

APPLICATION OF *Aspergillus wentii* ENZYME IN CLEANING CRUDE OIL POLLUTED SOIL

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Abstract

Microorganisms have a great metabolic diversity, which allows their ubiquity. Because of their ubiquitous nature, the biotechnological potential of microorganisms is virtually endless, with many possible applications. One of these applications is the utilization of microorganisms or their enzymes in petroleum bioremediation approaches. The contamination of the habitats by crude oil poses major public health and socio-economic hazards. Some fractions of crude oil are toxic to living organisms. The leakage of crude oil into soil damages the biota residing in the soil, including microorganisms and plants, and becomes increasingly toxic beyond a concentration. The extracted enzyme was added to the log of Polluted soil preparation in replicates as Rep 1, Rep2 and Rep3. Control sample was the polluted soil sample without addition of crude enzyme. Each sample was analyzed for residual hydrocarbon at 4 days interval for 12 days. The result is expressed in (mg/kg) in this research the cleaning of crude oil polluted environment using *Aspergillus wentii* was carried out on a polluted site. In the table above, day 0 has residual hydrocarbon of the fungi (*Aspergillus wentii*) presented on the table Rep 1, Rep 2 and Rep 3 has a sample of average 9442.82mg/kg and control of 9543.62mg/kg. In day 4 the residual hydrocarbon of Rep 1, Rep 2 and Rep 3 on the table has a sample average of 5586.87mg/kg and a control of 8703.78 mg/kg In day 8, Rep 1, Rep 2 and Rep 3 has sample average of 2670.42 mg/kg and control of 7937.85 mg/kg. In day 12, the residual hydrocarbon of the fungi *Aspergillus wentii* has a sample average of 1599.55 and control of 7239.32 mg/kg. The sample average has a standard deviation of 3509.53 mg/kg and the control of 992.17mg/kg.

Keyword: fungi, Microorganisms, *Aspergillus wentii*, Pollution, Crude Oil

Introduction

Microorganisms are everywhere; they have colonised diverse environments for thousands of years, including those that, for most organisms, are considered "extreme." In addition to colonising the environment, microorganisms colonise other organisms and are essential to life on our planet. In fact, microorganisms are key components of food webs and biogeochemical cycles and in the maintenance and survival of plants, animals, and other organisms through symbiotic relationships.

Collectively, microorganisms have a great metabolic diversity, which allows their ubiquity. Because of their ubiquitous nature, the biotechnological potential of microorganisms is virtually endless, with many possible applications. One of these applications is the utilization of microorganisms or their enzymes in petroleum bioremediation approaches. Biocatalysis can

provide alternative ways to improve petroleum bioremediation approaches; the screening for enzymes for this purpose is necessary. This paper presents some enzymatic applications for the degradation of petroleum toxic compounds and a discussion about improvements that could be used in petroleum enzymatic bioremediation.

A fungus is simply any member of the group of eukaryotic organisms that includes yeasts and molds, as mushrooms as well. Generally, they are classified as fungi. A fungus (plural fungi) can be single celled or very complex multi-cellular organisms. They are common and can live in any habitat but mostly lived on land, which is mainly in soil or on plant material rather than in sea or fresh water. There is a group called the decomposers, they grow in the soil or on dead plant matter and play an important role in carbon cycling as well as other elements (Carris et al., 2012).

Fungi are more adapted than plants to terrestrial habitats as indicated by their ability to grow at high negative water potentials. They also show wide temperature requirements that range from - 15°C, in psychrophilic (cold-growing) species, to 60°C for thermophilic- heat-growing.

Some are parasitic in nature, and dwells mostly on plants causing diseases such as mildews, rusts, scabs or canker.

Generally in crops, fungal diseases can lead to significant monetary loss to the farmer. In contrast to plants, it is only a very small number of fungi that causes diseases in animals. In humans for example include skin diseases such as athletes' foot, ringworm and thrush.

Many fungi are single-celled, while others are multi-cellular. Single-celled fungi are called yeast. Some fungi intermediate between single-celled yeast and multi-cellular forms which happens to be a function of their current stage of life. Cell walls of fungi do contained chitin, which is a hard substance also found in the exoskeletons of insect's and. arthropods such as crustaceans. There is absence of cellulose, which is usually a common component of plant cell walls.

More importantly, multi-cellular fungi have many hyphae (singular: hypha), which are branching filaments. This hyphae have tubular shape and are split into cell-like compartments by walls that are known as septa. There can be more than one nucleus in these cells, and nuclei and other organelles can move in between them. There have been and there is still debate where or not multi-cellular fungi are truly multi-cellular, due to the fact that organelles and cytoplasm have the tendency of moving from one cell to the other in a process called cytoplasmic streaming. Though they are commonly known as multi-cellular, but are not multi-cellular in the same way as plants and animals, which have enclosed cells. A fungus's network of hyphae is called a mycelium.

Fungi are heterotrophs; that is to say they cannot make their own food and must obtain nutrients from organic material. To do so, their hyphae is used, which elongate and branch off rapidly, allowing the mycelium of the fungus to quickly increase in size. Some fungi hyphae even form root-like threads called Rhizomorphs, which help tether the fungus to the

substrate that it grows on while allowing it to quickly obtain more nutrients from other sources.

Fungi are opportunists, by that it means that they can obtain nutrients from a wide variety of sources and thrive in a wide range of environmental conditions. Some fungi obtain nutrients from dead organic matter; the one that falls within this category are called saprobes and are decomposers, they break down and get rid of dead organisms. Other fungi parasitize plants and are responsible for plant diseases like Dutch elm disease.

Oil (commonly called Petroleum) is a naturally occurring, yellow-to- black liquid found in geological formations beneath the Earth's surface. It is commonly refined into various types of fuels. Components of petroleum are separated using a technique called fractional distillation i.e. separation of a liquid mixture into fractions differing in boiling point by means of distillation, typically using a fractionating column.

It consists of hydrocarbons of various molecular weights and other organic compounds. The name petroleum covers both naturally occurring unprocessed crude oil and petroleum products that are made up of refined crude oil. A fossil fuel, petroleum is formed when large quantities of dead organisms, usually zooplankton and algae, are buried underneath sedimentary rock and subjected to both intense heat and pressure.

Petroleum has mostly been recovered by oil drilling (natural petroleum springs are rare). Drilling is carried out after studies of structural geology (at the reservoir scale), sedimentary basin analysis, and reservoir characterisation (mainly in terms of the porosity and permeability of geologic reservoir structures) have been completed. it is refined and separated, most easily by distillation, into a large number of consumer products, from gasoline (petrol) and kerosene to asphalt and chemical reagents used to make plastics and pharmaceuticals. Petroleum is used in manufacturing a wide variety of materials, and it is estimated that the world consumes about 95 million barrels each day (Guerriero et al 2011; Guerriero et al, 2012).

The use of fossil fuels, such as petroleum, has a negative impact on Earth's biosphere, damaging ecosystems through events such as oil spills and releasing a range of pollutants into the air including

ground-level ozone and sulfur dioxide from sulfur impurities in fossil fuels. The burning of fossil fuels plays a major role in the current global warming.

Petroleum Hydrocarbons degradation is a very hard method that influenced mainly in the amount and nature of the PHs present PHs divided in four categories which are as follows:

aliphatics, aromatics, resins (carbazoles, sulfoxides, pyridines, quinolines and amides) and asphaltenes (phenols, ketones, esters, porphyrins and fatty acids,) (Steliga, 2012). Numerous studies have reported that different environmental factors influence the biodegradation of PHs (Cooney et al. 1985). The limited availability of microorganisms in the environment is the of the most significant factors that restrict biodegradability of oil contaminants. Bioremediation of sites polluted with crude oil are oftentimes limited because of the poor biodiversity of local microbes or the scarcity of local specialized microbes with supplementary substrate properties required for the degradation of various hydrocarbons present in contaminated sites (Ron and Rosenberg, 2014). Some of the PAHs with a high molecular weight are probably not degraded at all.

Degradation of Microbial is a major and a final natural mechanism which can help to clean-up PH contaminants in the environment (Juhasz and Naidu, 2000), Bacteria and filamentous fungi participate in the PH biodegradation (Rahman et al, 2003). In the past few years the biodegradation of ligninolytic fungi has been studied.

Major enzymes in the lignin system including lignin peroxidase, manganese-dependent peroxidase, phenol oxidase (laccases and tyrosinases) and 1-1202-producing enzymes. have been proven to degrade PAHs (Lee et al., 2015). Many species of fungi have been

Statement of the Problem

The wide spread of crude oil in most arable lands, creeks, swamps and natural source of water with crude oil particularly in the Niger Delta region of Nigeria, is due to an increasing petroleum exploration, refining and other related industrial activities. The contamination of the habitats by crude oil poses major public health and socio-economic hazards. Some fractions of crude oil are toxic to living organisms. The

leakage of crude oil into soil damages the biota residing in the soil, including microorganisms and plants, and becomes increasingly toxic beyond a concentration.

Aim and Objectives of the Study

Aim of the Study

The aim of the study is the application of *Aspergillus wentii*

in cleaning crude oil polluted soil.

Objectives of the Study

The following objectives of the study are to be considered:

1. To isolate *Aspergillus wentii* in polluted soil.
2. To extract and apply *Aspergillus wentii* enzyme
3. To make recommendations from the findings.

Significance of the Study

The study will be beneficial to those agency in charge of cleanup process as it enlighten them about the use of enzyme from fungi to restore the environment from crude oil degradation.

The study will be of immense benefit to people resident around crude oil polluted environment, the study would enlighten them about how to use enzyme from fungi to the remediation process of crude oil impacted site.

Scope of the Study

This research work is based on the application of *Aspergillus wentii* enzyme in cleaning crude oil polluted environment.

Limitation of the Study

More than reasonable efforts were made toward this research work to collate sufficient materials to satisfy the content of this work. But just like any other project work, the work is not without constraints in the aspect of literature and energy.

Definition of Terms

Aspergillus wentii: *Aspergillus wentii* is an asexual, filamentous, endosymbiotic fungus belonging to the mold genus *Aspergillus*.

CRUDE OIL: Crude oil is a naturally occurring, unrefined petroleum product composed of hydrocarbon deposits and other organic materials.

BIOREMEDIATION: Bioremediation is a waste management technique that involves the use of organisms to neutralize pollutants from a contaminated site.

FUNGUS: Fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms.

Petroleum and Environmental Pollution

Various activities of the oil industries ranging from exploration, exploitation, transportation, distribution and usage have been reported to affect the environment (Anyanwu et al., 2014). These activities can cause environmental damages (land, air and water pollutions). This is because they are capable of causing immediate physical, chemical and biological damage to the affected ecosystem. These problems arise mainly from the processing and distribution of crude and refined petroleum products in the oil producing areas and to areas needed (Ayotamuno et al., 2006). Petroleum hydrocarbon products affect the fertility status of the soil as most nutrients needed by plants for normal functioning have been reported to be deficient in soil polluted by crude oil (Abii and Nwosu, 2009). Significantly reduction in nitrogen and organic carbon contents of soil has been reported by Agbogidi et al., (2007); Wyszowski and Ziolkowska. (2008). Crude oil reduces water infiltration into the soil (Michael and Ojha. 2006).

Bioremediation

Bioremediation is an approach that facilitates the natural biodegradation process of hydrocarbons through the provision of nutrients and oxygen required by microbes. Bioremediation technologies are cost-effective and resource conservative approaches (Susarla et al., 2002; Lim et al, 2016). The technique was developed in the 1940s and has since been tested on different spill sites. For example in 1994, the effectiveness of a biosurfactant called PES-5 1 for the removal of weathered crude from contaminated sand was investigated in Eon Valdaz oil spill in La Touche Island. The results indicated 70% of the semivolatile components were removed from the sample (Tumeo et al., 1994). In other studies, for example, Prince et al, (2003) demonstrated similar enhanced results through

the addition of fertilizers or external microbes for the removal of contaminants from soil. While the addition of nutrients or microbes may not necessarily increase the rate of contaminant removal (Venosa et al, 1996), the addition of water soluble nutrients without supplements of microbial population for the degradation of contaminants on the shoreline of Delaware Bay did not reflect significant increase in biodegradation rate compared to' natural remediation of contaminants. This was however attributed to multiple factors such as high levels of nitrogen already existing in soil levels significant to influenced contaminant biodegradation (Lim et al., 2016). Thus, extant literature shows that biodegradation demonstrate high potential for effective and successful remediation of hydrocarbon. Three distinctive approaches are adopted in the context of bioremediation, namely, bioaugmentation, bio stimulation and bioventilation.

Bioaugmentation, Biostimulation and Bioventilation

Bioaugmentation is targeted at enhancing the performance of the microbial population through the addition of bacterial with specific catabolic activities, strains or enrichment consortia to increase the rate of contaminant degradation (Abdulsaltn et al, 2011; Lim et al., 2016). This implies an import of some contaminant degrading microbes to the already existing microbial population at the intervention area to quicken the rate of contaminant degradation. One challenge of this approach is that there is no single strain of bacteria that has the requisite metabolic capacity to degrade all oil components. Thus, studies recommend different types of bacteria strains and fungi for the remediation of hydrocarbon contaminants (Lim et al, 2016). The adjustment of environmental parameters such as nutrient introduction, biopolymers and biosurfactants is described as biostimulation (Prince et al, 2003; Jiang et al, 2016). The adjustment of these parameters could stimulate the growth of oil degrading microbes and thus the rate of responsive degradation by the microbes. Available evidence suggest that hydrocarbon degradation at contaminated sites proceed naturally (Odu, 1981; Zuofa et al; 1985). Hofman et al. (2004), however, observed that though the number of soil micro-organisms increased in PHC polluted soils, species richness often decrease

overtime. Similarly, Toogood, et al., 1977 observed that the addition of oil to soils increased the microbial population several fold and this in turn increase the organic matter content of soil. The effect of soil on soil nutrient status is reported to be related to the energy and carbon obtained by soil microbes from decomposition of the soil (McGill, 1976). The influence of nutrient amendments had been demonstrated (Chaineau et al., 2005). The study investigated biodegradation of crude oil in different soil samples including fertilized and unfertilized soil for a period of 150 days. The results indicated that the fertilized soil where nutrients including nitrogen, phosphorus and potassium in the ratio 170:17:48 were added had 62% biodegradation while the unfertilized soil had 47% biodegradation (Chaineau et al., 2005). Bioventilation on the other hand involves the addition of oxygen to the soil voids to stimulate the growth of microbes. Oxygen is a necessity and often the limiting factor in the process of biodegradation as it enhances microbial metabolism of organic matter and generate more energy (Lim et al., 2016). Bioventing has been shown to be effective and efficient in the remediation of a blend of diesel and biodiesel fuel with a higher remediation rate compared to natural attenuation (Thomé et al., 2014). They reported 85% contaminant degradation efficiency compared to 64% observed in natural attenuation after 60 days.

Generally, bioremediation has been researched extensively with success in hydrocarbon removal from soil in laboratories and afield (Dadrasnia and Agamuthu, 2014; Jung et al., 2014; Abed et al., 2015; Lim et al., 2016). Bioremediation has been shown to degrade oil contaminants permanently and successfully and leaves little or no adverse effects on the environment. It is also cost effective and eliminates wastes safely without threatening the wider environment (Lim et al., 2016). However, bioremediation requires longer treatment durations (up to several years) to achieve satisfactory contaminant removal. Comparatively, thermal remediation approaches (e.g. thermal desorption) are quick and could achieve 99% contaminant removal however, they are expensive; utilize excessive energy and produce by-products and unwanted air pollution (Lim et al., 2016).

Concept of *Aspergillus wentii*

Aspergillus wentii is an asexual, filamentous, endospore-forming fungus belonging to the mold genus *Aspergillus*. It is a common soil fungus with a cosmopolitan distribution, although it is primarily found in subtropical regions (Domsch, et al (1980). Found on a variety of organic materials. *A. wentii* is known to colonize corn, cereals, moist grains, peanuts and other ground nut crops. It is also used in the manufacture of biodiesel from lipids and is known for its ability to produce enzymes used in the food industry (Samson, Robert. et al, (2004).

Growth and morphology of *Aspergillus wentii*

Aspergillus wentii produces single-celled, globose, conidia (singular conidium) in unbranched, filamentous chains. Young asexual conidia (also called spores) start off smooth, colourless, and ellipsoidal before maturing into rough, globose spores approximately 4.5–5 µm in diameter. *Aspergillus wentii* conidia can appear anywhere from dark yellow to brown in colour when mature and have a single wall, unlike related species *Aspergillus tamarii* whose conidia have a double wall membrane. The elongating chains of conidia are dispersed through slightly pigmented, vase-shaped structures known as phialides that are around 6–8 µm. The phialides sit on top of almond-shaped structures known as metulae that are about 10–20 µm in length and also slightly pigmented. Together, these metulae and phialides structures radiate outward from a spheroid structure known as the vesicle, layering around its entire surface area. The vesicle can grow to a diameter of 80 µm, with a completely fertile spheroid surface area. Collectively, this large globose complex made up of the vesicle at the centre with metulae and phialides radiating outward is called the conidial head. The conidial head can vary from tan-yellow to darker coffee-coloured brown and grow as big as 500–800 µm in diameter. The conidial head is affixed atop of a thick, aseptate stalk known as a stipe. *Aspergillus wentii* stipes are notable for being interspersed and longer than average *Aspergillus* stalks (Onions, Allsopp, and Eggins, (1981). The stipe and conidial head together form a translucent, rod-shaped structure collectively known as the conidiophore that in turn, extends from the hyphal tip. The conidiophore

can grow anywhere between 3—5 millimeters in length. has a glassy appearance (described as hyaline) and typically have a smooth texture, although granular conidiophores have been observed. *Aspergillus wentii* produces aerial hyphae, white or sometimes yellow in colour that can grow to a few millimeters in length. *Aspergillus wentii* foot cells have dense walls and are branched Domsch, et al (1980),

Overall, *Aspergillus wentii* colonies appear dense, floccose [fluffy] to cottony, and are white in colour. Colonies can grow up to 2-3.5 cm in diameter on Czapek agar when grown under controlled conditions for a span of 7 days. Optimal growth of *Aspergillus wentii* in culture occurs on glucose media at pH 6.0 at a temperature of 30°C for a duration of 7 days.

Reproduction of *Aspergillus wentii*

Aspergillus wentii is an asexual fungus with no known sexual state. Although *Aspergillus wentii* is currently a mitotic fungus, vestigial remnants found in the hyphae of *A.*

wentii are evidence that ancestral *Aspergillus* once had the ability to sexually reproduce by meiosis. Morphological similarities observed between hyphal masses in *Aspergillus wentii* and young sexual structures (cleistothecia) found in *Chaetosartorya chrysellata* are further vestigial evidence of meiotic ability in ancestral *Aspergillus*. Phylogenetic analysis of DNA sequences revealed a strong phylogenetic relation between the obligate asexual species *Aspergillus wentii* and meiotic (sexual) species *Chaetosartorya chrysellata*, suggesting that the two species are close relatives, having recently diverged from the same sexually-reproducing ancestor.

Physiology of *Aspergillus wentii*

Aspergillus wentii is a filamentous fungus. In culture, optimal growth of *Aspergillus wentii* occurs on glucose media at pH 6.0 at a temperature of 30 °C. *Aspergillus wentii* grows well on carbon-based media supplemented with mannitol, fructose, galactose, sucrose, lactose or maltose. Generally, *Aspergillus wentii* exhibits the highest growth rates in carbon-based media, although it can be grown on nitrogen-based media with lower growth yields. *Aspergillus wentii* does not grow well on creatine

sucrose agar (CREA) and produces sterile hyphae on malt extract agar.

Aspergillus wentii is moderately xerophile, able to tolerate very dry conditions with low water activity (with an a_w of 0.73—0.79 for growth and germination). In its natural environment, *Aspergillus wentii* is aerobic, able to grow, replicate, and produce metabolites optimally in an oxygen-rich environment. Under light exposure, *Aspergillus wentii* cultures have been observed to produce white aerial mycelium (at times expressing a pink hue) in large masses that can often expand to fit entire volumes of test tubes or culture plates. *Aspergillus wentii* mycelia have an 8-9 % glucosamine content and have an average doubling time of 4—8 hours in liquid culture Samson, Robert, Ellen and Frisvad, (2004).

Like many *Aspergillus* fungi, *Aspergillus wentii* is resistant to amphotericin B and itraconazole. Thermal death time for *Aspergillus wentii* occurs after 25 minutes at a temperature of 63 °C. Conditions of 100% oxygen pressurized at 10 atm will also cease *A. wentii* fungal growth.

Habitat and Ecology of *Aspergillus wentii*

Aspergillus are collectively classified as indoor mold fungi. *Aspergillus wentii* is typically found as mold on various decomposing vegetable and organic material and is notorious for causing food spoilage in corns, cereals, ground nuts and peanuts. *Aspergillus wentii* can also be isolated from tobacco. As a common soil fungus and endosymbiont, *Aspergillus wentii* often lives in symbiosis with species in rhizospheres (an area of soil populated with roots and home to many microorganisms). Within these rhizospheres, *Aspergillus wentii* can be found amongst cottonseed, olives, barley, rice, pineapple, oats, Brazil nuts, pecans, groundnuts, wheat, fir tree leaf matter, and more. Not only limited to plant and vegetative sources, *A. wentii* has also been associated with bird and gerbil nests.

Distributed in many different parts of the world, *Aspergillus wentii* has been found in countries such as China, Peru, Argentina, Japan, South Africa, France, Pakistan, Guyana, Turkey, India, Spain, Italy, Israel, the Bahamas, the United States and more. *Aspergillus wentii* is most commonly found in warm, subtropical areas such as South America.

Aspergillus wentii has a tendency to colonize dry soils, especially in deserts and warm climates. However, *A. wentii* has been isolated from a variety of cultivated and uncultivated soil types including grassland soils, forest soils, clay isolated from caves and even alkaline soils. It is also common to find *Aspergillus wentii* near water sources such as in seawater, sediments of estuaries (partially enclosed coastal bodies), peat bogs, waste stabilization ponds, water treatment plants and in fresh water sources. In Hawaii, one study found that *Aspergillus wentii* only colonized roots of pineapple plants in regions with higher rainfall and lower soil pH. In addition to moist environments, *Aspergillus wentii* was also found to colonize dry plant stems of *Coptis japonicus* in soil. As an aerobic organism, *Aspergillus wentii* was cited as a rare, trivial component of spora found in the air in Europe.

Application of *Aspergillus Wentii* Enzyme in Cleaning Crude Oil Polluted Environment

According to Hawrot and Nowak (2006) and Anene and Chika (2011), the rate and efficiency of biodegradation depends on the occurrence of adequately numerous and active microflora in the contaminated environment. Organisms that have been reported with potential to degrade hydrocarbons include *Atcaligenes*, *Acme* to bacter spp, *FLavobacterium* spp, *Micrococcus* spp, *Bacillus* spp, *Pseudomonas* spp, *Trichoclerma* spp, *Candida* spp, *Aspergillus* spp, *Rhizopus* spp (Anene and Chika, 2011, Stephen and Egene, 2012, Stephen et al., 2013). According to Okereke et al. (2007) and Chikere et al. (2009), *Aspergillus* species especially *A. niger*, *A. ftercus*, *A. Wentii* and *A. fumigatus* have been found in oil spilled site. Watkinson and Morgan (1990) reported that *Aspergillus* spp are capable of initiating the degradation of n-alkanes by subterminal oxidation, hence their relative abundance in hydrocarbon polluted soil. Ijah and Abioye (2003) reported the presence of *Bacillus* spp on kerosene polluted soil while Stephen et al. (2013) reported the presence of *Bacillus* spp on diesel polluted soil undergoing biodegradation. Okwuote and Ijah (2014) also reported the presence of *Aspergillus niger* and *Bacillus* spp in palm oil mill effluent (POME) polluted soil undergoing biodegradation. Abduhadi and Kawo (2006) reported that spent lubricating oil refers to any lubricating oil

that has served its service properties and considered not fit for its initial purpose. Huge amount of spent lubricating oil are produced worldwide (Khaled et al., 2012). All types of lubricants become contaminated and lose their performance due to changes in their properties (Shakirullah et al., 2006)

The materials and methodology used for the study is outlined in this chapter.

Study Area

The study area for this study is the Kira campus of Kenule Beeson Saro-Wiwa Polytechnics.

Sample Collection

Garden soil was collected from Kira Campus of Kenpoly. The soil was sieved to remove particulate materials. 1 Kg of the sieved soil was weighed and placed in sterile screw cap bottle and conveyed to the laboratory for further analytical procedures.

Sample Preparation -

A measurement of 500ml of Bonny-Light crude oil was done and added to 1Kg of previously sieved soil followed by proper homogenization, *Aspergillus Wentii* was isolated using minimal salt media in a semi-solid state. Biotin and vitamins were added to stimulate fast growth and spore production. Spore suspension was prepared and sub cultured twice to ensure pure cultures of *Aspergillus Wentii*. The addition of crude oil was applied in the vapour phase to ensure the growth of the organism that is capable of utilizing hydrocarbon properly and incubated for 6 days at ambient temperature.

Enzyme Extraction

The semisolid media with *Aspergillus Wentii* was transferred into a suitable beaker and 50ml distilled water added in preparation for centrifuge. The preparation was centrifuged in two steps and at 12000 rpm for 15min the enzyme was separated and decanted. Further filtration of the supernatant with 0.45µm and 0.2µm Millipore membrane filter was done. The enzyme was stored at 4°C.

Application of *Aspergillus Wentii* Enzyme

The extracted enzyme was added to the log of Polluted soil preparation in replicates as Rep 1, Rep2 and Rep3. Control sample was the polluted soil sample without

addition of crude enzyme. Each sample was analyzed for residual hydrocarbon at 4 days interval for 12 days.

RESULT

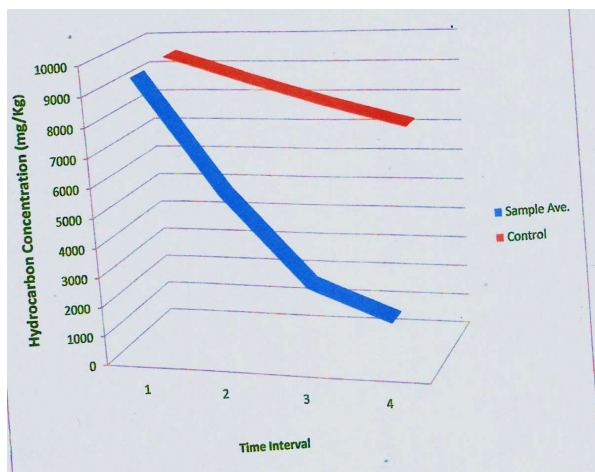
The result obtained from this study is presented in table and in figures.

The data obtained from the residual hydrocarbon are presented in table 4.1 and sample average and control are presented in figure 4.1.

Table 4.1: Initial and Residual Hydrocarbon concentration (mg/Kg) during the study.

Duration	Residual hydrocarbon (mg/kg)					
	Rep 1	Rep 2	Rep 3	Total	Sample Ave	Control
Day 0	9648 .32	9261 .24	9418 .91	28328 .47	9442 .82	9543 .62
Day 4	5210 .09	5829 .46	5721 .06	16760 .61	5586 .87	8703 .78
Day 8	2813 .45	2599 .38	2598 .44	8011 .27	2670 .42	7937 .85
Day 12	1519 .26	1526 .31	1753 .08	4798 .65	1599 .55	7239 .32
SD					3509 .53	992 .17

Comparison of Residual Hydrocarbon in Sample and Content



Key 1 = day 0
2 = day 4
3 = day 8
4 = day 12

Fig. 1. Comparison of Residual Hydrocarbon in Sample and Control

Discussion

Table 4.1 shows the result obtained from this research. The result is expressed in (mg/kg) in this research the cleaning of crude oil polluted environment using *Aspergillus wentii* was carried out on a polluted site, In the table above, day 0 has residual hydrocarbon of the fungi (*Aspergillus wentii*) presented on the table Rep 1, Rep 2 and Rep 3 has a sample of average 9442.82mg/kg and control of 9543. 62mg/kg.

In day 4 the residual hydrocarbon of Rep 1, Rep 2 and Rep 3 on the table has a sample average of 5586.87mg/kg and a control of 8703.78 mg/kg In day 8, Rep 1, Rep 2 and Rep 3 has sample average of 2670.42 mg/kg and control of 7937.85 mg/kg -

In day 12, the residual hydrocarbon of the fungi *Aspergillus wentii* has a sample average of 1599.55 and control of 7239.32 mg/kg.

The sample average has a standard deviation of 3509.53 mg/kg and the control of 992.17mg/kg

Conclusion

Crude oil spill in the environment has been a consistent challenge as long as crude oil exploration takes place oil spillage is bound to occur. This study therefore concluded that *Aspergillus wentii* will be effective, inexpensive and environmentally friendly, oil spillage remediation organism. Thus *Aspergillus wentii* can be applied in cleaning crude oil polluted site.

Recommendation

Based on the effectiveness of *Aspergillus wentii* in the reduction

crude oil impacts on the study sample. This recommended that

Aspergillus wentii

1. Should be utilized in the treatment of refinery effluents before discharging it into the environment.
2. Should be used in crude oil cleaning since it is environmentally friendly.

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