



Isolation and characterization of bacterial diversity from soils supplemented with electrical transformer fluids

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ABSTRACT

Repetitive enrichment of soils samples from an agricultural land and newly marked dumpsite on electrical transformer fluid yielded six bacterial species that have the capacity to utilize electrical transformer fluids (askarel) as carbon and energy source. Bacterial species namely: *Micrococcus*, *Arthrobacter*, *Pseudomonas putida*, *Pseudomonas* spp, *Norcadia* and *Corynebacterium* were identified using morphological and biochemical characteristics. The abilities of these bacterial species to utilize the electrical transformer fluids as carbon source in minimal salt medium (MSM); sub cultured in concentrations of 5, 10, 15 and 20 μ L of electrical transformer fluids were investigated for twenty-one days period. Physiological changes in terms of biomass increase were monitored by measuring the pH and optical density of the culture medium. From the results obtained, there was observed a general decrease in the pH and increase in Optical density (O.D). The mean pH and O.D readings ranged between (4.34-6.13 and 0.073-0.386) respectively. The decreased pH could justify for the acidic metabolites produced in the course of utilization of askarel as growth substrates. This study suggested that, the tropical ecosystems can provide exotic bacterial species that are capable of degrading or mineralizing polychlorinated biphenyls and their derivatives from dumpsites and agricultural soils.

1. Introduction

Polychlorinated biphenyls and their derivatives are among widespread environmental pollutants, having being detected in virtually all environments. Not only are they persistent, but also toxic. Polychlorinated biphenyls (PCBs) are widely studied pollutants because of the severe ecotoxicological effects. They are among the persistent organic pollutants which are a wide class of semi-volatile, toxic hydrophobic compounds. Examples include aldrin, chlordane, dichlorodiphenyl trichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated dibenzo-p-

dioxins (dioxins) polychlorinated dibenzofurans (furans) and polycyclic aromatic hydrocarbons (PAHs) (Hernan et al., 2008). PCBs have multiple isomers with different degrees of chlorination; however owing to its toxicity, several countries had banned its use. (USEPA, 2009). Unfortunately, PCBs has been found in all environments, and relatively large quantities have been released due to inappropriate disposal methods, accidents, leakage from industrial facilities, and a lot of it is still being released through diffuse emissions of industries (Fiedler, 1997). The environmental transport of PCBs is complex and nearly global in scale

(Urbaniak, 2007). In Nigeria, several fish samples from the Lagos lagoon, were analyzed for the presence of polychlorinated biphenyls (PCBs). The fish species analyzed include: *Tilapia zilli* (red belly Tilapia), *Ethmalosa fimbriata* (Bonga shad) and *Chrysichthys nigrodigitatus* (catfish). Eight PCB congeners were identified and quantified in muscle of the fish species analyzed. The study showed that concentrations of PCBs were higher in adult than in juvenile of most of the fish, and there was no correlation between fat content and total concentration of PCBs (Adeyemi et al., 2009). PCBs have accumulated in soils and the most contaminated sites are near plants where they were used. PCB concentrations can reach several grams per kg, while the maximum permissible concentration in soil is 0.01–30 mg/kg, depending on country and land use (Galina et al., 2009). Decontamination of polluted soils becomes imperative because they are secondary source of contamination of the atmosphere, plants, animals, and humans. The two most common methods for treating PCB-contaminated soils are the land burial and incineration. These methods are costly, energy-consuming, and may negatively impact the environment (Piver and Lindstrom, 1985). Studies had shown that women who were exposed to relatively high levels of PCBs had babies that weighed slightly less when compared with babies from women who did not have these exposures (Buck et al., 2000; Stewart et al., 2000). Some of the health effects associated to PCBs exposure include: problems with motor skills and a decrease in short-term memory, altered estrogen levels which result to reproduction problems. For instance, in the womb, males can be feminized or the baby may be intersex, neither a male nor a female; were, both sets of reproductive organs may develop. In the aquatic environment, animals at the top of the food chain, like whales, polar bears, dolphins and humans, can store PCBs at highly concentrated levels. Biological magnification of PCBs in polar bears and whales result to development of both male and female sex organs; and as such the males cannot reproduce. This effect is known as endocrine disruption (Diamanti-Kandarakis et al, 2009). Endocrine Disrupting Chemicals (EDC's) pose a serious threat to reproduction in top-level predators. The chemicals, which take many years to bioaccumulate, pass easily through the lipid portions of cell membranes and are readily absorbed into mammalian fat tissue. According to (Adebusoye et al., 2008), the beliefs of PCBs as immutable and completely refractile to microbial deg-

radation has been shattered due to isolation of exotic microorganisms capable of utilizing PCBs as carbon source. Thus, bioremediation using microorganisms presents as best alternative. *Burkholderia xenovorans* strain LB400 and members of the genus *Dehalococcoides* have been identified as one of the bacteria capable of degrading PCBs. (Pieper and Seeger, 2008). Several novel transformations of PCBs by bacterial species had been noted these include *Rhodococcus* sp. Strain RHA1, *Corynebacterium* sp MB1, *Archromobacter*, *Arthrobacter*, *Bacillus*, *Alcaligenes odorans* and *Alcaligenes denitrificans* have been used to degrade dichlorobiphenyls. (Jung-Ho et al., 1996; Masashi et al., 1995). Others include: *Pseudomonas*, *Vibrio*, *Aeromonas*, *Micrococcus*, *Acinetobacter*, *Bacillus*, and *Streptomyces* that degrade mono-, di-, tri-, and some tetra chlorinated PCBs by meta-cleavage of unchlorinated 2,3-carbons. Some strains are exceptional PCB degraders' e.g *Pseudomonas* LB400, *Alcaligenes eutrophus* H850, *Corynebacterium* MB1 and *Acinetobacter* P6. For other cultures, a consortium of bacteria is necessary to completely mineralize PCBs e.g. a co-culture of strain *Acinetobacter* P6 and *Acinetobacter* strain 4CB1, isolated on 4-chlorobenzoate were able to completely degrade 4, 4'-dichlorobiphenyl. *Alcaligenes* ACA, enriched on acetophenone, could cometabolically degrade several chloroacetophenones. All these are actually by-products from the oxidation of PCBs, during aerobic degradation. (Bedard, 1986). However these achievements, the species with exotic abilities are yet to be fully isolated and characterized. Thus authors sought to isolate and characterize bacterial diversity from non polluted soils supplemented with askarel and their degradative abilities compared. This is because pollution from non-polluted source is more difficult to control, much more were regulatory policies are in non-existent.

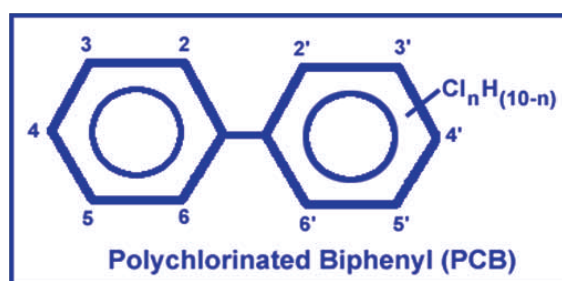


Figure 1: Structure of Polychlorinated biphenyls.
Source: Mueller (2009).

2. Materials and Methods

2.1. Chemicals and Reagent

All chemicals and reagents were of analytical grade. The (PCB blend) Askarel was generously provided by Power Holding Company of Nigeria. All other chemicals and reagents were obtained from Sigma- Aldrich Chemicals Co Ltd England.

2.2. Sample Collection

The soil samples were collected from Covenant University new refuse dumpsite in Ogun State and Agbede Agricultural Farm Land in Edo State, Nigeria. The soil samples were collected randomly, at depth of about 2-9cm deep using a sterile hand trowel. The soil samples were placed in separate sterile jars and transported back at ambient temperatures for further studies. The trowel surface was sterilized with 70% ethanol prior to collection of each sample.



Figure 2: Map of Nigeria showing the states where the organisms were isolated.

Source: Google Maps, 2011

2.3. Isolation and Enrichment of Aerobic Indigenous Bacterial Strains

The enrichment and degradation potential of askarel oil were conducted in Minimal salt medium containing in CaCO_3 (2mg/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1g/L), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1.44mg/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.25mg), H_3PO_4 (0.06mg/L), HCl (51.3 μL) and askarel oil (PCB blend) about 100ppm as carbon source. The pH was adjusted to 7.0. Cultures were incubated in Universal tubes containing a liquid volume of 30ml with mouth plugged with sterile cotton wool and incubated at room temperature (25°C) for a period of three weeks. For the bacterial isolation from enrichment culture, transfers to fresh askarel minimal salt medium using about 10% of inoculum from the previous enrichment was done weekly

and incubated at 25°C. This procedure was repeated for four successive transfers. Pure cultures were isolated from enrichments by plating out on nutrient agar sprayed with Askarel. Discrete single colonies were selected and inoculated on Minimal agar medium sprayed with Askarel. The process was repeated severally to obtain pure cultures capable of growth on Askarel oil (Liu et al., 2002; Nwinyi et al., 2008; Nwinyi, 2010; Nwinyi, 2011).

2.4. Identification and characterization of the Isolated Bacterial Strains:

The pure bacterial strains were identified on the basis of their morphological and biochemical tests. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests such as color, shape, elevation, consistency, margin, Catalase test, MRVP (methyl red-voges proskauer test), fermentation of sugars, Kovacs citrate, indole, hydrolysis of starch, and sensitivity tests. In order to determine the identity of bacteria isolates, results were compared with standard references of Bergey's Manual of Determinative Bacteriology 2nd edition (Buchanan and Gibbon, 1974; Olutiola et al., 1991).

2.5. Determination of Growth Profile in Different Concentration of Askarel Blend:

The isolates were inoculated into different concentrations of 5 μL , 10 μL , 15 μL , 20 μL askarel oil minimal salt medium and control (minimal salt medium and the bacterial isolates only). This was done to determine the tolerance level, degradation/ transformation of askarel oil, where it serves as carbon source. The cultures were then incubated at ambient temperature for three weeks. The cell biomass was monitored by measuring weekly the turbidity at 540nm using standardized Hanna H198703 Turbidity meter and pH by Hanna microprocessor pH meter.

3. Results

The bacterial species obtained in this study have the capacity to degrade askarel and other PCB compounds. *Micrococcus spp.*, *Arthrobacter spp.*, *Pseudomonas putida*, *Pseudomonas Corynebacterium* and *Norcadia* were identified using morphological, cultural and biochemical tests the results obtained are summarized in Table 1a and 1b. Population density (growth) of the isolates increased significantly in the preceding weeks with concomitant increase in turbidity and blue coloration of the tubes containing the *Pseudomonas* species. In the control tubes with the askarel, there was no signifi-

cant increase in the optical density nor the pH. This shows that the changes that occurred in the tubes in terms of the turbidity and color change were due to biodegradation of the askarel.

The result for the biochemical tests, colonial morphology on all the bacteria that can degrade the askarel can be seen in the tables below.

4. Discussion

The use of indigenous bacterial species as a bioremediation tool for effective remediation of PCB – contaminated sites is crucial in the campaign for sustainable green environment. In recent times, bioremediation has been used for effective clean-up of environment contaminated with oil products. It provides the most ecofriendly means of ensuring

	A1	A2	B1	B2	C1	C2
Shape	Cocci	Rods	Rods	Rods	Rods	Rods
Color	Yellow	Cream	White	White	Cream	Cream
Form	Effuse	Filiform	Filiform	Filiform	Filiform	Effuse
Pigmentation	No Pigment	No Pigment	Green Pigment	Green Pigment	No Pigment	No Pigment
Optical Characteristics	Transparent	Opaque	Translucent	Translucent	Translucent	Translucent
Abundance of growth	Moderate	Large	Large	Large	Moderate	Moderate

Table 1a: Morphological characteristics of the Bacterial isolates.

	A1	A2	B1	B2	C1	C2
Gram's Reaction	+ cocci in clusters	+ rods in chains	- short rods	- short rods	+ rods	+ rods
Motility Test	-	-	+	+	-	-
Acid- Fast Stain	-	-	-	-	+	+
Spore Stain	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+
Citrate test	+	+	+	+	-	+
Urease test	+	+	+	+	+	-
Indole test	-	-	-	-	-	-
MR-VP test	-	-	-	-	-	-
Growth in 5% NaCl	+	+	+	+	+	+
Growth at 35°C	+	+	+	+	+	+
Growth at pH at 6.0	+	+	+	+	+	+
Starch Hydrolysis	-	+	+	-	-	+
*Glucose	-	-	-	-	-	-
*Lactose	-	-	-	-	-	-
*Maltose	-	-	-	-	-	-
*Sucrose	-	-	-	-	-	-

Probable org. *Micrococcus spp.* *Arthrobacter spp.* *Pseudomonas putida* *Pseudomonas* *Corynebacterium* *Norcadia*

Table 1b: Biochemical characteristics of the bacterial isolates.

+ : Positive reaction, - : Negative reaction

Covenant University dumpsite location A include: *Micrococcus spp* and *Arthrobacter spp.*

Covenant University dumpsite location B include: *Pseudomonas putida* and *Pseudomonas spp.*

Agbede in Edo State, Nigeria location C include: *Corynebacterium spp* and *Norcadia spp.*

sustainable use of lands for agriculture. Thus the search of bacterial species for complete removal of PCB –contaminated sites particularly in the developing world is necessary, particularly due to lack of monitoring and unregulated importation of banned PCBs and PCB items in such regions. According to (Adebusoye *et al.*, 2008), there may be presence of efficient and unique metabolic capacity that may be widespread among microorganisms found in tropical soils.

In this study, we isolated six bacterial species that have the capacity to utilize askarel at varying concentrations from a tropical soil. Isolates A1, A2, B1, B2 were isolated from a new dumpsite in Covenant University while C1 and C2 were from an Agricultural land in Edo State. In Fig 5, the degradation of biphenyls is initiated by an enzyme called biphenyl 2-3-deoxygenase, and this enzyme cleave the 2, 3 carbons of the PCB. (Neilson, 1990; Gibson and Parales, 2000). This process occurs during the removal of chlorine from the biphenyl ring followed by cleavage and oxidation of the resulting com-

pound. In our study, the utilization of askarel was expressed by the increase in turbidity (cell mass) by the degrading bacteria, and decrease in pH. The decrease in pH is agreeable because the course of degradation will result in the production of acids and its intermediates, either organic or inorganic depending on what is produced from the degradation pathway, though in some cases they are organic acids with carboxyl groups, and increase in cell mass shows the utilization of carbon (askarel) as the principal energy source. In addition, the increase in the turbidity and decrease of pH of broth medium depicts microbial growth, as in the general knowledge of microbial substrate utilization in batch fermentation. In our study, there was a color change of the PCB blend (askarel) minimal medium, from clear to light blue, with variations in the shades of the color, depending on the action of the bacteria, and the type/ effect of the enzymes produced during the degradation process. This color change was observed in some culture bottles containing the different concentration of askarel. From literature, the blue color change is common among

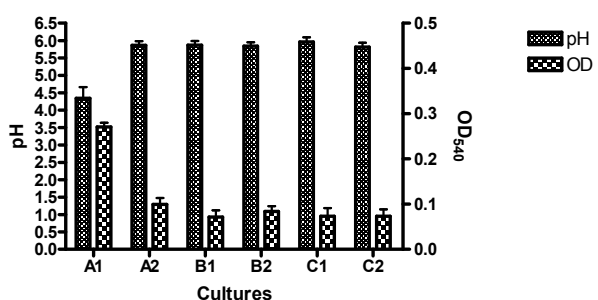


Figure 3. Growth profile (O.D and Turbidity) of the bacterial cultures at 5uL concentration of the askarel minimal salt medium. The data show bar graph of mean values for pH and optical density (OD) of the bacterial isolates at (5uL) concentration of askarel in minimal salt medium for 21 days. Initial pH and O.D reading for the control sample were 5.94 and 0.034 respectively.

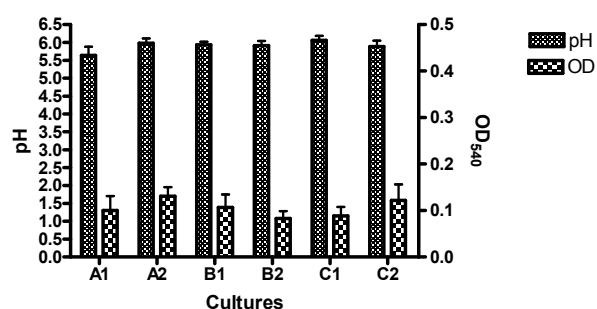


Figure 4. Growth profile (O.D and Turbidity) of the bacterial cultures at 10uL concentration of the askarel minimal salt medium. Data show bar graph of mean values for pH and optical density (OD) of the bacterial isolates at (10uL) concentration of askarel in minimal medium for 21 days.

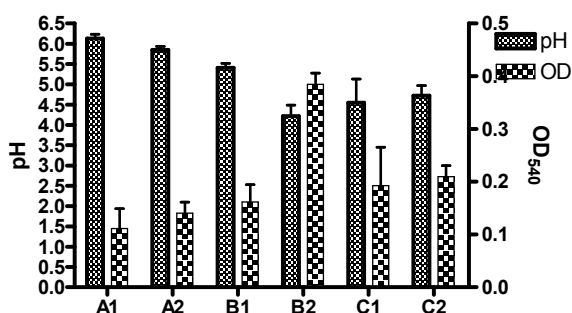


Figure 5. Growth profile (O.D and Turbidity) of the bacterial cultures at 15uL concentration of the askarel minimal salt medium. The bar graph shows the mean values for pH and optical density (OD) of the bacterial isolates at (15uL) concentration of askarel in minimal medium for 21 days.

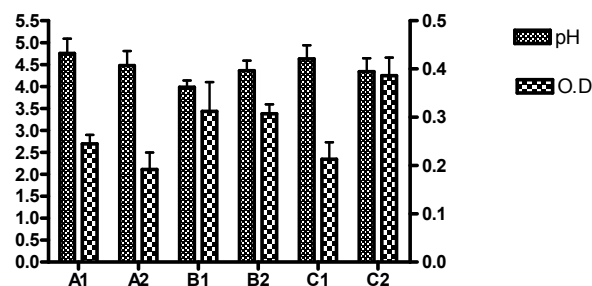


Figure 6. Growth profile (O.D and Turbidity) of the bacterial cultures at 20uL concentration of the askarel minimal salt medium. The bar graph depicts the mean values for pH and Optical Density (OD) of the bacterial isolates at (20uL) concentration of askarel in minimal medium for 21 days.

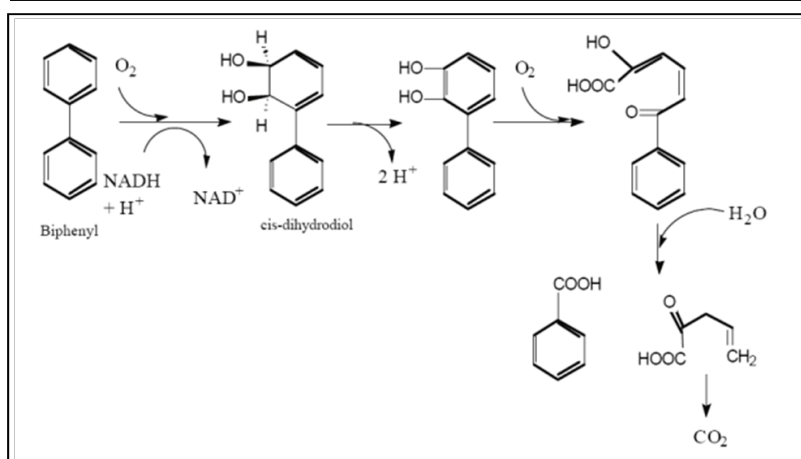


Figure 7: Showing degradation of biphenyl using 2,3-deoxygenase enzyme. Source: (Neilson, 1990).

the *Pseudomonas* spp. And from results obtained, only the isolated strains of *Pseudomonas* spp exhibited this feature. Several bacterial species have utilized organic pollutants these include: *Acinetobacter*, *Alcaligenes eutrophus*, *Pseudomonas*, *Norcadia*, *Camomonas*, *Frateruia*, and *Delftia*. They were able to utilize PCBs at different concentrations and tolerance levels. (Liu et al., 2002). Bacteria like *Corynebacterium* sp MB1, *Archromobacter*, *Arthrobacter*, *Bacillus*, *Alcaligenes odorans* and *Alcaligenes denitrificans* have been used to degrade dichlorobiphenyls. (Jung-Ho et al., 1996). From the obtained results, our isolates were also able to utilize askarel (PCB blend) which is also a toxic compound. It was observed that the isolated *Micrococcus* (A1) were able to utilize the askarel (PCB blend) in the minimal medium as its carbon source at a concentration of 5 μ L, were 0.271, was recorded for turbidity and 4.34 for pH of the medium. However, at higher concentrations, it did not perform well. For the *Corynebacterium* species, (C1) we recorded low pH value (4.55 and 4.97) in the 15 and 20 μ L concentration of the PCB blends. This shows that *Corynebacterium* can thrive in relatively high concentration of askarel. *Arthrobacter* (A2) was able to utilize the askarel (PCB blend), as illustrated in the bar graph of fig: 6, were the askarel (PCB blend) concentration was 20 μ L PCB. The pH reading was (4.47), while the turbidity was (0.192). *Norcadia*, (C2) is usually morphologically like *Actinomycetes*, but has few differences. It was the second best organism that utilized the askarel (PCB blend), with pH (4.34) and turbidity (0.386) at 20 μ L concentration. Thus the bacterial specie performed better at higher concentrations, as it also performed well at 15 μ L concentration with pH (4.72), and OD (0.210). *Pseudomonas putida* (B1) utilized the askarel mini-

mal medium at the highest concentration of 20 μ L, resulting in the lowest pH values (3.99), also increased turbidity (0.312), though not as high as *Norcadia* (0.386). Also, another *Pseudomonas* species (B2) was able to utilize the PCB blend askarel at 15 μ L (4.21) and OD (0.385). This further supports the fact that different species or strains of a particular organism respond differently to organic pollutants. From studies, bacteria belonging to the *Pseudomonas* species are capable of degrading toxic persistent organic pollutants. From this study *Pseudomonas*

putida, showed the highest potential in utilizing askarel as carbon and energy source. In conclusion, dumpsites and arable agricultural lands in tropical ecosystem which are predisposed to PCBs and other organic pollutant contamination by means of improper disposal methods, accidents or even effluent run-offs contain exotic bacterial species that can use aerobic and anaerobic conditions in biodegradation of PCBs in soil and sediments. This further validates the reports of (Adebusoye et al., 2008) that tropical soils contain an array of exotic metabolic capacities. Thus our isolated bacterial species *Arthrobacter* spp., *Norcadia* spp., *Pseudomonas putida*, *Corynebacterium* spp. *Micrococcus* spp and strains of *Pseudomonas* spp which are ubiquitous in non polluted soils can remediate such soils upon pollution with polychlorinated biphenyls and other organic pollutant.

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