

**BIOREMEDIATION OF EFFLUENT FROM KADUNA REFINERY AND
PETROCHEMICAL COMPANY USING *CLADOSPORIUM* SPECIES**

BY

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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
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APRIL, 2017

DECLARATION

I declare that the work in this thesis entitled “**BIOREMEDIATION OF EFFLUENT FROM KADUNA REFINERY AND PETROCHEMICAL COMPANY USING *Cladosporium* Species**”, has been carried out by me in the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

KWAJI JOSEPH MAGDALINE

Signature

Date

CERTIFICATION

This thesis entitled “BIOREMEDIATION OF EFFLUENT FROM KADUNA REFINERY AND PETROCHEMICAL COMPANY USING *Cladosporium* Species” by MAGDALINE JOSEPH KWAJI meets the regulations governing the award of degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Lord Jesus Christ the source of all wisdom.

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ABSTRACT

This study investigated the potential bioremediation ability of *Cladosporium* species in hydrocarbon contaminated wastewater. Hydrocarbon contaminated wastewater samples were collected from the wastewater reservoir of the Kaduna Refinery and Petrochemical Company, Kaduna and the concentrations of the heavy metals, total hydrocarbon and physicochemical parameters of the samples were determined. Some of the physicochemical parameters of the hydrocarbon contaminated water such as Electrical conductivity (15.74-25.70 μ S/cm), TDS (8.00-8.50 mg/l), dissolved oxygen (0.60-0.75 mg/l) and BOD (0.20-0.52 mg/l) were lower than their respective WHO (2011) permissible limits while Ammonium nitrates (0.12-0.8 mg/l), Turbidity (2.63-10.30 NTU) and pH (7.40-12.00) were higher before bioremediation. The concentration of Zinc (1.97-2.86mg/l) and Copper (0.35-1.49 mg /l) were within their respective WHO (2011) acceptable limits while Manganese (0.65-2.0 mg/l), Cadmium (0.38-0.56mg/l) and Lead (14.42-15.09mg/l) exceeded the acceptable standards before bioremediation. Nickel was not detected in all the sampling points. The highest total hydrocarbon concentration observed before bioremediation was 1.87mg/l. *Cladosporium* species was isolated from the study area, identified and characterized on the basis of their macroscopic and microscopic morphologies using standard taxonomic guides. The *Cladosporium* isolate was used in the bioremediation of the samples. The concentrations of some physicochemical parameters such as Electrical conductivity (25.70 -14.95 μ S/cm), TDS (8.50-0.03 mg/l), D.O (0.75-0.30 mg/l) and pH (12.00-6.90) decreased after bioremediation with *Cladosporium* species. A decrease was also observed in the concentration of some heavy metals such as Zinc (2.86-0 mg/l), Cadmium (0.56-0 mg/l), and Copper (1.49-0 mg/l) after bioremediation. The total hydrocarbon concentration decreased for both the sterilized (1.874-0.450 mg/l) and non-sterilized samples (1.874-0.703 mg/l) after bioremediation. However, the concentrations of Lead (14.19-33.98 mg/l) and Manganese (0.65-2.86 mg/l) increased after bioremediation. This study shows that *Cladosporium* species has potential for bioremediation of hydrocarbon contaminated wastewater.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Bioremediation is the treatment of contaminated environment by using biological mechanisms (Adamet *et al.*, 2017). Biodegradation is defined as the biologically catalyzed reduction in the complexity of chemical compounds (Alexander, 1973). These substances are transformed through metabolic or enzymatic process. This transformation is based on two processes namely growth and co-metabolism. In growth, organic pollutants are used as sole source of energy and carbon while growth substrate is used in co-metabolism. The process results in a complete degradation called mineralization (Roling *et al.*, 2004;Fritscheand Hofritcher, 2008).

Biodegrading microorganisms are those that mineralize complex organic molecules into simpler ones. Hydrocarbons are complex and heterogeneous groups of organic molecules that are made up of carbon and hydrogen. Mycoremediation is a form of bioremediation where fungi are used to degrade or sequester contaminants in the environment (Margesin and Shinner 2001).The decomposition by the fungi is usually performed by the mycelia .The mycelia secretes extracellular enzymes and acids that break down the organic matter (Medina-Beliver *et al.*, 2005).

The growth in petroleum hydrocarbon exploration in Nigeria and the world over has led to increased oil pollution in the environment. Petroleum refining produces large amount of effluents that are toxic and results in environmental pollution of receiving bodies of water and soils. The exact composition cannot however be generalized as it depends on the refinery and the units that are in operation at any specific time. Such contaminated habitats lose their

capability to support both plants and animal lives and thus constitute public health and socio-economic hazards as well as pose serious aquatic toxicity problems (Okerentugba and Ezeronye, 2003). The Kaduna Refinery and Petrochemical Company (KRPC) was designed to refine two types of crude oils namely the imported heavy crude and Nigerian light crude. The increasing demand for petroleum and its products in the last ten decades has resulted in petroleum spills. Despite fluctuations in its prices, oil will remain a major source of energy in the next several decades because a reliable alternative has not yet been found (Adieze *et al.*, 2003).

The impacts of crude petroleum, prospecting and production operations on oil producing countries of the world had resulted in enormous pollution of air, water and particularly agricultural land for food production (Ward *et al.*, 1980; Atlas, 1981; Tealet *et al.*, 1992; Amund *et al.*, 1993; Wang, *et al.*, 2011). Such contamination may result in an extensive damage to the natural environments and these effects may be long term and extended over many seasons.

Generally speaking, effluents discharge into salt marsh ecosystems impart potential damage to their physical and ecological integrity even in minimal spill level, let alone catastrophic accidents like the Exxon Valdez in 1989 (36, 000 tons of crude oil covered approximately 500 kilometers of shoreline) (Miller, 1999). The removal of oil that has been accidentally or purposely spilled into the environment is therefore of great concern to the petroleum industry (Sanni and Ajisebutu, 2003). Previous investigations have shown that crude oil and other related organic pollutants can be degraded (Okpokwasili and Okorie, 1988; Ijah and Ukpe, 1992; Philips and Stewart, 1994; Ekpo and Ekpo, 2006). To remediate petroleum contaminants in these environments, bio-stimulation and bio-augmentation are generally considered as environmentally friendly techniques. However, the use of extrinsic microorganism is unlikely to be acceptable to the public or introduction of a single species has a tendency to use limited

compounds, and had low survival rates due to predation, parasitism and competition with indigenous species in the limited environment. Predominant hydrocarbons are theoretically degradable but the components are rather complicated. It contains aliphatic and polycyclic aromatic hydrocarbons (PAH), for example, crude oil consists of paraffins 15 - 60%, naphthenes 30 - 60%, aromatics 3-30% and asphaltic 6% by weight (Hyne, 2001).

Microbial degradation presents one of the major routes through which petroleum hydrocarbons can be removed from contaminated environments (Prince, 1993; Venossa *et al.*, 1996). Minas and Gunkel (1995) observed that since hydrocarbons are natural products, it is not surprising to find organisms that are able to degrade these energy rich substrates. Microbial degradation appears to be the most environmentally friendly method for removal of oil pollutant since other methods such as surfactant, washing and incineration lead to introduction of more toxic compounds to the environment. Hydrocarbon-degrading microorganisms are widely distributed in marine, freshwater, and soil ecosystems (Atlas and Bartha, 1998). The ability to isolate oil-degrading microorganisms from an environment is commonly taken as evidence that the isolated organisms are active degraders in that environment (Okerentugba and Ezeronye, 2003).

1.2 Statement of Research Problem

Refinery effluents are recalcitrant molecules toxic to living organisms and persist in the environment for many years depending on the nature and quality of the oil (Ward *et al.*, 1980; Teal *et al.*, 1992). The effluents are composed of heavy metals, oil and grease along with many other toxic organic compounds. The frequency of oil spill and untreated waste water discharged into water bodies and the attendant negative impact on the aquatic and terrestrial

eco-systems is well known and well documented over the years (Nduka and Orisakwe, 2009; Uzoekwu and Oghosanine, 2011).

Treatment of effluents from Kaduna Refinery and Petrochemical Company may not be enough to remove pollutants from the the waste water completely, therefore the need for determining the physicochemical parameters and bioremediation potential of the waste water cannot be overemphasized. Mbaneme *et al.* (2013) reported that direct effluent discharge from nearby Refining Company into aquatic ecosystem resulted into persistent organic pollutants such as petroleum aliphatic hydrocarbons to be among the most serious and important classes of pollutants. Other than oil and grease contamination, phenol and its derivatives are also among the most important contaminants present in the refinery effluents. Phenol is one of the major organic pollutants encountered in waste water produced by industrial and refinery activities. Phenols have been reported as highly toxic and hazardous to living organisms (Moschella *et al.*, 2005).

1.3 Justification for the Study

Biological treatment technologies for the remediation of water contaminated with organopollutants are widely because of their environmentally friendly impact combined with low cost compared to other treatment alternatives (Saseket *et al.*, 1998).

Natural attenuation is an alternative to bioaugmentation and biostimulation, natural attenuation can favor contaminant degradation when dealing with historically or heavily contaminated sites (Volgel, 1996). Natural attenuation is connected with the degradation activities of indigenous microorganisms. This method avoids damaging the habitat, allows ecosystem revert to its original condition and enables detoxification of toxic compounds. Natural attenuation using fungi is able to perform the breakdown of a wide range of organopollutants (Field *et al.*, 1993). Fungi display a high ability to immobilize toxic metals by either insoluble metals oxalate formation, biosorption or chelations into melanin-like polymers (Baldrian 2003). The use of *Cladosporium* to bioremediate KRPC effluents will play a great role in solving the problem of refinery effluent contamination. Therefore, *Cladosporium* isolate is a reliable bioremediation tool that can be used to effectively treat contaminants in refinery effluents.

1.4 Aim of the Study

The aim of this study was to bioremediate hydrocarbon contaminated water using *Cladosporium* species.

1.5 Specific Objectives

The specific objectives of this study were to;

1. Determine the physicochemical properties, heavy metals and total hydrocarbon concentration of hydrocarbon contaminated water samples before bioremediation.
2. Isolate and characterize *Cladosporium* spp from hydrocarbon contaminated water samples.
3. Utilize the isolated *Cladosporium* spp to bioremediate the hydrocarbon contaminated water samples.
4. Determine the physicochemical parameters, heavy metals and total hydrocarbon concentration of the bio remediated water samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Cladosporium*

Cladosporium is a pigmented mold most often found to be air borne or on rotten organic matter, it is found both indoors and outdoors. Colonies range from dark green to black colour. They are relatively slow growing. The dark spores are usually one to two celled and occur in long, branching chains that arise from a dark conidiospore. The youngest spores are usually found on the top of the chain as described by Ellis (1976). The most effective way to distinguish the genus is by the prominent scars on the spores where the adjacent spores were attached. *Cladosporium* colonies are rather slow growing, mostly olivaceous-brown to blackish brown but also sometimes grey, buff or brown, often becoming powdery due to production of abundant conidia. Vegetative hyphae, conidiospores are more or less distinct from the vegetative hyphae, erected straight or flexuous, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia are 1-4 celled, smooth, and verrucose or echinulate, with a distinct dark hilum and are produced in branched acropetal chains. The term blastocatenate is often used to describe chains of conidia where the conidia are at the apical or distal end of the chain. The conidia closest to the conidiophore and where the chains branch, are usually 'shield-shaped'. The presence of shield-shaped conidia, a distinct hilum, and chains of conidia that readily distarculate, are diagnostic for the genus (Domsch *et al.*, 1980). *Cladosporium* species are frequent isolates in air samples taken throughout the year and distributed in countries worldwide (Peterne *et al* 2004). This genus comprises over 30 species, of which several are colonizers of food products and organic

materials or of clinical importance as pathogens. *Cladosporium* species are a dematiaceous (pigmented) mold generally characterized microscopically by darkened spore scarring at points of attachment, irregular to sub-regular shapes, and single to multicellular conidia in readily disarticulating chains (Domsch *et al.*, 1980). Depending on the conditions for growth, *Cladosporium* colonies may be olivaceous brown, green, black, grey, or greenish black, with a powdery or velvety visible texture. Fungi similarly identified as *Cladosporium* species are of the genus *Cladophialophora*, which demonstrate varying clinical, pathogenic and ecological significance.

Samples can be quickly identified to the Genus and Species level (Although, this is conditional) for *Cladosporium* and various other fungal types by bright field compound light microscopy through tape lifts, airborne spore traps, and bulk material collections with suspected fungal growths (Holme, 2006).

2.2 Classification of *Cladosporium*

The result of a study done by San-Blas Gioconda showed that these cells of the genus *Cladosporium*, identified by Link for the first time in 1815, are characterised by the absence of a sexual proliferation phase; therefore, it is classified into the Fungi Imperfecti (Deuteromycota) group. This genus belongs to the mitosporic Ascomycotic phylum, subphylum Pezizomycota, class Dothideomycetes, family Mycosphaerellaceae and contains approximately 500 species (De Hoog *et al.*, 1995). *Cladosporium* species are most frequently found in outdoor and indoor environments, spoiled organic matter and are considered as food important contaminants (De Hoog *et al.* 2000). Additionally, some *Cladosporium* species can develop even on the surface of glass fibres and inside water pipes. These fungi can utilise

different growth substrates, such as, wood plants, dead plants, food, soil, straw and textile. Several species of this genus have been associated with fish diseases. The common ancestor has been identified for only 15 species of *Cladosporium*. The most isolated species are *Cladosporium sphaerospermum*, *Cladosporium cladosporioides*, *Cladosporium herbarum* and *Cladosporium elatum*. In contrast, many species of *Cladosporium* are also able to produce some secondary metabolites such as, antibiotics which are inhibitors of *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* (Gallo *et al.*, 2004).

2.3 Morphological Features of *Cladosporium*

Cladosporium colonies are slow growing, mostly olivaceous-brown to blackish-brown but also sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia. *Cladosporium* constitute vegetative hyphae, conidiophores and conidia are equally pigmented. Conidiophores are more or less distinct from the vegetative hyphae, being erect, straight or flexuose, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia are produced in branched acropetal chains, being smooth, verrucose or echinulate, one to four-celled, and have a distinct dark hilum (Ellis 1971, 1976; Domsch *et al.*, 1980; Crous *et al.*, 2007; Schubert *et al.*, 2007; Zalar *et al.*, 2007; Bensch *et al.*, 2010, 2012). Species with solitary conidia on the host usually have the capacity to produce conidia in chains in culture. The formation of the conidia in chains or solitary is a useful feature to differentiate particular species, but it is not attainable at the generic level. Conidial chains within the genus *Cladosporium* are acropetal, sympodial and often profusely branched. The term ramoconidia has been used by several authors (Ellis 1976; McKemmy and Morgan-Jones, 1991; David, 1997) for those conidia at the base of branches having more than one distal scar. Kirk *et al.*

(2001) provided a definition of the term “ramoconidium”, describing it as a branch of a conidiophore, which functions as a conidium, which means that it represents a detached conidiogenous cell. In *Cladosporium*, they are characterised by having a truncate or slightly convex, unthickened base, without any dome or raised rim, which could be confirmed by light and scanning electron microscopy (Schubert, 2005).

2.4 Hydrocarbons

Petroleum is a complex mixture of hydrocarbons and other organic compounds, including some organometallo-constituents. Petroleum constituents represent: saturates, aromatics, resins and asphaltenes. Saturates are defined as hydrocarbons containing no double bonds. They are categorized according to their chemical structures into alkanes (paraffins) and cycloalkanes. Saturates represent the highest percentage of crude oil constituents. Aromatic hydrocarbons with one or several aromatic rings are usually substituted with different alkyl groups. In comparison to the saturated and aromatic fractions, the resin and asphaltenes contain non-hydrocarbon polar compounds. Resins and asphaltenes have very complex and mostly unknown carbon structure with addition of many nitrogen, sulfur and oxygen atoms (Harayama *et al.*, 2004).

Hydrocarbon is an organic compound consisting entirely of hydrogen and carbon. Hydrocarbons are the main pollutants from oil refineries and oil spills. Hydrocarbons differ in their susceptibility to microbial attack and, in the past, have been generally ranked in the following order of decreasing susceptibility: n-alkanes, branched alkanes (low molecular weight aromatics) and cyclic alkanes (high molecular weight aromatics) (Leahy and Colwell, 1990).

Petroleum recovered from different reservoirs varies widely in compositional and physical properties (Malatova, 2005). The composition of a particular petroleum product ranges from the very low molecular weight hydrocarbons to the very high molecular weight hydrocarbons. Hydrocarbons' chemical structure affects its biodegradation in two ways. First, the molecule may contain groups or substituent that cannot react with available or inducible enzymes. Second, the structure may determine the compound to be in a physical state where microbial degradation does not easily occur. Usually, the larger and more complex the structure of a hydrocarbon, the more slowly it is oxidized (Navarre, 2014) Also the degree of substitution affects the degradation properties.

Hydrocarbons differ in their solubility, from polar compounds, such as methanol to very low compounds that contain amine, methoxy and sulfonate groups, linkages, halogens and branched carbon chains are generally persistent. Adding aliphatic side-chains increases the susceptibility of cyclic hydrocarbons to microbial attack (Riser-Roberts, 1992). Hydrocarbon composition affects the physicochemical solubility of non-polar compounds, such as high molecular weight polynuclear aromatic hydrocarbons. Solubilization is not the only factor determining the degradation of hydrocarbons. Many microorganisms, such as *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus laterospor* excrete emulsifiers that increase the surface area of the substrate. On the other hand, these microorganisms modify their cell surface to increase its affinity for hydrophobic substrates and, thus facilitate their absorption (Cybulski *et al.*, 2003; Carvalho and Fonseca, 2005). Hydrocarbons can be very viscous and very volatile or relatively non-volatile. Viscosity of polluting oils is an important property. It determines the spreading and dispersion of the hydrocarbon mixture and also the surface area available for microbial attack

(Leahy and Colwell, 1990). Variability in the physicochemical character of hydrocarbons causes changes in the behavior of individual hydrocarbons as well as mixtures (Malatova, 2005).

The concentration of organic compounds in the environment also affects the level of tolerance. At low concentrations, all fractions are likely to be attacked. However, at high concentrations, only those fractions most susceptible to degradation will be broken down. Also the concentration of contaminants will affect the number of organisms present. It has been shown that the higher concentrations of gasoline in contaminated water were related to higher counts of microorganisms (Doong and Wu, 1995).

2.5 History of Oil Contamination in Nigeria

In 1956, Shell British Petroleum (now Royal Dutch Shell) discovered crude oil at a village Oloibiri in Bayelsa State located within the Niger Delta of Nigeria (Anifowose, 2008; Onuoha, 2008) and commercial production began in 1958. Human activities and those of oil exploration and exploitation raise a number of issues such as depletion of biodiversity, coastal and riverbank erosion, flooding, oil spillage, gas flaring, noise pollution, sewage and wastewater pollution, land degradation and soil fertility loss and deforestation, which are all major environmental issues. Oil exploration and exploitation has been ongoing for several decades in the Niger Delta. A major 1970 oil spill in Ogoni land (Bomu II) in the south-east of Nigeria led to thousands of gallons being spilt on farm lands and rivers, ultimately leading to 26 million dollar fine for Shell in Nigerian courts 30 years later. According to the Nigerian Government, there were more than 7,000 spills between 1970 and 2000 (Anifowose, 2008; Onuoha, 2008).

In 1990, the government announced a new round oil field licensing, these largest since the 1960s. Non-violent opposition to the oil companies by Ogoni people in the early 1990s over the contamination of their land and lack of financial benefit from the oil revenues attracted international attention (Anifowose, 2008; Onuoha, 2008).

There are no consistent figures of the quantity of crude oil spilled in Niger Delta but it is widely believed that an estimated 13 million barrels (1.5 million tons) of crude oil have been spilled since 1958 from over 7000 oil spill incidents; a yearly average of about 240,000 barrels (Anifowose, 2008; Onuoha, 2008).

2.6 Environmental Factors Affecting Hydrocarbon Biodegradation.

Hydrocarbon biodegradation can occur over a wide pH and temperature range. The biodegradation rate generally increases from the psychrophilic to mesophilic temperatures. The optimum temperature for biodegradation has been reported in the range of 25–40°C (Van Hamme *et al.*, 2003). Temperature influences hydrocarbon biodegradation by affecting the physical state and chemical composition of oil as well as the metabolic activities and composition of the microbial community (Crawford and Zhou, 1995; Walworth and Reynolds, 1995). *Bacillus* sp. was reported to be capable of growing on petroleum hydrocarbons in the range of 45–70°C (Klug and Markovetz, 1971; Sorkhoh *et al.*, 1995). A psychrophilic strain of *Rhodococcus* sp. was found to mineralize short-chain alkanes to a greater extent than long-chain alkanes at low temperatures of 0 and 5°C (Whyte *et al.*, 2002). Low temperatures also reduce the rates of volatilization of low molecular weight hydrocarbons.

Studies on the feasibility of bioremediation as a treatment option for a chronically diesel-oil-polluted soil in an alpine glacier area at an altitude of 2,875 m above sea level for three

summer seasons indicated that at moderate temperatures (20–40°C), there were both greater abiotic losses and higher rates of hydrocarbon metabolism (Margesin and Schinner, 2001).

Nutrient availability plays an important role in adaptation of microbes and their growth on hydrocarbons. Two major nutrients, nitrogen and phosphorus, are considered to be the most important, as they are required for incorporation of carbon into the biomass. Nitrogen and phosphorus often become limiting factors in hydrocarbon biodegradation, as was found to be the case in Prince William Sound after the spill from Exxon Valdez (Pritchard and Costa, 1991), and the high C:N and/or C:P ratio became unfavourable for microbial growth (Lancelot and Billen 1985). Oil-sludge biodegradation was found optimal at C: N and C:P ratios of 60:1 and 800:1 respectively (Dibble and Bartha, 1979). The importance of oxygen for hydrocarbon degradation is realized by the fact that the major degradative pathways in saturated, cyclic and aromatic hydrocarbons in bacteria and fungi involve oxygenases for which molecular oxygen is required (Cerniglia 1984; Atlas, 1995). To maintain metabolic activities of microbial cells, the oxygen supply rate must match the overall oxygen consumption rate under equilibrium conditions (Huesemann and Truex, 1996).

Anaerobic degradation of petroleum hydrocarbons has also been reported, but the degradation rates were found to be very slow for a practical bioremediation system (Parisiet *al.*, 2009). It has been demonstrated that sulphate-reducing bacteria can utilize aliphatic and aromatic hydrocarbons directly under anoxic/ anaerobic conditions (So and Young, 1999). Bolingaet *al.* (1999) have shown that petroleum hydrocarbon mineralization in anaerobic aquifers was linked to the consumption of oxidants such as O₂, NO₃ or others, with subsequent production of corresponding reduced species. Denitrifying microorganisms also carry out degradation of alkane and alkylbenzenes under anaerobic conditions (Rabus *et al.*, 1999; Ehrenreich, *et al.*,

2000). Reduced bioavailability of hydrocarbons can limit biodegradation, particularly in aged soils that have been contaminated for many years and during the final stages of a soil bioremediation treatment processes (Alexander, 1973). Mass transfer of hydrocarbons into microbial cells is a significant determinant of biodegradation rates and extent. Hydrocarbon bioavailability and subsequent degradation can be improved by addition of chemical surfactants and biosurfactants. However, depending on the physiology of the microbes involved, mass transfer may be affected positively, negatively, or not at all in the presence of a biosurfactant (Das Mukherjee, 2007). In a study, a biosurfactant was found to greatly increase desorption of four-ring PAHs from soil and enhance biodegradation by *Pseudomonas alkaligenes* PA-10, possibly due to a combination of increased solubility and increased biomass, given that the biosurfactant could also serve as a carbon source (Hickey *et al.*, 2007). Thus, microorganisms that overproduce biosurfactants may have an important role in the hydrocarbon degradation process.

2.7 Effects of Refinery Effluents on the Environment

The refining process generates wastewater (0.4-1.6) times the volume of crude oil processed (Coelho *et al.*, 2006). Petroleum and its products are the dominant components of modern industrial society. However, the refining of these fuels poses inevitable environmental risks (Peña-Castro *et al.*, 2006). The refining process consumes large amounts of water. Consequently, significant volumes of wastewater are generated (Coelho *et al.*, 2006) and globally this wastewater has been recognized as hazardous industrial waste as it contains toxic substances such as phenols, hydrocarbons, metal derivatives, surface active substances, sulphides, naphthalene acids, and other chemicals (Suleimanov, 1995; Zhao *et al.*, 2006; Ghulam *et al.*, 2013).

A consequence of processing crude oil in petroleum refineries is the generation of these toxic effluents, which is estimated at 33.6 million barrels per day worldwide (Diya'uddeen *et al.*, 2011). Petroleum refinery effluents (PRE) are wastes originating from industries primarily engaged in refining crude oil and manufacturing fuels, lubricants and petrochemical intermediates (Harry, 1995). These effluents are a major source of aquatic environmental pollution (Wake, 2005). Therefore, discharge of untreated petroleum refining wastewater into water bodies results in environmental and human health effects due to release of toxic contaminants (hydrocarbons, phenol and dissolved minerals (Muneron *et al.*, 2000; Jo *et al.*, 2008; Diya'uddeen *et al.*, 2011).

The refinery effluents could increase the potential of the receiving river to be contaminated with petroleum hydrocarbon pollutants including the aliphatic. Virtually every refinery processes, from primary distillation to final treatment, contains various fractions of oils and other hydrocarbon compounds in their waste waters. The oil and grease in this waste water may appear as free oil, dispersed oil, emulsified oil, soluble oil or as a coating or suspended matter (Singh *et al.*, 2013). Hydrocarbons (benzene, toluene, ethyl benzene and xylene (BTEX) are of serious concern due to their toxicity and as carcinogenic compounds (Irwin, 1997; Farhadiah *et al.*, 2007). High exposure for long periods to these compounds can cause leukemia and tumors in multiple organs (Muneron de Mello *et al.*, 2000; Ishak and Isah, 2012). Phenol and dissolved minerals are also toxic to aquatic life and lead to liver, lung, kidney and vascular system infection (Baristein-Cardoso *et al.*, 2000; Tang *et al.*, 2008). Therefore, according to Environmental Protection Agency (USEPA), petroleum refining waste water have to be sufficiently treated for quality to meet the established regulations (USEPA, 2008). However, Physical and chemical methods of treating Petroleum

refining wastewater are costly due to excessive amounts of sludge production. Thus, biological methods are preferred due to simple, cheap and environmentally friendly operations (Muneronet *al.*, 2000; Dania, 2010). Clearly, there is no universal approach to handling and treating refinery wastewater streams. There have been reports from fishermen of tainted fish tissues development and they attributed to possible pollution of the water body with improperly treated refinery effluents. Already, refinery effluents have been classified by several authors, including Garrettson (2001), Ogbuagu *et al.* (2011) and Okoli *et al.* (2011) as detrimental to public health, especially where they contain petroleum components such as the aliphatic hydrocarbons.

There are many different ways of testing the toxicity of different compounds but there are two main types of tests. Firstly, the acute lethal test which is usually lasts for 96 hours. The aim of this type of test is to find out the lethal concentration of a substance. Secondly, there are sub-lethal tests. These can take many forms but basically test for any sub-lethal reactions that a substance may cause a problem for the individual and / or the population over a long period of exposure (Wake, 2005). Measurements of sub-lethal effects that are often used are respiration rate, growth rate, reproductive success and behavioural changes.

The toxicity of oil refinery effluent is dependent on a number of factors. The volume, quality, salinity and variability of the discharge, the siting of the outfall, the physical and chemical conditions of the discharge area, the proximity of other effluents and pollutants, and the biological condition of the discharge area (Wake, 2005). Some of the different components of the refinery effluent such as pH, temperature, total dissolved solid, total suspended solids, turbidity, biological oxygen demand, phenol, ammonia, sulphides, nitrates and phosphate,

phenol, total hydrocarbon concentration, heavy metals and faecal coliform can have varying effects and toxicities (Wake, 2005).

Toxicity from hydrocarbon exposure can be thought of as different syndromes, depending on which organ system is predominately involved. Organ systems that can be affected by hydrocarbons include the pulmonary, neurologic, cardiac, gastrointestinal, hepatic, renal, dermatologic, and hematologic systems (Mbaneme *et al.*, 2013). The pulmonary system is the most commonly involved system (Lifshitz *et al.*, 2003). Pulmonary complications, especially aspiration, are the most frequently reported adverse effect of hydrocarbon exposure. While most aliphatic hydrocarbons have little gastro-intestinal absorption, aspiration frequently occurs, either initially or in a semi-delayed fashion as the patient coughs or vomits, thereby resulting in pulmonary effects. Once aspirated, the hydrocarbons can create a severe pneumonitis (Mbaneme *et al.*, 2013).

The toxic action of phenol is always associated with loss of cytoplasmic membrane integrity. This results in disruption of energy transduction, disturbance of membrane barrier function, inhibition of membrane protein function and subsequent cell death (Collins and Daugulis, 1997; Yap *et al.*, 1999).

Hydrocarbon pneumonitis results from a direct toxic affect by the hydrocarbon on the lung parenchyma. The type II pneumocytes are most affected, resulting in decreased surfactant production. This decrease in surfactant, results in alveolar collapse, ventilation-perfusion mismatch, and hypoxemia. Hemorrhagic alveolitis can subsequently occur, which peaks 3 days after ingestion (Gross *et al.*, 1963). The end result of hydrocarbon aspiration is interstitial inflammation, intra-alveolar hemorrhage and edema, hyperemia, bronchial necrosis, and

vascular necrosis. Rare pulmonary complications include the development of a pneumothorax, pneumatocele, or bronchopleural fistula (Rodricks *et al.*, 2003).

Prolonged abuse of hydrocarbons can result in white matter degeneration (leukoencephalopathy) and atrophy (Borne *et al.*, 2005). In addition, prolonged exposure to certain hydrocarbons (eg, n -hexane or methyl-n -butyl ketone ([MnBK]) can result in peripheral neuropathy, blurred vision, sensory impairment, muscle atrophy, and Parkinsonism (Garrettson, 2001). Exposure to hydrocarbons can result in cardiotoxicity (Klein and Simon, 1986). Vomiting has been reported in up to one third of all hydrocarbon exposures. The chlorinated hydrocarbons, in particular carbon tetrachloride, are hepatotoxic. Usually, the hepatotoxicity results after the hydrocarbon undergoes phase I metabolism, thereby inducing free radical formation. These free radicals subsequently bond with hepatic macromolecules and ultimately cause lipid peroxidation. This metabolite creates a covalent bond with the hepatic macromolecules, thereby initiating lipid peroxidation.

Aromatic compound is water soluble and highly mobile and as such is likely eventually to reach downstream drinking water sources where, even at very low concentrations, can cause severe odour and taste problems (Nwanyanwu and Abu, 2012).

The Presence of pollutants in normal water alters the quality and often poses serious threats to aquatic life. Various studies have shown positive correlation between pollution from petrochemical and refinery effluents and the health of aquatic organisms (Otokunefor and Obiukwu, 2005). Previous observations suggested a correlation between contaminants of water and sediments with aromatic hydrocarbon from refinery effluents and compromised fish health (Kuehn *et al.*, 1995; Uzoekwe and Oghosanine 2011).

Due to the ineffectiveness of purification systems, wastewaters may become dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystems (Beg *et al.*, 2001; Beg *et al.*, 2003; Otokunefor and Obiukwu, 2005). This indicates the need for a more efficient, cheaper and eco-friendly method of cleaning up wastewater as suggested by Bako *et al.* (2008).

2.8 Bioremediation Using Fungi.

White-rot fungi digest lignin by the secretion of enzymes and give a bleached appearance to wood from undissolved cellulose, hence their name. In contrast, brown-rot fungi degrade cellulose, leaving lignin as a typically brownish deposit. These fungi can also cause checked, cubical cracking and shrinking in wood, which is frequently apparent on felled conifer trees (Stamets, 2005). It has been estimated that some 30% of the literature on fungal bioremediation is concerned with white-rot fungi (Singh, 2006). There are particular mechanisms implicit to white-rot over other kinds of fungi, which offer advantages, e.g. over the use of bacteria, as a means for bioremediation. In particular, bacteria need to be pre-exposed to the particular pollutant they are intended to degrade, in order to induce those enzymes that are required to accomplish the task. There is, furthermore, a pollutant concentration level below which the enzymes are not expressed in bacteria, thus limiting the technology (Adenipekun and Lawal, 2012). A very large range of organic molecules are susceptible to the actions of various strains of white-rot fungi, to varying degrees, and even normally highly intractable and persistent substances, including polyaromatic hydrocarbons (PAH), may be degraded by them (Singh, 2006).

The white-rot fungus *Phanerochaete chrysosporium* is an ideal model for bioremediation by fungi, since it is more efficient than other fungi or microorganisms in degrading toxic or insoluble materials. It presents simultaneous oxidative and reductive mechanisms which permit its use in many different situations, regarding the type of contamination, its degree, and the nature of the site itself. A number of other white-rot fungi also can degrade persistent xenobiotic compounds, e.g. *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, *Irpex lacteus*, *Agaricus bisporus*, *Pleurotus tuber-regium*, *Pleurotus*

pulmonarius(Singh, 2006; Adenipekun and Lawal, 2012). Soils may also be decontaminated from crude oil, with the requirement that lignocellulosic substrates (e.g. sawdust straw and corn cob) are also provided, to support the growth of fungal species in the soil.

Other toxic materials that have been successfully degraded using white-rot fungi are: polychlorinated biphenyls and dioxins, pesticides, phenols and chlorophenols, effluents from pulp and paper mills and dyestuffs (Spadora *et al.*, 1992; Singh, 2006; Chander and Arora, 2007; Kaushik and Malik, 2009). It has been proposed that fungi might be deployed in the bioremediation of sites that are polluted by complex mixtures of (polycyclic aromatic hydrocarbons) PAH, for example from creosote, coal tar and crude oil (Loske *et al.*, 1990). However, it has been shown that the degradation of Benzo[a]pyrene by *Pleurotus ostreatus* is strongly influenced by the presence of heavy metal cations and mediators such as vanillin and 2, 2'-azinobis-(3- ethylbenzothiazoline-6-sulfonate). A 15 M concentration of copper was found to best enhance the degradation (74.2%), which was progressively worsened as the Cu concentration increased. The extent of degradation was increased to 83.6 % when 5m of vanillin was included in the medium (Bhattacharya *et al.*, 2014). The possibility is offered, therefore, that the presence of vanillin (a breakdown product of lignin) might augment the process of mycoremediation using white-rot fungi in actual field-applications. It has been demonstrated (Isikhuemhen *et al.*, 2011) that *L. squarrosulus* can degrade cornstalks significantly after 30 days, with a maximum lignocellulolytic enzyme activity being achieved on day 6 of cultivation, to generate exopolysaccharides. Thus, *L. squarrosulus* might prove very effective in the industrial pretreatment and bio delignification of lignocellulosic biomass. The main reason that white-rot fungi are active to such a wide range of compounds is their release of extra-cellular lignin modifying enzymes, with a low substrate-specificity, so they

can act upon various molecules that are broadly similar to lignin (Adenipekun and Lawal, 2012). The enzymes present in the system employed for degrading lignin include lignin-peroxidase (LiP), manganese peroxidase (MnP), various H₂O₂ producing enzymes and laccase, although these types of enzymatic activity are not present in all lignolytic fungi (Kirk and Farrell, 1987).

2.9 Bioremediation Methods

Bioremediation is eco-friendly, non-invasive, cheaper than conventional methods, and it is a permanent solution that can end with degradation or transformation of environmental contaminants into harmless or less toxic forms (Garbisu *et al.*, 2003; Kulik *et al.*, 2006; Perelo, 2010; Xu and Lu, 2010). Soil bioremediation can be carried out at the place of contamination (*in situ*), or in a specially prepared place (*ex situ*).

2.9.1 *In situ* bioremediation

In situ technology is used when there is no possibility to transfer polluted soil, for example when contamination affects an extensive area. However, challenges exist with in-situ treatment. A major challenge with all in-situ treatment methods is to achieve uniform remediation throughout the treatment area. As contaminated soils are usually heterogeneous, a uniform delivery of chemicals to the whole area is very difficult to achieve (Xu and Lu, 2010; Tomei and Daugulis, 2013; Angelucci and Tomei, 2016).

There are three basic methods of in situ bioremediation with microorganisms: natural attenuation, biostimulation, and bioaugmentation (Perelo, 2010; Pimmata *et al.*, 2013; Suja *et al.*, 2014;).

2.9.1.1 *Natural Attenuation*

Natural attenuation is connected with the degradation activities of indigenous microorganisms. This method avoids damaging the habitat, allows ecosystem revert to its original condition and enables detoxification of toxic compounds. Removal of contamination by the natural attenuation takes a long time because degrading microorganisms in soil represent only about 10% of the total population (Perelo, 2010; Simarro *et al.*, 2013).

2.9.1.2 *Biostimulation*

To accelerate *in situ* bioremediation processes, biostimulation is used in order to modify the physical and chemical parameters of the soil. For this purpose, compounds such as nutrients (e.g. biogas slurry, manure, spent mushroom compost, rice straw and corncob) or electron acceptors (phosphorus, nitrogen, oxygen, carbon) are introduced into the soil (Hamdi *et al.*, 2007; Kauppi *et al.*, 2011; Pimmata *et al.*, 2013; Suja *et al.*, 2014).

2.9.1.3 *Bioaugmentation*

Bioaugmentation is an *in situ* process in which the specific degraders are introduced into the soil (Pimmata *et al.*, 2013, Simarro *et al.*, 2013). This method is applied when the indigenous microflora are unable to break down pollutants, or when the population of microorganisms capable of degrading contaminants is not sufficiently large. To make the process of bioaugmentation successful, microorganisms introduced into the polluted environment as a free or immobilized inoculum should be able to degrade specific contamination and survive in a foreign and unfriendly habitat, be genetically stable and viable, and move through the pores in the soil. Microorganisms can be previously isolated from the contaminated soil and propagated, or their functional ability can be enhanced in the laboratory. Nonindigenous strains or genetically modified microorganisms (GMM) can also be incorporated into the remediated soil (Hamdi *et al.*, 2007; Ueno *et al.*, 2007; Alisi *et al.*, 2009; Simarro *et al.*, 2013).

However, the result of bioaugmentation depends on the interaction between exogenous and indigenous populations of microorganisms because of the competition, mainly for nutrients (Simarro *et al.*, 2013).

2.9.2 *Ex situ* bioremediation

Ex situ methods allow more efficient removal of pollutants, by controlling the physico-chemical parameters, resulting in a shortening of the total time of reclamation. These advantages outweigh *ex situ* methods' disadvantages such as additional cost and risk connected with the possibility of dispersion of the contamination during transport. During the *ex situ* processes contaminated medium is excavated or extracted and moved to the process location. *Ex situ* bioremediation can be done through different methods such as land farming, composting, biopiles and slurry reactors (EPA, 2006; Xu and Lu, 2010; Georgieva *et al.*, 2010; Tomei and Daugulis, 2013).

2.9.2.1 *Landfarming*

Landfarming is one of the most widely used soil bioremediation technologies. In this technology, excavated contaminated soils are spread out in a thin layer on the ground surface. Aerobic microbial activity within the soil is stimulated through the aeration and addition of minerals, nutrients and moisture (Paudyn *et al.*, 2008; Silva-Castro *et al.*, 2015). Landfarming is a relatively simple technology however it is inexpensive and effective for easily biodegradable contaminants only at low concentration (Maila and Cloete 2004; EPA, 2006; Paudyn *et al.*, 2008; Tomei and Daugulis, 2013; Silva-Castro *et al.*, 2015).

2.9.2.2 *Composting*

Composting is a controlled biological process that treats agricultural and municipal solid wastes and sewage sludge using microorganisms under thermophilic and aerobic conditions

(EPA, 2006; Tomei and Daugulis, 2013). Through composting, it is possible to reduce the volume of residues in landfills.

2.9.2.3 *Biopiles*

Biopiles are a more advanced form of composting that are more expensive but enable more effective control of the process and its higher efficiency (Tomei and Daugulis, 2013). It is possible as the aerated composted piles are equipped with the dissolved oxygen, moisture and nutrient control systems and the proper aeration is forced by vacuum or injection system. This technology has been used for remediation of petroleum contaminated soil (EPA, 2006; Golodyaev *et al.*, 2009; Tomei and Daugulis, 2013)

2.9.2.4 *Slurry bioreactors*

Bioremediation processes in slurry bioreactors can be performed under aerobic or anaerobic conditions (Tomei and Daugulis, 2013). These systems utilize naturally occurring microorganisms or strains possessing specific metabolic capabilities to transform toxic compounds (Angelucci and Tomei, 2016). Slurry bioreactors are one of the best applied technologies used in the bioremediation of contaminated soils because they work under controlled operating conditions. It allows for the enhancement of microorganisms activity (Prasanna *et al.*, 2008; Mohan *et al.*, 2009; Angelucci and Tomei, 2016).

2.9.3 Electro Remediation

Electro kinetic remediation has been used to remove metals and polar organic compounds from soils, sludges and sediments (Virkyute *et al.*, 2002). Electrokinetic remediation methods use electrodes with a low-level direct current electric field (usually $<10 \text{ V cm}$ or mA cm^2) installed into the contaminated soil. The current mobilizes and transports charged chemicals in the soils liquid phase towards the electrodes. Negatively charged anions and organic

compounds will move to the anode, whereas positively charged chemicals, such as metals, will move towards the cathode. Contaminants can be removed from the electrodes with several methods including electroplating, adsorption onto the electrodes, pumping near the electrodes, precipitation or coprecipitation at the electrodes, complexing and capturing the contaminants in reactive permeable barriers (Virukyte *et al.*, 2002). The main phenomena affecting the movement of contaminants in electro kinetic remediation are electro-migration and electro-osmosis. Water can also transport uncharged organic and inorganic compounds. Electro-migration is independent of the pore size of the soil, therefore, applicable to both coarse- and fine-grained soils. However, electro-osmosis is ineffective in coarse-grained soils (Virukyte *et al.*, 2002).

Metals are usually present in soil as cations, therefore migrating to the cathode. This migration is further enhanced by the electro-osmotic flow. A number of studies have presented the feasibility of removing metals from soil with electro-kinetics. The most common contaminant metal studied is Lead (Kim *et al.*, 2005). Although, electro remediation of organics is not as widespread as treating metals, positive results have encouraged its implementation.

2.9.4. Phytoremediation

Phytoremediation is the name given to a set of technologies that use different plants as a containment, destruction, or an extraction technique. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites (Susarla *et al.*, 2002; Pulfor and Watson, 2003; Jadia and Fulekar, 2009; Zhang *et al.*, 2009). Phytoremediation is an emerging technology that uses

various plants to degrade, extract, contain, or immobilize contaminants including metals, pesticides, hydrocarbons, and chlorinated solvents from soil and water. Phytoremediation can be classified into different applications, such as phytofiltration or rhizofiltration, phytostabilization, phytovolatilization, phytodegradation (Long *et al.*, 2002), and phytoextraction (Jadia and Fulekar, 2008).

2.9.4.1 *Phytoextraction*

This technology refers that plants absorb metals from soil, sludges and translocate them to the harvestable shoots where they accumulate. The roots and shoots are subsequently harvested to remove the contaminants from the soil. Zhang *et al.* (2009) expressed that as Cd phytoextraction is observed by maize, the percentage of exchangeable form of Cd decreased in the planted soil. Similar finding of decrease in Cd level in soil planted with maize has also been reported by Mojiri (2011).

2.9.4.2 *Phytostabilization*

Phytostabilization referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges (United States Environmental Protection Agency, USEPA, 2000). It is the use of plant roots to limit contaminant mobility and bioavailability in the soil. In phytostabilization, plants are responsible for reducing the percolation of water within the soil matrix, which may create a hazardous leachate, inhibiting direct contact with polluted soil by acting as barrier and interfering with soil erosion, which results in the spread of toxic metals to the other sites (Raskin and Ensley, 2000). Phytostabilization can be used to remediate Cd, Cu, As, Zn and Cr from the soil. Some of the advantages associated with this technology are

that the disposal of hazardous material/biomass is not required (USEPA, 2000) and it is very effective when rapid immobilization is needed to preserve ground and surface waters (Chhotu *et al.*, 2009).

2.9.4.3 *Rhizofiltration*

Rhizofiltration is primarily used to remediate extracted groundwater, surface water and wastewater with low contaminant concentrations (Ensley, 2000). Rhizofiltration involves the use of plants to clean various aquatic environments. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots (USEPA, 2000). Sunflower, Indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove lead from water, with sunflower having the greatest ability (Chhotu *et al.*, 2009).

2.9.4.4 *Phytodegradation*

Phytodegradation is the use of plants and micro-organisms to uptake metabolize and degrade the organic contaminant. In this process, plant roots are used in association with microorganisms to detoxify soil contaminated with organic compounds (Garbisu and Alkorta, 2001). Some plants are able to decontaminate soil, sludge, sediment, and ground and surface water by producing enzymes. This approach involves organic compounds, including herbicides, insecticides, chlorinated solvents, and inorganic contaminants (Pivetz, 2001). Phytodegradation is the breakdown of organic contaminants within plant tissue. Plants produce enzymes, such as dehalogenase and oxygenase that help catalyze degradation. It appears that both the plants and the associated microbial communities play a significant role in attenuating contaminants. It is referred to the degradation or breakdown of organic

contaminants by internal and external metabolic processes driven by the plant (Prasad and Freitas, 2003).

2.9.4.5 *Phytovolatilization*

Phytovolatilization is the use of green plants to extract volatile contaminants, such as Hg and Se, from polluted soils and to ascend them into the air from their foliage (Karami and Shamsuddin, 2010). In other word, Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere (USEPA, 2000). Banuelos (2000) perceived that some plants were able to transform Se in the form of dimethylselenide and dimethyldiselenide in high-selenium media. Unlike other remediation techniques, once the contaminants have been removed via volatilization, one has no control over their migration to other areas.

2.10 Advantages and Disadvantages of Bioremediation

Human effort in cleaning oil spills are more productive, yet man's best effort do more harm than good. Chemical dispersants interfere with natural processes that break down oil, but bioremediation enables the water to activate a self-cleansing process without a negative side effect. Bioremediation had been aimed towards complete degradation of pollutants or at least transformation of hazardous materials into innocuous substances. Some chemical technologies were also developed and applied but due to their cost-sensitivity, technological complexity and increased risk exposure to contaminants for both workers at the site and nearby residents, these approaches were not widely accepted. Hence bioremediation seems to be only better option for the remediation of polluted site in terms of cost effectiveness and environmental friendliness (Robb and Hoggat, 1995). Bioremediation is therefore cost effective, simple and

have minimal risk exposure compared to other technologies e.g incinerators. Low capital expenditure is required in bioremediation. This bioremediation process requires (mycoremediation) less energy and supervision (Robb and Hoggat, 1995).

In a typical waste water treatment process, the goal of effluent treatment is to reduce or remove organic matter (Hydrocarbons), solids, nutrients, disease causing organisms and other pollutants from the treated wastewater before it is discharged into water bodies. Bioremediation is far more disruptive for the environment, it has better public acceptance, and it avoids the need for excavation and heavy traffic as done in the case of incineration

The process is slow. Time required is in days to months. This is because it is a natural process. Substantial gaps exist in understanding of microbial ecology, physiology and genetic expression and site engineering. A stronger scientific base is required for rational designing of process and success.

2.11 Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) By *Cladosporium sphaerospermum*

The ability of *Cladosporium sphaerospermum*, previously isolated from soil of an aged gas manufacturing plant, to degrade polycyclic aromatic hydrocarbons was investigated by Potin *et al.*(2004). In liquid culture, this strain degraded rapidly benzo (a) pyrene during its early exponential phase of growth (18% after 4 days of incubation). Among extracellular ligninolytic enzyme activities tested, only laccase activity was detected in liquid culture in the absence or in presence of benzo (a) pyrene. *C. sphaerospermum* might be a potential candidate for an effective bioremediation of aged PAH-contaminated soil. Sites in the vicinity of gas manufacturing plants have frequently been contaminated with compounds such as polycyclic aromatic hydrocarbons (PAHs), which are produced by incomplete combustion

processes of organic carbon-based material. PAHs are hydrophobic compounds and their persistence in the environment is chiefly due to their low water solubility. This class of compounds is of increasing interest because of their toxic, mutagenic and carcinogenic properties (White, 1986). Consequently, the US Environmental Protection Agency has listed 16 PAHs as priority pollutants. Clean-up of contaminated sites is therefore desirable in order to avoid public health hazards.

Although PAHs may undergo chemical oxidation, photolysis and volatilization, microbial degradation is the major process affecting PAH persistence in nature (Cerniglia, 1993). Bioremediation, expected to be an economic and efficient alternative method to other remediation processes such as chemical or physical ones, has been developed as a soil clean-up technique. However, the success of PAH bioremediation projects has been limited by the failure to remove high molecular weight PAH (Wilson and Jones, 1993). The recalcitrance of PAHs to microbial degradation has been related to their hydrophobic nature. These compounds are thus regularly bound to soil particles, resulting in low bioavailability to microorganisms (Weissenfels *et al.*, 1992; Loehr and Webster, 1996). This phenomenon is especially enhanced in aged contaminated soils, so many biodegradation studies have focused on isolating microorganisms and evaluating their degradative ability on high molecular weight compounds (Field *et al.*, 1995; Haemmerli *et al.*, 1986; Hammel *et al.*, 1986; Pointing, 2001).

Among *Cladosporium* species known to degrade hydrocarbons, *Cladosporium (Amorphotheca) resinae* has received much attention in the last three decades. It was named the 'kerosene' or 'creosote' fungus because of its occurrence in aviation fuel causing damage by clogging filters and corroding pumps and tanks (Parbery, 1969; Neihof and May, 1982). Few studies reported on *Cladosporium sphaerosperum* degradation. This species was first

isolated from a biofilter that had been used to remove toluene from waste gases and was also demonstrated to use toluene, ethyl benzene and styrene as the sole source of carbon and energy (Prenafeta-Boldu *et al.*, 2006). The ability of *C. sphaerospermum* to degrade high molecular weight PAHs suggests potential use for bio augmentation of PAH-contaminated soil, and this isolate was deposited at the CNCM (Collection National de Cultures de Microorganismes Institute Pasteur, Number 1-2255) for potential use for bioremediation processes (Baud-Grasset and Potin, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study area is the wastewater reservoir of the Kaduna Refinery and Petrochemical Company (KRPC). The refinery is located to the west of Kachia Road in Kaduna and occupies a land area of 2.89 square kilometres next to Ungwan Tanko approximately 15km Southeast of Kaduna city (KRPC, 2004). Its location has an elevation of approximately 615m above mean sea level. Kaduna Refinery (Plate 3a) was constructed by the Chiyoda Chemical Engineering and Construction Company (now Chiyoda Corporation) and was commissioned in 1980 with an initial capacity of 100,000 barrels per stream day (BPSD) as the third Refinery in Nigeria in order meet the tremendous and growing demand for petroleum products (KRPC,2004).

3.2 Collection of Samples

The wastewater samples were collected from three different points in the wastewater reservoir (Plate 3b) in Kaduna Refinery and Petrochemical Company. These points were designated A, B and C respectively. The samples were collected into four litre plastic bottles and stored in an ice block cooler and transferred to the laboratory immediately for analysis. All the collected samples were preserved in accordance with guidelines and International Standards. All other quality assurance and quality control procedure relevant to samples

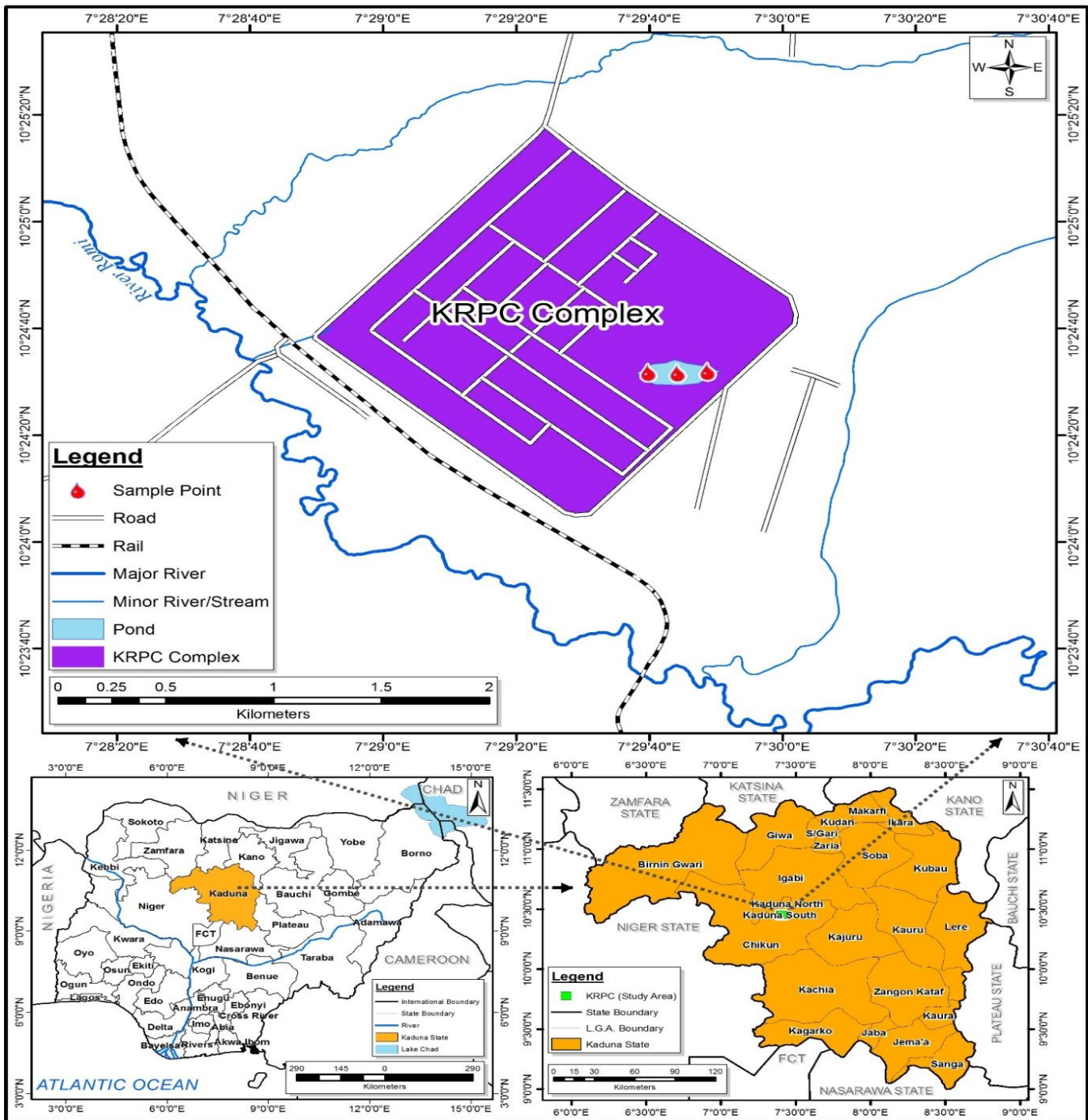


Plate 3a: Map of Kaduna Refinery and Petrochemical Company showing Sampling Points
 Source: Modified from Administrative Map of Kaduna State



Plate 3b: Waste water Reservoir of the Kaduna Refinery and Petrochemical Company

collection, custody and analyses were strictly adhered to for the determination of physicochemical parameters (Ademoroti, 1996; APHA, 1998).

3.3 Determination of Physicochemical Parameters of the Samples before bioremediation using *Cladosporium* Isolate

3.3.1 Determination of pH

The pH was measured using a digital pH meter (Harch Seion). The pH meter and associated electrodes were standardized with phosphate buffer solutions pH 5 and 10. The water sample was filled into a beaker (100ml) and the electrode was placed in the beaker containing the water sample, and readings were taken from the pH meter and recorded appropriately (APHA, 1998).

3.3.2 Determination of Dissolved oxygen

A portion (250 ml) of the sample was collected in a clean sterile bottle and 2ml of manganese sulphate solution was added. A portion (2ml) of Alkali-iodide-azide reagent was added well below the surface of the liquid. It was stoppered with care to exclude air bubbles and mixed by inverting the bottle several times. The precipitate was allowed to settle, leaving a clear supernatant above the floc and shaken again. The setting was allowed to produce 100ml clear supernatant and 2ml of concentrated H_2SO_4 was added. It was restoppered and mixed by gentle inversion. A portion (200ml) of the solution was removed for titration. It was titrated with 0.025N $Na_2S_2O_3 \cdot 5H_2O$ to a pale straw colour. Starch solution (2ml) was added. It was titrated until the first disappearance of the blue colour. The difference between the initial reading and the final reading was taken and recorded as dissolved oxygen (APHA, 1998).

3.3.3 Determination of biochemical oxygen demand (BOD)

The BOD was determined using standard methods described by Ademoroti (1996). A portion (100ml) of the samples was incubated for five days at 20°C in BOD bottles. The dissolved oxygen (DO) content of the samples was determined by adding a portion (2ml) of Alkali-iodide-azide reagent below the surface of the liquid. The setting was allowed to produce 100ml clear supernatant and 2ml of concentrated H₂SO₄ was added. It was restoppered and mixed by gentle inversion. The solution was titrated with 0.025N Na₂S₂O₃.5H₂O to a pale straw colour. Starch solution (2ml) was added. It was titrated until the first disappearance of the blue colour. The difference between the dissolved oxygen of the fifth day and the first day was recorded as the biochemical oxygen demand.

3.3.4 Determination of Electrical Conductivity

Conductivity of the water samples was measured using a digital conductivity meter (HANNA, H198130, Denver, USA). The measuring cell was thoroughly cleansed and rinsed with distilled water and thereafter rinsed with some of the samples to be measured. The cell was filled with the sample and the connecting cable was plugged into the measuring cell socket. The temperature dial was set to the temperature of the sample and the selection switch turned on. The activator button was held down and the measuring dial was slowly rotated until the balance indicator moved to the centre scale. The dial setting at which it occurred was multiplied by the range factor. The result was written in micro seimens per centimetre (μS/cm) (Ademoroti, 1996).

3.3.5 Determination of Turbidity

Turbidity was measured using a digital turbidimeter (2100AN HARCH Model). A portion (100 ml) of the sample was shaken and filtered to disperse the solid contents. The filtered sample was then poured into the turbidimeter tube. The turbidity was observed and recorded accurately based on the manufacturer's instruction (Ademoroti, 1996).

3.3.6 Determination of Total Dissolved solids

Gravimetric method was used to determine the Total Dissolved Solid by evaporation in an oven. The petri dish was weighed and the 100ml of the sample was filtered using filter paper. The filtrate was poured into the petri dish and placed in a boiling water bath to evaporate to dryness. The petri dish was reweighed. The increase in the weight over the empty dish represents the total dissolved solid (APHA, 1998).

3.3.8 Determination of Ammonium Nitrate

A portion (50ml) of sample was measured into a 250 ml beaker. Five (5) drops of Rochelle salt solution was added and mixed. Two millilitres of Nessler's reagent was added and mixed. It was allowed to stand for 10mins for colour development. The Nessler tube was filled and inserted inside a Lovibond comparator to determine the colour intensity (APHA, 1998).

3.4 Heavy Metal Analysis

The samples for Atomic Absorption Spectroscopy (AAS) analysis were filtered using Whatman 0.45 μm filter paper into a clean plastic container and acidified with 3 ml of Concentrated HNO_3 per litre of water (Ramos *et al.*, 1999) and frozen before analysis to prevent loss of metals by surface adsorption (Kahraman *et al.*, 1976).

Furthermore, digestion was carried out by adding a mixture of conc. Nitric acid (HNO_3) and conc. Perchloric acid (HClO_4) (4:1) to 50ml of the samples. The mixture was heated gently on

a hot plate until it was clear and white fumes appeared. This was cooled, filtered and analyzed using the Atomic absorption spectrophotometer (Perkin-Elmer TM 300 USA) for Manganese, Zinc, Cadmium, Copper, and Lead.

3.5 Determination of Total Hydrocarbon Concentration

To obtain the total hydrocarbon concentration, the sample was properly mixed by shaking the bottle containing the sample and 50ml of the sample was transferred into a beaker. The sample was then transferred into a 100ml graduated separatory funnel and 20ml of tetrachloroethylene was added to it. The separatory funnel was shaken vigorously and the cap was removed at intervals to release pressure build up. The separatory funnel was kept standing to allow the content to settle and separate, all emulsions were allowed to break before drawing the extract and the bottom layer was transferred into a clean 50 ml volumetric flask. Another 20 ml of tetrachloroethylene was added into the sample in the separatory funnel. Thereafter, the sample was fed into the quartz cell reagent to establish background spectra (American Society for Testing Materials, ASTM, 1985).

3.6 Isolation and Characterization of *Cladosporium* Sp from Hydrocarbon Contaminated Water Samples (Khan *et al.*, 2013)

The microbiological analysis of the samples was carried out the same day of sample collection to avoid microbial deterioration of the samples. Briefly, the samples were kept to stand at room temperature ($28\pm 3^{\circ}\text{C}$) on a sterile laboratory work bench. 9ml of the sample was aseptically dispensed in sterile centrifuge tubes and centrifuged at a speed of 250rpm for 10 minutes to concentrate the samples. After decanting the supernatant, 0.1ml of the residue of each sample was spread-plated on the sterile solidified laboratory prepared media (Potato

Dextrose Agar plates and Sabouraud Dextrose Agar plates)in duplicates containing 500mg/l of Streptomycin,using sterile bent glass rod(Harrigan and McCance, 1990).

Pure cultures of the fungal isolate were obtained by aseptically subculturing fungal cultures into fresh plates and incubated until the fungus began to sporulate and produce hyphae, followed by subsequent subculturing to obtain pure cultures consisting of only one type of fungal isolate.The pure isolates were identified appropriately using a microscope and Atlas.

3.6.1 Cultural Morphology

Colony growth was observed visually 3 days post incubation from the PDA and SDA plates using the method described by Larone (2002). The colonies were observed daily for colour, shape, and texture (fluffy appearance and tubelike hyphae).

3.6.2 Microscopic morphology

Isolates subjected to further subculture on SDA and PDA plates were later observed after three days of incubation (3days) under the microscope at 40x magnification for hyphae (branched or unbranched, septate or non septate conidiospore)(Antonella *et al.*, 2005).

3.7 Bioremediation of Hydrocarbon Contaminated Water Sample Using *Cladosporium* Isolate

Bioremediation was carried out at room temperature using the method of Singh (2014).The effluents collected were initially sterilized by autoclaving at 121°C for 15 minutes, the samples were removed after cooling. The isolate was then introduced into the water sample using a sterile inoculation loop with internal diameter of 4cm where each of the sampling bottles was inoculated with 3 loop full of the isolates, and these strains were allowed to grow

respectively under white fluorescent light (to enhance spore formation) within the period of 10 days. *Cladosporium* isolates completely colonized the hydrocarbon contaminated water sample within 10 days of bioremediation. Physicochemical Parameters and total hydrocarbon concentration were determined to monitor the progress of bioremediation after 10 days.

3.8 Determination of Physicochemical Parameters, Heavy Metals and Total Hydrocarbon Concentration after Bioremediation

3.8.1 Determination of physicochemical parameters of the bioremediated water samples

Hydrocarbon contaminated water samples were analyzed after bioremediation. Turbidity, Total Dissolved Solids (TDS), Biological Oxygen demand (BOD), Dissolved Oxygen (D.O), Electrical Conductivity (E.C) were determined after bioremediation treatment to determine the bioremediation potential of the fungal isolate. These were carried out using the earlier described method in section 3.4 of the present study.

3.8.2 Determination of heavy metal content of the bioremediated water sample

Heavy metal content was again determined after bioremediation. This was carried out using the steps earlier described in section 3.5 of the present study.

3.8.3 Determination of total hydrocarbon content of the bioremediated water samples

Total hydrocarbon content in water sample was determined by analyzing the concentrations of total hydrocarbons in the treated and untreated hydrocarbon contaminated water sample following the steps described earlier in section 3.6 of the present study.

3.9 Data Presentation

All data obtained were clearly presented in Tables and Plates.

CHAPTER FOUR

4.0 RESULTS

4.1 Determination of Physicochemical Parameters, Heavy Metals and Total Hydrocarbon Concentration of the Samples before and after Bioremediation

4.1.1 Physicochemical parameters

In this study, Electrical conductivity (E.C) was highest at point C (25.7 $\mu\text{S}/\text{cm}$) and lowest at point A (15.74 $\mu\text{S}/\text{cm}$) before bioremediation, but highest at point A (15.40 $\mu\text{S}/\text{cm}$) and lowest at point C (14.95 $\mu\text{S}/\text{cm}$) after bioremediation as shown in Table 4.1a. The percentage removal was highest at point C (26%) and lowest at point A (1%). Turbidity was highest at point A (10.30 NTU) with point C having the lowest value (2.63 NTU) before bioremediation, but was found to be highest at point B (38.10mg/I) and lowest at point A (12.80mg/I) after bioremediation. No percentage removal was observed for turbidity at all the sampling points. The highest concentration of Total Dissolved Solids (TDS) was found at point B (8.50 mg/I) and lowest at point C (2.23 mg/l) before bioremediation but was found to be highest at point C (0.05 mg/I) and lowest at point A (0.03 mg/I) after bioremediation. Similarly, the percentage removal was highest at point A (99.25%) and lowest at point C (95.61%). Table 4.1a further shows that the concentration of Ammonium Nitrates (NH_3N) was highest at point A (0.80mg/l) and lowest at point B (0.12mg/l) before bioremediation but the concentrations of Ammonium Nitrate was highest at point B (0.24 mg/l) and lowest at points A and C (0.20 mg/l) after bioremediation. The percentage removal of Ammonium Nitrate was observed at point A

Table 4.1a Physicochemical Parameters before and after Bioremediation Using *Cladosporium* Species

Parameters	Before	After	%Removal	WHO (2011)
E.C (μS/cm).		1500 μS/cm		
A	15.74	15.40	1	
B	16.59	15.30	4	
C	25.70	14.95	26	
Turbidity(NTU)				10 NTU
A	10.30	12.30		
B	10.00	38.10		
C	2.63	14.10		
TDS (mg/l)		1000 mg/l		
A	8.00	0.03	99.25	
B	8.50	0.04	99	
C	2.23	0.05	95.61	
NH₃N(mg/l)		0.2 mg/l		
A	0.80	0.20	60	
B	0.12	0.24		
C	0.20	0.20		

μ S/cm-micro-Siemens per centimeter,E.C-ElectricalConductivity,NH₃N-Ammonia Nitrate,TDS- Total Dissolved Solids,NTU-Nephelometric turbidity units, mg/l- milligramme per litre and A, B, and C refer to the sampling points

(60%) but was not observed at points B and C. The concentration of Dissolved Oxygen (D.O) before bioremediation was highest at point B (0.75 mg/l) with point C having the lowest concentration(0.60 mg/l) but was found to be highest at point B (0.40 mg/l) and lowest at A and C (0.30 mg/l) after bioremediation(Table 4.1b). The percentage removal of D.O was highest at point A (40%) and lowest at point B (30%). Similarly, Biochemical Oxygen Demand (BOD) was highest at point A (0.52 mg/l) with point C having the lowest (0.20 mg/l) before bioremediation but was found to be highest after bioremediation at point B (0.50 mg/l) and lowest at point C (0.30 mg/l). The percentage removal was highest at point C (20%) and lowest at point C (13%).The highest pH before bioremediation was observed at B (12.00) and lowest at point C (7.40) while the pH after bioremediation was observed to be highest at point C (7.30) while the lowest pH was observed at point A (6.90). The percentage removal for pH was highest at point B (25%) and lowest at point C (0.7%) (Table 4.1b).

Table 4.1b: Physicochemical Parameters before and after Bioremediation using *Cladosporium* Species

Parameters	Before	After	%Removal	WHO (2011)
D.O(mg/l)				5 mg/l
A	0.70	0.30	40	
B	0.75	0.40	30	
C	0.60	0.30	33	
BOD(mg/l)		6 mg/l		
A	0.52	0.40	13	
B	0.50	0.50	0	
C	0.20	0.30	20	
Ph				6.5-8.5
A	10.20	6.90	19	
B	12.00	7.10	25	
C	7.40	7.30	0.7	

DO- Dissolved Oxygen, BOD-Biochemical Oxygen Demand, mg/l- milligramme per litre.A, B, and C refer to the sampling points

4.1.2 Heavy metals

Manganese was highest at point A (2.00 mg/l) while point B had the lowest concentration (0.65 mg/l) before bioremediation, but was observed to be highest at point C (2.86mg/l) and lowest at point A (0.90mg/l) after bioremediation. The percentage removal was highly positive at point A (38.1%) but was not observed at point B and C (Table 4.2). Zinc was observed to be highest at point C (2.86 mg/l) while point B had the lowest concentration (1.97 mg/l) before bioremediation, but highest at point A (0.30mg/l) and lowest at point B and C (0 mg/l and 0.02 mg/l respectively) after bioremediation as shown in Table 4.2. The percentage removal of Zinc was highest at point B (100%) and lowest at point C (77%). The highest concentration of Cadmium before bioremediation was observed at point A (0.56 mg/l) while point C had the lowest concentration (0.38mg/l), but was observed to be highest at point B (0.07 mg/l) and lowest at point B (0.00mg/l) after bioremediation. The percentage removal of cadmium was highest at point C (100%) and lowest at point A (86.60%).The highest concentration of Copper was observed at point A (1.49 mg/l) while point C had the lowest concentration (0.35 mg/l) before bioremediation, but highest at point A (0.13mg/l) and lowest at point B and C (0.00mg/l) after bioremediation. Likewise the percentage removal was highest at point B and C(100%) and lowest at point A (84.00%).The highest concentration of Lead was observed at point C (15.09 mg/l) and the lowest concentration at point B (14.19 mg/l) before bioremediation but was highest at point C (33.98mg/l) and lowest at point A (30.09mg/l) after bioremediation. There was no percentage removal for Lead at all the sampling points (Table 4.2).

Table 4.2 Heavy Metal Concentration before and after Bioremediation using *Cladosporium* Species

Metal (mg/l)	Before	After	%Removal	WHO (2011)
Mn				0.4 mg/l
A	2.00	0.90	38	
B	0.65	1.97		
C	0.98	2.86		
Zn				3 mg/l
A	2.33	0.30	77	
B	1.97	0.00	100	
C	2.86	0.02	98	
Cd				0.003 mg/l
A	0.56	0.04	86	
B	0.44	0.07	72	
C	0.38	0.00	100	
Cu				2 mg/l
A	1.49	0.13	84	
B	0.35	0.00	100	
C	0.38	0.00	100	
Pb				0.01 mg/l
A	14.42	30.09		
B	14.19	32.50		
C	15.09	33.98		

mg/l- milligramme per litre. A, B, and C refer to the sampling points

4.1.3 Total hydrocarbon concentration

The highest concentration was recorded at point A (1.874 mg/l) with the lowest concentration at point C (0.400 mg/l) before bioremediation, but was highest at point A (1.405 mg/l) and lowest at point C (0.450 mg/l) for the sterilized sample and highest at point B (0.937) and lowest at point A (0.703) for the unsterilized samples after bioremediation. The highest percentage removal was observed at point B (45%) and lowest at point A (14%) for the sterilized sample. The percentage removal observed for the non-sterilized samples was at point A (56%) and none was observed for points B and C.

Table 4.3 Total Hydrocarbon Concentration (mg/l) in Contaminated Water Before and After Bioremediation using *Cladosporium* Species

Sample	A	B	C
Before	1.874	1.639	0.400
After			
S	1.405	1.171	0.450
PR (%)	14	45	16
NS	0.703	0.937	0.800
PR (%)	56		

mg/l- milligramme per litre. A,B, and C refer to the sampling points, NS=Non Sterilized, S=Sterilized. PR (%) =Percentage removal

4.2 Isolation and Characterization of *Cladosporium* sp from Hydrocarbon Contaminated Water Sample

4.2.1 Cultural Morphology

Colonies ranging from grey, black to brown in colour with distinct and erected elongations were isolated on SDA as shown in Plate 4.1a while grey to brown colonies were isolated as shown in Plate 4.2a. Pure Black colonies with distinct and erected elongations after subculture on SDA plates were observed as shown in Plate 4.3a

4.2.2 Microscopic Morphology

The observation of the isolate under microscope revealed spores with hilum (Plate 4.4b) while tangled masses of thin stalks (hyphae) with spongy appearance were observed as shown on Plate 4.5b at 40x objective.

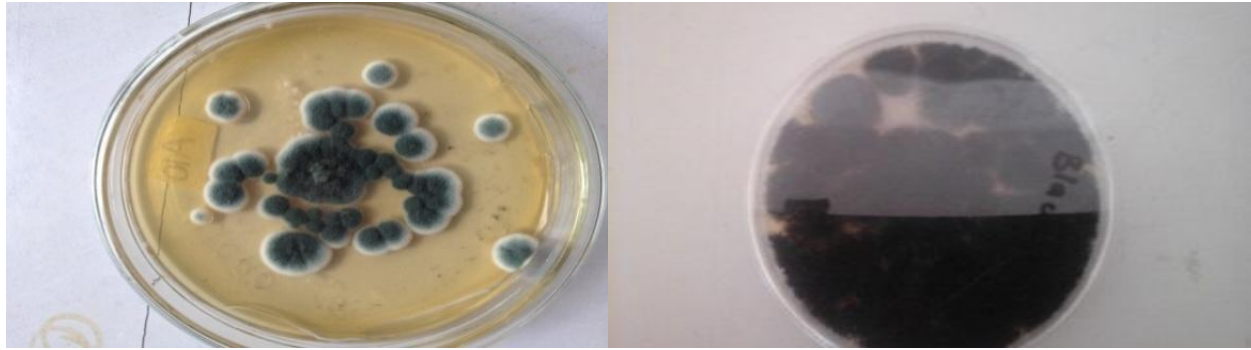


Plate 4.1a *Cladosporium* isolates subculture **Plate 4.2a** *Cladosporium* isolates subculture



Plate 4.3b *Cladosporium* sp **Plate 4.4b** *Cladosporium* sp **Plate 4.5b** *Cladosporium* sp

A= Surface characteristics of sub-culture b=Microscopic characteristics from sub-culture

Table 4.4 Characterization of Fungal Isolate

Cultural Morphology	Microscopic Morphology	Inference
Round, rapidly, growing wooly colony with black and grey areas and black reverse coloration on SDA (Plate 4.1a.).	The observation of the isolate under microscope revealed spores with scars hilum (Plate 4.3b)	<i>Cladosporium</i> Isolate
Pure Black colonies with distinct and erected elongations on SDA (Plate 4.2a).	Non septate, vegetative hyphae, spongy mycelium, conidiospores, scars on spores (Plate 4.4b).	<i>Cladosporium</i> Isolate
Pure black colonies with distinct and erected elongations on SDA (Plate 4.2a).	Erected enlongations with mycelium consisting of black spores at the root (Plate 4.5b)	<i>Cladosporium</i> Isolate

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Parameters, Heavy Metals and Total Hydrocarbon Concentration before Bioremediation

In this study, samples were collected from the wastewater reservoir of the Kaduna Refinery and Petrochemical Company (KRPC) Kaduna. The samples were collected from three points (A, B, and C).

The electrical conductivity (15.74 $\mu\text{S}/\text{cm}$ – 25.70 $\mu\text{S}/\text{cm}$) was below the WHO (2011) standard (1500 $\mu\text{S}/\text{cm}$). This is lower than the electrical conductivity of 158.06 $\mu\text{S}/\text{cm}$ - 1270 $\mu\text{S}/\text{cm}$ reported by Eunice *et al* (2017). This low EC values observed may be as a result of the hydrocarbons not being present in ionic form in the effluent at the time of study. The turbidity values obtained ranged from 2.63 NTU to 10.30 NTU. Turbidity is the measure of relative clarity of a liquid. It is an optical characteristic of water and is an expression of the amount of light that is scattered by material in the water when light is shined through the water sample. This is due to fine particles suspended in the water, causing cloudiness. The higher the intensity of scattered light the higher the turbidity and material responsible for making water to be turbid include soil runoffs. The slight variation of turbidity values across the sampling points may be as a result of natural dilutions down the water course. Turbidity exceeded the World Health Organization (2011) permissible limit at point A. The values of total dissolved solid in the untreated wastewater may be attributed to prolonged accumulation in the sewer without proper dilution. The Total Dissolved Solids (TDS) was within the World Health Organization (2011) acceptable limits of 0-1000 mg/l in all the sampling points. The most highly oxidized form of nitrogen compounds is commonly present in surface and

groundwater because it is the end product of aerobic decomposition of organic nitrogenous matter. Unpolluted natural waters usually contain only minute amounts of nitrate (Jaji *et al.*, 2007). In this study, the values of ammonium nitrate obtained were below the recommended limit by WHO (2011) except for point A where a high concentration of 0.80 mg/l was observed and this may have been as a result of inorganic fertilizers from agricultural runoff.

Dissolved oxygen is an important factor used for water quality control. The effect of waste discharge on a surface water source is largely determined by the oxygen balance of the system and its presence is essential in maintaining biological life within a system (Department for International Development, DFID 1999). Dissolved oxygen concentrations in unpolluted water normally range between 8 and 10 mg/L and concentrations below 5 mg/L adversely affect aquatic life (Department for International Development, DFID, 1999; Rao, 2005). DO standard for drinking purpose is 6 mg/L whereas for sustaining fish and aquatic life is 4-5mg/l(Rao, 2005). The D.O values obtained in this study were below the World Health Organization (2011) acceptable standard of 5 mg/l. Biochemical oxygen demand (BOD) test is useful in determining the relative waste load and higher degree therefore indicates the presence of large amount of organic pollutant and relatively higher level of microbial activities with consequent depletion of oxygen content. The BOD values obtained in this study (0.20-0.52 mg/l) was lower than the BOD values reported by Eunice *et al* (2017). All the BOD values obtained in this study were below the WHO (2011) permissible guideline of 6 mg/l. Result from this study showed that pH values from the effluent before bioremediation at points A (10.20) and B (12.00) were above the permissible limit of 7.0 - 8.5 as specified by WHO (2011). The values obtained in this study were higher than the values of 6.26 - 6.90 reported by Uzoekwe and Oghosanine (2011). The high pH observed could have resulted

from the continuous discharge of the effluent into the wastewater reservoir. The results of the analysis indicated that the Refinery wastewater was polluted, and that if not properly treated before being discharged into receiving water bodies could be a major cause of environmental pollution.

The concentration of Manganese (0.65 - 2.04 mg/l) in all the sampling points exceeded the World Health Organization (2011) permissible limit of 0.4mg/l. The concentration of Zinc (1.97-2.86 mg/l) falls within the World Health Organization (2011) permissible limit of 3 mg/l. The concentrations of Cadmium (0.38-0.56 mg/l) exceeded the World Health Organization (2011) Standard limit of 0.003mg/l while concentration of Copper (0.35-1.49 mg/l) recorded in all the sampling point was below the WHO (2011) permissible limit of 2.0 mg/l. Nickel was not detected in all the sampling points, which indicated that Nickel was absent. The concentration of Lead recorded (14.19-15.09 mg/l) exceeded the permissible limit of 0.01 mg/l set by the WHO (2011). The high concentration of these metals in the untreated wastewater could be attributed to slow dilution due to accumulation of the metals. It may also have originated from feedstock, corrosion products of equipment and pipes and chemical additives used in downstream processes (Beddri and Ismail, 2007; Stuart and Milne, 2008).

Petroleum hydrocarbons have been observed to be toxic to aquatic life. The total hydrocarbon concentration observed ranged from 0.400 to 1.874 mg/l. It has been observed (Pollino *et al.*, 2003) that a water accommodated fraction of crude oil or dispersed crude oil water accommodated fraction increased the activity of gill citrate synthase at a concentration of 14.5mg/l. Lipophilic hydrocarbons have been observed to accumulate in the membrane lipid bilayers of microorganisms and interfering with their structural and functional properties (Sikkema *et al.*, 1995). Uzoekwe and Oghosanine (2011) noted that hydrocarbons when

discharged into water body can cause depletion of dissolved oxygen due to transformation of organic components into inorganic compounds, loss of that is important in food chain and eutrophication. Short term toxicity in fishes includes lymphocytosis, epidermal hyperplasia and haemorrhagic septicaemia (Beeby, 1993).

5.2 Isolation and Characterisation of *Cladosporium* Isolate

Cladosporium sp was isolated from the wastewater sample. This is due to the fact that the organism is mostly associated with oil contaminated water. This agrees with the findings of April *et al.* (2000) and Kingsley *et al.* (2015) in their study on the isolation and characterization of microorganisms from oil polluted soil in Kwata, Awka South, Nigeria which recorded the isolation of *Cladosporium* sp among other organisms.

Microscopy revealed colonies with the same colour as the primary culture. Vegetative hyphae and spores were visible using (40x) objective. Spores were distinct from the vegetative hyphae, straight and were branched in the apical region with elongations while the hyphae were non septate. This is in agreement with the findings of Kingsley *et al.*(2015) where conidiophores with dark hyphae and sparsely branching long chains of conidia were observed.

5.3 Bioremediation of Hydrocarbon Contaminated Water Using *Cladosporium* Isolate

Bioremediation of the hydrocarbon contaminated water sample was achieved given the values of physicochemical parameters, heavy metals and total hydrocarbon concentration obtained after bioremediation. *Cladosporium* isolates exhibited degrading potential for hydrocarbons.

5.4 Physicochemical Parameters, Heavy Metals and Total Hydrocarbon Concentration after Bioremediation

Dissolved oxygen (DO) decreased after bioremediation (0.70-0.30 mg/l for A, 0.75-0.40 mg/l for point B and 0.60-0.30 mg/l for C). Bioremediation is an oxidation process which utilizes dissolved oxygen for the degradation of the organic matter. This process is more intense in the cases of organic molecules with high oxygen demand such as petroleum hydrocarbons (Pichtel, 2007). This demand for oxygen could have resulted to the decrease in dissolved oxygen after bioremediation.

Electrical conductivity of water samples is used as an indicator of how salt free, ion free or impurity free the sample is. It is very important for the control of wastewater pollution level. Electrical conductivity values decreased after bioremediation. This result is in agreement with the findings of Nwaichi *et al.* (2013). Turbidity increased after bioremediation. This could have resulted from the *Cladosporium* activity during bioremediation.

Total dissolved solids and ammonium nitrate decreased after bioremediation. The decrease in concentration could be attributed to the bioremediation effect of the *Cladosporium* isolate. The isolates could have utilized ammonium nitrate for the degradation of the hydrocarbon.

The BOD₅ concentration at point A decreased after bioremediation and point B remained unchanged. However, an increase was observed at point C from 0.20mg/l to 0.30 mg/l. The decrease could be as a result of high oxygen demand required by the organism for the biodegradation of hydrocarbons. There was a drop in pH level from 12 to 7.1. This could be as a result of accumulation of acidic metabolites in the medium. It can also be inferred from this work that bioremediation occurred in alkaline environment and have resulted to change in the pH level of the hydrocarbon contaminated water. The pH is obtained after bioremediation is in agreement with the findings of Tanee and Kinako (2008).

The concentration of some heavy metals decreased after bioremediation (Zinc, 0.30-2.33 mg/l for A, 0-1.97 mg/l for B and 0.02-2.86 mg/l for C; Cadmium, 0.40-0.56 mg/l for A, 0.07-0.44 mg/l for B and 0-0.38 mg/l for C; Copper, 1.13-1.49 mg/l for A, 0-0.35 mg/l for B, 0-0.38 mg/l for C). The success of bioremediation for these metals is reflected in their high percentage removal (77-100% for Zinc, 72-100% for Cadmium and 84-100% for Copper). These results imply that the introduction of *Cladosporium* species into the water sample have led to a decrease in the concentrations of the heavy metals (Zinc, Cadmium, and Copper) for all the samples after bioremediation. Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Zhou and Kiff, 1991; Fourest and Roux, 1992; Andreset *et al.* 1993; Hussein *et al.*, 2001). The decrease in the concentration of Zinc, Copper and Cadmium could be as a result of fungal bioremediation which is in agreement with the report of Silva *et al* (2009).

However, concentrations of Lead (14.42-30.09 mg/l for A, 14.19-32.50 mg/l for B and 15.09-33.98 mg/l for C) and Manganese (0.65-1.97 mg/l for B and 0.98-2.86 mg/l for C) increased after bioremediation; This could be that the organism was unable to take up Lead and Manganese or that other microorganisms present might have antagonized the *Cladosporium* and prevented it from taking up the heavy metals. It is also possible that the concentration of these metals were toxic to the organism which led to an increase in their concentration. Fungi can be used as excellent source of various extracellular enzymes which influences nanoparticle synthesis (Saxena *et al.*, 2014). Studies have shown the synthesis of nanoparticles of Cadmium either intracellularly or extracellularly by fungal species (Saxena *et al.*, 2014). All the heavy metals were bioremediated with the introduction of *Cladosporium* with the exception of Lead and Manganese which increased after the bioremediation process.

The Total hydrocarbon concentration decreased for both the sterilized and non-sterilized sample after bioremediation. Point A showed a decrease in concentration from 1.874 mg/l to 1.405 mg/l and 1.874 mg/l to 0.703 mg/l for sterilized and unsterilized samples respectively. Similarly, a decrease was observed in point B with sterilized samples from 1.639 mg/l to 1.171 mg/l and 1.639 mg/l to 0.937 mg/l for non-sterilized samples. It can be inferred from these observations that *Cladosporium* sp has potential to degrade hydrocarbons and it is in agreement with similar findings reported by Potin *et al.* (2004). The decrease was higher with unsterilized samples than sterilized samples which indicates that the fungal isolate (*Cladosporium* sp) could have a higher biodegrading potential in unsterilized samples than sterilized sample. It is also possible that *Cladosporium* functions more effectively in the presence of other microorganisms since it has more effect in non-sterilized sample than the sterilized sample as clearly shown in the percentage removal of the hydrocarbon. This suggests that sterilization of samples before bioremediation could inhibit the potential of *Cladosporium* species in bioremediating hydrocarbon contaminated water which is in agreement with the findings of Jha *et al.* (2014). The decrease in total hydrocarbon concentration in samples from point A was higher than the decrease in samples from point B. This could be as a result of the use of chemical dispersant which might have affected the hydrocarbons and in turn the biodegradation of *Cladosporium* sp. At point C, the total hydrocarbon concentration increased after bioremediation from 0.400 mg/l to 0.450 mg/l for sterilized sample and 0.400 mg/l to 0.800 mg/l for non-sterilized samples). This could be as a result of other fungi which were able to synthesize the hydrocarbons in the unsterilized sample. It is also possible that as the *Cladosporium* sp was breaking down the hydrocarbons,

other microbial activities occurred such as the synthesis of hydrocarbons by other hydrocarbon synthesizing microbial consortium (Saxena *et al.*, 2014).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In comparison to WHO (2011) standards, some of the physicochemical parameters of the hydrocarbon contaminated water such as Electrical conductivity (15.74-25.70 μ S/cm), TDS (8.00-8.50 mg/l), dissolved oxygen (0.60-0.75 mg/l) and BOD (0.20-0.52 mg/l) were lower than their respective permissible limits while Ammonium nitrates(0.12-0.8 mg/l), Turbidity (2.63-10.30 NTU) and pH (7.40-12.00) were higher before bioremediation.

The concentration of Zinc (1.97-2.86mg/l) and Copper (0.35-1.49 mg /l) were within their respective WHO (2011) acceptable limits while Manganese (0.65-2.0 mg/l), Cadmium (0.38-0.56mg/l) and Lead (14.42-15.09mg/l) exceeded the acceptable standards before bioremediation. Nickel was not detected in all the sampling points. The total hydrocarbon concentration was high at point A (1.87mg/l) when compared with point B (1.64mg/l) and C (0.40mg/l) before bioremediation.

Cladosporium sp was isolated and identified from the wastewater reservoir of Kaduna Refining and Petrochemical Company.

Bioremediation of the hydrocarbon contaminated water sample was achieved which was indicated by the decrease in the concentration of some of the physicochemical parameters. The study showed a decrease in Electrical conductivity, TDS, DO and pH after bioremediation. The BOD concentration only showed a slight decrease at point A (0.52-0.40 mg/l) after bioremediation but there was no observed decrease at point B and an increase was observed at point C. The Ammonium nitrate (NH₃N) concentration only decreased at point A (0.80-0.20

mg/l) but no decrease in concentration was observed at points B and C. Bioremediation did not decrease the turbidity across all the sampling points.

A decrease was observed in the concentration of some heavy metals after bioremediation. The concentration of Zinc, Cadmium and Copper decreased after bioremediation. This implies that the organism bio remediated these heavy metals in the samples. A decrease in Manganese concentration was only observed at point A (2.00-0.90 mg/l) but no decrease was observed at points B and C. Similarly no decrease was observed in the concentration of Lead across all the sampling points.

The total hydrocarbon concentration decreased for both the sterilized and non-sterilized sample after bioremediation with points A for sterilized and unsterilized samples, point B for sterilized samples and non-sterilized samples respectively. However, an increase was observed at point C for sterilized sample and non-sterilized samples. *Cladosporium* sp showed potential to bioremediate hydrocarbon contaminated water as shown in this study.

6.2 Recommendations

It is therefore recommended from the findings in this study that;

1. Further study should be carried out on the bioremediation of hydrocarbon contaminated water using *Cladosporium* sp at various (improved) environmental conditions to enhance the bioremediation potential of *Cladosporium* species.
2. Government should strengthen the implementation of the application of biological control measures to tackle the menace of water body contamination as result of oil spills.
3. Regulatory agencies should impose direct charges on industrial effluents, as well as continuous monitoring and surveillance in order to ensure the protection of water resources from further degradation.
4. *Cladosporium* species was highly effective for heavy metals and other parameters such as TDS in this study. It is therefore recommended that *Cladosporium* can be used for bioremediation of heavy metals in hydrocarbon contaminated water. Similarly, *Cladosporium* was effective in the bioremediation of hydrocarbons for non-sterilized sample, this implies that the sterilization of effluents before treatment can affect the bioremediation ability of *Cladosporium* for hydrocarbons relatively. Therefore, bioremediation of hydrocarbon contaminated water using *Cladosporium* should be carried out and allowed to take place naturally.

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