medium with 30mL crude oil in a conical flask, 5 ml of harvested cells was inoculated into 50 g of sterilized polluted soil in plastic container with 5ml of basal medium, 3 ml of silver particles produced was inoculated into 30 ml crude oil in a conical flask with 10ml basal medium and 5ml into 50 g of sterilized polluted soil in plastic container with 5ml of basal medium. Each of the conical flasks were plugged with sterile cotton wool wrapped with aluminium foil so as to ensure maximum aeration and prevent cross-contamination. All the conical flasks were then incubated in a shaker for 37°C for 30 days, N-hexane was used to extract the oil during which degradation of crude oil was monitored by reading the OD at 540 nm at five day interval. The experiment was done in replicates. The unsterilized soil and soil without inoculum and silver particles served as control. Also, crude oil

without inoculum and silver particles served as control [12,13].

2.8 Statistical Analysis of Data

Data obtained were subjected to analysis of variance and means were compared using Duncan's New Range Test (DNMRT) with the aid of SPSS software version 17 at $p \leq 0.05$ level of significance.

3. RESULTS

3.1 Physicochemical Analysis of Crude Oil Contaminated Soil

A comparatively analytical study was done to assess the physico-chemical properties of the crude oil contaminated soil samples, 170(g kg-1) clay, 180 (g kg-1) Silt, 650 (g kg-1) Sand. The pH value of the soil was 8.09, nitrogen content (0.15mg/kg), available phosphorus (9.0mg/kg) and total hydrocarbon content of 39.61(Table 1).

Table 1. The physicochemical properties of the crude oil contaminated soil

Properties	Crude oil contaminated soil
Clay (g kg-1)	170
Silt(g kg-1)	180
sand(g kg-1)	650
pH	8.09
Nitrogen (mg kg-1)	0.15
Phosphorus (mg kg1)	9.0
Total hydrocarbon (THC)	39.61

3.2 Microbial Loads

The microbial loads of the soil obtained from different soil rhizosphere of timber, cocoa and orange were evaluated and compared with one another. The results (Tables 2 and 3) revealed that *Moellerella wisconsens, Enterobacter agglutinate* were present in rhizosphere of timber, *Citrobacter freundii, Yersinia pseudotuberculosis, Edwardsiella tarda were* present in rhizosphere of cocoa and *Serratia marcescens, Providencia stuatii* were present in rhizosphere of orange.

3.2.1 Identity of bacterial isolates

The identification of bacteria isolates was based on their colony appearance on the selective/differential and general purpose media of the gram negative bacteria which are cultured on Nutrient agar and Mineral salt agar. All the bacteria isolated from the respective selective/differential media were further confirmed by their reaction to Gram stain, specific biochemical tests and morphological characteristics [4,14]. The total viable counts of the bacterial isolates were represented in (Table 3).

Table 2. The total viable counts (cfu/ml) of bacteria colonies obtained on plates

Isolates	Nutrient Agar ($\times 10^3$)	Mineral salt Agar ($\times 10^3$)
S. marcescens	2	4
Enterobacter sp	1	2
Providencia sp	1	1
Citrobacter freundii	3	2
Yersinia sp.	2	3
E. tarda	1	1
M. wisconsensis	2	4

3.2.1.1 Biochemical characteristics of bacteria isolated from crop rhizosphere

Seven bacteria were isolated and identified from soil rhizosphere. Four of these bacterial isolates were rod in shape and three cocci. All of the bacterial isolates were Gram negative. These organisms were tested based on their ability to utilize sugar and production of gas. Three isolates were positive and four were negative for citrate test and all were positive for catalase test. The isolates were all negative for indole test except *S. marcescens* and *E. tarda* which was positive (Table 4).

3.2.2 Synthesis of silver nanoparticles at different concentration in dark room and incubator shaker

The following bacteria isolates were able to produce silver particles through colour changes from bright-yellow to dark-brown at 10 millimole and 1millimole (hr) in both dark room and incubator shaker which was used to bioremediate polluted soil; *E. agglutinate, S. marcescens, Y. species, C. freundii and P. stuatii* (Tables 5 and 6).

3.2.3 Test for degradation

The microbial loads of the soil obtained from different soil rhizosphere of timber, cocoa and orange were evaluated and compared with one

another and the ability of each organism to degrade crude oil in the laboratory and polluted soil, including the organisms ability to produce silver particles which were used to bioremediate polluted soil sample which is core the of this project work. The result revealed that *M. wiscosensis, P. stuatii*, *C. freundii*, *Yersinia* sp, *E. tarda, S. marcescens, Enterobacter sp* were dominant Gram-negative bacteria isolated from rhizosphere of Cocoa, Orange, Timber which were able to degrade crude oil and the polluted soil and *Serratia* sp, *Enterobacter* sp, *Providencia* sp, *Citrobacter* sp, *Yersinia* sp were isolates that produce silver particles which was used to bioremediate polluted soil (Tables 5, 6 and 7) respectively.

4. DISCUSSION

Recent studies have revealed that releasing of petroleum into the environment whether accidentally or due to human activities is a main cause of soil pollution. Soil contaminated with petroleum has a serious hazard to human health and causes environmental problem as well. Petroleum pollutants, mainly hydrocarbon, are classified as priority pollutants. The application of microorganism or microbial processes to remove or degrade contaminants from soil is called bioremediation.

In this study, microorganisms capable of degrading crude oil were isolated from rhizosphere soil samples of Cocoa, Timber, Orange (Table 8). As shown in Table 1 the nitrogen content, phosphate and total organic carbon of crude oil contaminated soil sample from Zion town in Ese-Odo at Ilaje, Ondo State were relatively higher. Anthropogenic impacts, such as changes in nutrient composition, have the potential to directly or indirectly affect the

Table 3. Biochemical and cultural characteristics of bacterial isolates

H2S	Mr	VP	Suc	Glu	Lac	Ure	Gas	Cat	Ind	Cit	Sta	Suspected organisms
-	+	+	-	+	-	+	+	+	+	+	-	*S. marcesens*
-	+	+	-	+	-	+	-	+	-	+	+	*E. species*
-	-	-	-	-	-	-	-	+	-	+	-	*P. stuatii*
-	-	-	+	+	-	-	+	+	-	-	-	*C. freundii*
-	-	-	+	+	-	+	-	+	-	-	-	*Yersinia* sp
+	-	-	+	+	-	-	+	+	+	-	+	*E. tarda*
-	-	-	+	+	-	-	-	+	-	-	-	*M. wisconsen*

Keys: H2S- hydrogen sulphide, MR- methyl red, VP- vogesproskauer, Suc- sucrose, Glu- glucose, Lac- lactose, Ure- urea, Cat- catalase, Ind- indole, Cit- citrate, Sta- starch

Table 4. Production of silver particles at different concentration in static position (dark-room)

Isolates	At 10mmolhr			At 1mmolhr		
	24	48	72	24	48	72
Moellerella sp	-	-	-	-	-	-
Providencia sp	-	-	-	-	-	-
Serratia sp	-	-	-	-	-	+
Enterobacter sp	-	-	-	-	-	+
Yersinia sp	-	-	-	-	-	+
Edwardsiella sp	-	-	-	-	-	+
Citrobacter sp	-	-	-	-	-	+

Table 5. Production of silver particles at difference concentration in incubator shaker

Isolates	At 10mmolhr			At 1mmolhr		
	24	48	72	24	48	72
Moellerella sp	-	-	-	-	-	-
Providencia sp	-	-	-	-	-	-
Serratia sp	-	-	-	-	-	+
Enterobacter sp	-	-	-	-	-	+
Yersinia sp	-	-	-	-	-	-
Edwardsiella sp	-	-	-	-	-	-
Citrobacter sp	-	-	-	-	-	+

Table 6. Degradation of crude oil polluted soil by bacteria cells

Selected isolates	Optical density at different days in litres (mole × cm)						
	1st day	5th day	10th day	15th day	20th day	25th day	30th day
Control	2.32±0.01^{b}	2.32±0.01^{b}	2.32±0.01^{b}	2.30±0.00^{a}	2.30±0.01^{a}	2.31±0.00^{a}	2.32±0.01^{a}
Moellerella sp	2.29±0.00^{c}	2.29±0.00^{c}	2.29±0.01^{c}	2.28±0.00^{b}	2.27±0.00^{b}	2.26±0.00^{a}	2.26±0.01^{a}
Providencia sp	2.32±0.01^{b}	2.32±0.01^{b}	2.28±0.01^{a}	2.29±0.00^{a}	2.28±0.01^{a}	2.27±0.00^{a}	2.27±0.00^{a}
Serratia sp	2.30±0.00^{b}	2.30±0.00^{b}	2.29±0.00^{b}	2.29±0.00^{b}	2.28±0.00^{a}	2.27±0.00^{a}	2.27±0.00^{a}
Enterobacter sp	2.30±0.00^{d}	2.29±0.00^{c}	2.26±0.02^{c}	2.23±0.01^{c}	2.20±0.00^{c}	2.07±0.73^{b}	1.59±0.14^{a}
Yersinia sp	2.31±0.01^{d}	2.29±0.00^{c}	2.29±0.01^{c}	2.28±0.01^{b}	2.27±0.00^{a}	2.26±0.00^{a}	2.26±0.00^{a}
Edward sp	2.31±0.01^{c}	2.29±0.00^{b}	2.29±0.00^{b}	2.29±0.00^{b}	2.28±0.00^{a}	2.28±0.00^{a}	2.28±0.00^{b}
Citrobacter sp	2.30±0.01^{c}	2.30±0.01^{c}	2.29±0.00^{c}	2.28±0.00^{b}	2.28±0.00^{b}	2.27±0.00^{a}	2.27±0.03^{a}
Control b	2.30±0.01^{e}	2.29±0.00^{e}	2.28±0.00^{d}	2.32±0.00^{f}	2.10±0.01^{d}	1.89±0.00^{b}	1.59±0.01^{a}

Data are represented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (p<0.05)

Table 7. Degradation of crude oil polluted soil by silver particles

Selected isolates	Optical density at different days in litres (mole × cm)						
	1st day	5th day	10th day	15th day	20th day	25th day	30th day
Control	2.31±0.01^{a}	2.31±0.01^{a}	2.31±0.00^{a}	2.31±0.00^{a}	2.30±0.00^{a}	2.31±0.00^{a}	2.30±0.00^{a}
Enterobacter sp	2.30±0.00^{e}	2.23±0.01^{e}	2.18±0.00^{e}	2.10±0.10^{d}	1.89±0.00^{c}	1.57±0.00^{b}	1.21±0.01^{a}
Serratia sp	2.30±0.00^{g}	2.28±0.00^{f}	2.25±0.00^{e}	2.21±0.01^{d}	2.08±0.00^{c}	1.56±0.00^{b}	1.26±0.01^{a}
Yersinia sp	2.31±0.1^{c}	2.28±0.00^{c}	2.25±0.01^{c}	2.10±0.00^{b}	2.04±0.09^{b}	1.31±0.01^{a}	1.26±0.01^{a}
Citrobacter sp	2.30±0.00^{g}	2.25±0.00^{f}	2.15±0.00^{e}	2.01±0.00^{d}	1.62±0.00^{c}	1.34±0.00^{b}	1.25±0.01^{a}
Providencia sp	2.31±0.00^{f}	2.26±0.00^{e}	2.19±0.00^{d}	1.81±0.01^{d}	1.56±0.00^{c}	1.31±0.01^{b}	1.25±0.01^{a}

Data are represented as Mean± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (p< 0.05)

Table 8. Degradation of crude oil by bacteria cells

Selected isolates	Optical density at different days in litres (mole × cm)						
	1st day	5th day	10th day	15th day	20th day	25th day	30th day
Control	2.26±0.03[a]	2.26±.00[a]	2.26±0.00[a]	2.27±0.00[a]	2.26±0.00[a]	2.26±0.00[a]	2.27±0.00[a]
Moelerella sp	2.27±0.01[a]	2.27±0.01[a]	2.26±0.00[b]	2.26±0.01[b]	2.25±0.00[a]	2.24±0.00[a]	2.25±0.06[a]
Providencia sp	2.26±0.01[a]	2.26±0.01[a]	2.25±0.00[a]	2.25±0.00[a]	2.25±0.00[a]	2.25±0.00[a]	2.25±0.00[a]
Serratia sp	2.26±0.00[c]	2.26±0.00[b]	2.26±0.00[c]	2.26±0.00[c]	2.25±0.00[b]	2.25±0.00[a]	2.25±0.00[a]
*Enterobacte*r sp	2.26±0.00[e]	2.26±0.00[e]	2.26±0.00[d]	2.25±0.00[d]	2.24±0.00[b]	2.23±0.00[b]	2.20±0.00[a]
Yersinia sp	2.27±0.01[b]	2.27±0.01[b]	2.27±0.00[b]	2.26±0.03[a]	2.26±0.00[a]	2.25±.00[a]	2.26±0.00[a]
Edwardsiella sp	2.27±0.00[c]	2.26±0.00[c]	2.26±0.00[b]	2.26±0.00[b]	2.25±0.00[b]	2.25±0.00[a]	2.25±0.00[a]
Citrobacter sp	2.27±0.00[b]	2.27±0.00[b]	2.27±0.00[b]	2.27±0.00[a]	2.26±0.00[a]	2.26±0.01[a]	2.26±.01[a]

Data are represented as Mean±S.E(n=3). Values with the same superscript letter(s) along the same column are not significantly different (p<0.05)

bacterial composition of the soil [15]. Likewise, more moisture favors microbial growth. The proportion of hydrocarbon content in the Zion town in Ese-Odo at Ilaje, Ondo State samples were 39.61%, respectively. It has been reported that population levels of hydrocarbon utilisers and their population within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons [16]. In unpolluted ecosystem, hydrocarbon utilizers generally constitute about 0.1% of the microbial community and in oil polluted ecosystems they can constitute up to 100% of the viable microorganisms [15]. The microbial populations quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contamination [17]. The low proportion of hydrocarbon utilizers compared to the total heterotrophic population indicates that the soil ecosystem from which the samples were obtained probably had not been exposed to heavy and consistent crude oil pollution. The rhizosphere soil samples used in this study harboured hydrocarbon utilizers which include *Serratia* sp, *Enterobacter* sp, *Citrobacter* sp, *Providencia* sp, *Edwardsiella* sp, *Yersinia* sp, *Moellerella* sp. The flora reflects the diverse heterotrophic bacteria present in rhizosphere soil and the diversity could be as a result of the varied sources of the refuse, dumped at the sites. Several hydrocarbon degrading organisms have been isolated from diverse environments; soil and aquatic sources which are the two major environments affected by hydrocarbon pollution [13]and their isolation is not restricted to hydrocarbon-bearing environments. In this study I focused on hydrocarbon utilizing isolates obtained which had varied degree of degradation however, *Enterobacter* sp, *Edwardsiella* sp, appeared to be the fastest growing species in crude oil followed by *Serratia* sp, *Providencia* sp, *Moellerella* sp.*Yersinia* sp, *Citrobacter* sp. And also *Enterobacter* sp, *Moellerella* sp, *Yersinia* sp, appeared to be the fastest growing species in polluted soil, followed by *Serratia* sp, *Providencia* sp, *Edwardsiella* sp, *Citrobacter* sp which are the slow degraders. While for nanoparticles bioremediation, *Enterobacter* sp, appeared to be the fastest on polluted soil followed by *Citrobacter* sp, *Providencia* sp, and *Serratia* sp, *Yersinia* sp.

However, several other workers also reported on *Micrococcus varians*, *Bacillus badius*, *Corynebacterium ulcerans* and *Corynebacterium amycolatum* appeared to be the fastest growing species in crude oil. The study of [18] identified the above genera among hydrocarbon degrading microorganisms.

The isolates showed different rates of growth in hydrocarbon liquid media and polluted soil likewise.

5. CONCLUSIONS

The result demonstrates the effects of rhizosphere Gram negative bacterial of timber, cocoa, orange in cleaning up crude oil polluted environment. Also, their importance and usefulness in production of silver particles poses a great effect in remediating crude oil polluted soil.

Bioremediation is the main natural mechanism than can clean up the petroleum hydrocarbon pollutants from the environment. A number of influencing degradation factors has been identified to reduce the toxicity of oil contamination in the environment by removing, degrading or transforming contaminants. Therefore, a successful bioremediation treatment requires understanding of those factors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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