Biodegradation of hydrocarbons in a crude-oil polluted soil using peroxidase from fungal di-culture of *Rhizopus* and *Saccharomyces* spp.

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The biodegradation of hydrocarbon contents in a crude-oil polluted soil using peroxidase from fungi di-culture (*Rhizopus* and *Saccharomyces* spp.) was assessed. The aim was to investigate its efficiency in the degradation of petroleum hydrocarbons from the contaminated soil. This was achieved by physicochemical analysis of soil followed by microbial isolation, enzyme assay, stability, and hydrocarbon biodegradation activity of peroxidase. Analysis of the soil sample revealed that it was acidic (pH 5.02) with a conductivity of 723 µs/cm. Heavy metals identified included Fe, Cd, Hg, Pb, and As. Fungi were isolated from polluted soil using standard microbiological and biochemical techniques. Emulsification analysis was carried out to screen the identified organisms for bio-surfactant production. Peroxidase was tested against varying concentrations of crude oil contaminated soil (5-20%, v/v). Peroxidase from the fungi di-cultures showed optimal degradation strength. Gravimetric evaluation of hydrocarbon biodegradation showed that the peroxidase enzyme degraded a total crude oil weight of 4.02 g, which is approximately 20% of the initial crude oil. The optimum pH for hydrocarbon degradation was 5.5 and the peak degradation was observed on day 12. The efficiency of oil degraded increased with an increase in enzyme concentration from 5-20%. This study has shown that peroxidase has great potential in the biodegradation of hydrocarbons present in crude oil-polluted soil. The results from the present study suggest that the enzyme is of ecotoxicological relevance to environmental remediation.

Keywords: biodegradation; heavy metals; peroxidase; *Rhizopus* sp.; *Saccharomyces* sp.

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Introduction

Environmental pollution from petroleum and petrochemical by-products is recognized as one of the most severe problems in developing countries due to the increasing use of petroleum products. Oil pollution has caused a huge impact in all spheres of life. Petroleum hydrocarbons are a big group of chemicals that have caused a major concern because of their wide distribution in the environment, bioaccumulation potential, harmful effects, and biodegradation resistance [1]. Crude oil-polluted soil is largely ascribed to hydrocarbons and oil effluents from oil refineries, petrochemical industries, human activities, and other sources. In Nigeria, within the coastal region, there are large surface areas contaminated with petroleum hydrocarbons mainly due to accidental spills or leaks from underground deposits [2]. This has caused

significant negative impacts and hazards for agro-ecosystems and human health. Biodegradation of petroleum hydrocarbons is complex and generally requires different microbial species or consortia with specific enzymatic capabilities that can accelerate the rate of petroleum degradation.

Microorganisms have been considered a treasure of useful enzymes. Fungi have a high tolerance to the toxicity of hydrocarbons due to their physiology and adaptations to such variations in the environment and have the mechanism for the elimination of oil from a polluted environment. The fungus is considered as an efficient candidate for potential degradation of hydrocarbons. Hundreds of different species of fungi inhabit the soil, especially near the soil surface where aerobic conditions prevail. Such fungi are active in degrading a wide variety of biological materials present in the soil [3]. Isolated fungi can use the hydrocarbons from polluted soil as substrates for growth by probably releasing extracellular enzymes and acids which are capable of breaking down the recalcitrant hydrocarbon molecules, by dismantling long chains of hydrogen and carbon, thereby converting petroleum into simpler forms or products that can serve as a nutrient for the fungi and absorbed for its growth [4]. The super-family of haem peroxidases from plants, fungi, and bacteria are a group of enzymes that utilize hydrogen peroxide to oxidize a second (reducing) substrate, often an aromatic oxygen donor. Peroxidases catalyze various oxidative reactions in which electrons are transferred to peroxide species (often H_2O_2) and substrate molecules are oxidized. These enzymes have been found in all living organisms, involved in a variety of biological processes [5]. The detoxification of toxic organic compounds by various bacteria and fungi through oxidative coupling is mediated by oxidoreductases such as peroxidase. During such oxidation-reduction reactions, the contaminants are reduced to harmless or less harmful compounds [6].

Peroxidase forms part of the defense system of living organisms against radical-mediated peroxidation of unsaturated lipids. They are ubiquitous in nature and are involved in various physiological processes in microorganisms. Various studies have shown that peroxidases from various sources especially microbial sources have remarkable properties in the degradation of varying concentrations of crude oil and detoxification of polluted environment [6-9], based on their ability to catalyze the reduction of peroxides and the oxidation of a variety of organic and inorganic compounds. It was reported in our previous study that peroxidase was produced and partially purified from a di-culture of *Rhizopus* and *Saccharomyces* spp. isolated from crude oil polluted soil exhibited thermostable properties and may have potentials for bioremediation processes [10]. Thus, this study was carried out to evaluate the biodegradation potential of this peroxidase enzyme isolated from the di-culture of *Rhizopus* and *Saccharomyces* species.

Materials and methods

Sample collection and physicochemical analysis

Soil samples were collected from Onne oil jetty site, a crude oil effluent disposal site located at Eleme, River State, Nigeria, and transferred to the laboratory using sterile polythene bags. The physicochemical parameters of the soil were analyzed as described in the proceedings of the Agency of Toxic substances and disease registry [11]. pH was checked using pH meter (model PHS-3C, Search Tech. Instrument, China) at 1:2.5 soil-water suspension. The electrical conductivity meter (HANNA HI 9933107, Hanna Instruments, Smithfield, RI, USA) was used to check the soil electrical conductance, and the temperature of the soil was extrapolated from it (1: 2.5 soil-water suspension).

Soil moisture content was determined by the oven-dry method. The gravimetric method was used for Sulphate ion. Chloride ion was determined using a method according to Mohr

(4500 B-Cl^-, Argentometric method). Total hardness and calcium hardness were determined using titration method and magnesium hardness was calculated from their values. Jenway PFP7 Flame photometer was used to determine potassium ion. Phosphate was determined by the amino acid method using HI83200 multi-parameter bench photometer at a wavelength of 525 mm. Total petroleum hydrocarbon (TPH) was determined using n-hexane and the spectrophotometric reading was taken at a wavelength of 420 nm after separation. The total organic carbon content (TOC) was determined based on the Walkey and Black chromic acid wet oxidation method. Total organic matter (TOM) was estimated by measuring the weight loss by an oven-dried soil sample when it was well heated (400°C). Iron was identified using a spectrophotometric assay. Heavy metals were determined using Atomic Absorption Spectrophotometer (AAS) Varian AA240 model (Agilent, Santa Clara, CA, USA). A gram of each sample was digested in a 250 mL conical flask by adding 30 mL of aqua regia (HCl) and heated on a hot plate until volume reached 12 mL. The digest was filtered using what-man filter paper and the volume made up to the mark in a 50 mL volumetric flask and was then stored in a plastic container for AAS analysis. The heavy metal was determined using appropriate calibration curves prepared in the same acid matrix with standard metal solutions for atomic absorption spectrophotometer

Isolation and identification of *Rhizopus* and *Saccharomyces* spp. from the crude oil polluted soil

Fungal from crude oil contaminated soil were isolated according to the method described by Ezeonu *et al*. [12]. 10 g of the soil sample was mixed with 100 mL of distilled water, shaken for 30 minutes, and filtered. 10 mL of the filtrate was pipetted into a 25 mL volumetric flask to which 10 mL of hydroxylamine hydrochloric acid was added to. The mixture was shaken for 5 minutes and 5 mL of sodium acetate was added followed with 4 mL of phenanthroline reagent and 30 mL of deionized water. An aliquot of 1 mL was taken and added into a cuvette. Serial dilution of soil sample was done and inoculated on Sabaround Dextrose Agar (SDA) media prepared according to the manufacturer's description. The inoculation plates were incubated at 30°C for 3-4 days for colony growth.

Colonies of different hydrocarbon utilizing fungi and hydrocarbon-degrading fungi were randomly picked and pure isolates were obtained following repeated sub-culturing on SDA. The spores of the isolated fungus were harvested and, aseptically transferred to the SDA slants and incubated at 30°C for 4 days. After three days of culture, the pure fungi isolates were morphologically examined and identified by microscopy. Microscopic slides were prepared using the three-day old pure cultures. A few mycelia were dropped on the slide and a drop of lactophenol blue was added. A coverslip was placed over it and examination performed under the light microscope at 40X magnification according to the method of Ezeonu *et al*. [12], and the features and micrographs were related to "Atlas of mycology" by Barnett and Hunter [13]. The fungal isolates were further identified and characterized using biochemical techniques as described by Cappuccino and Sherman [14] with some modifications. The total heterotrophic biomass from both the nutrient media and the mineral salt agar was counted from the grown media plate as follow:

CFU/g = colony observed × dilution factor × volume of inoculum

Where CFU/g is colony-forming unit per gram.

Screening of the isolates for bio-surfactant production

Prior to the screening for bio-surfactants, the isolates identified were inoculated into 10 mL of Bushnell Haas broth medium (Sigma-Aldrich, Saint Louis, MO, USA) containing 0.2 g/L of $MgSO_4$, 0.02 g/L of $CaCl_2$, 1.0 g/L of KH_2PO_4, 1.0 g/L of K_2HPO_4, 1.0 g/L of NH_4NO_3, 0.05 g/L of $FeCl_3$, 20.0 g/L of agar-agar, pH 7.0 each, and then incubated at 4°C for 72 h. The culture media was

centrifuged using benchtop centrifuge (PAC Pacific) at 3,000 rpm for 30 minutes. The supernatant was collected, and the cells discarded. The supernatant was used for the various bio-surfactant screening assays as described by Mbachu *et al*. [15]. The various emulsifications screening assays were carried out with the culture suspensions:

(1) The drop collapse assay:
The assay was done as described by Jain *et al.* [16]. Briefly, a drop of the culture supernatant was dropped on an oil-coated glass slide and observed after one minute. If the drop of supernatant collapsed and spread on the oil-coated surface, it signifies the presence of bio-surfactant (positive). But if the drop remains after one minute, it was documented as negative. This test was simultaneously carried out on distilled water as a control.

(2) Oil spreading assay:
The assay was done according to the methods of Morikawa *et al*. [17]. Using a micropipette, ten microliters (10 µl) of vegetable oil was added to the surface of 40 mL of distilled water into a petri dish to form a tin oil layer. 10 µl of the culture supernatant was gently dropped on the center of the oil layer. After one minute, a bio-surfactant was present in the supernatant if oil was displaced and a clear zone was seen.

(3) The emulsification capacity of bio-surfactant:
The assay was done according to the method of Cooper and Goldenberg [18]. Briefly, two (2 ml) of kerosene was added to 2 mL of the culture supernatant and the mixture was vortexed or shake at high speed for 2 minutes. The mixture was left for 24 hours; the height of the stable emulsion layer was measured. The emulsion index E24 was calculated as the ratio of the height of the emulsion layer and the total height of the liquid.

E24 = Height of emulsion ÷ total height × 100%

After the bio-surfactant assays, the isolates with maximum bio-surfactant potential were selected as the species for peroxidase production while others were eliminated. The methods for the production, purification, and characterizations of the peroxidase from the selected fungi species are described in our previous study [10].

Hydrocarbon biodegradation studies using peroxidase from the selected isolates (di-culture of *Rhizopus* and *Saccharomyces* spp.)
The ability of the partially purified peroxidase enzyme to degrade hydrocarbons in the polluted soil was assessed using the gravimetric method as described by Ferrera Cerrato *et al*. [19] and Mbachu *et al*. [15]. 20 g of the uncontaminated soil sample was dissolved in deionized water and polluted with 10% crude oil in a 250 mL conical flask and allowed to acclimatize for 7 days. Extraction was done using the n-hexane organic solvent. The hydrocarbon degradation assay was done using a sterile nutrient medium containing 5% crude oil, 5% enzyme suspension, 0.5% phosphate salt, and deionized water. 50 mL of the medium was added to a 200 mL reaction mixture which contained 98 mL of bonny crude oil, 98 mL of peroxidase extract, 2 mL of hydrogen peroxide, and 2 mL of stabilizer in 250 mL conical flasks. The concentration of petroleum hydrocarbon in the reaction mixture was varied from 5-20% (v/v). Given concentrations (5-20%, v/v) of the peroxidase were mixed equally varied in the medium. The reaction mixture was incubated for 20 days at a spinning rate of 180 rpm. The activity of the peroxidase was monitored, and the weight of hydrocarbon degraded was estimated spectrophotometrically (OD) at 660 nm and gravimetrically (equivalent weight loss) at each of the varied parameters. The control contained the same reaction mixture without the addition of peroxidase. During the degradation assessment, some physicochemical factors were varied to ensure optimum condition. The parameters varied included pH, incubation days, crude oil concentration, and enzyme concentration.

Statistical analysis
Microsoft excel worksheet (2010 version) was used for data analysis and plotting of graphs. The

average values of duplicate experiments were taken. Data were expressed as mean ± standard deviation. A difference was considered significant at $p<0.05$.

Results and discussion

Soil physicochemical analysis

The results of the physicochemical analyses of the soil samples obtained from the Onne oil Jetty site are shown in Table 1. The results obtained from the soil analysis of the crude oil contaminated soil showed that the soil contained relatively high hydrogen ion (H^+) concentration with a pH of 5.02 showing an acidic range. Higher soil temperature (33°C), conductivity (723 µS/cm), and soil acidity (11.2) and low moisture content (12.43) were observed in the contaminated soil compared to the control. This could be attributed to the presence of polycyclic aromatic hydrocarbons (PAHs) such as pyrene, naphthalene, etc. in the polluted soil that contains higher acidic contents as has been reported in the proceedings of Agency of Toxic substances and disease registry [11]. The polluted soil showed significantly higher concentration of potassium (12.52 mg/g), magnesium (11.27 mg/g), and chloride (1,151.61 mg/g) ions compared to the unpolluted soil ($p<0.05$). The presence of potassium (K), magnesium (Mg), and chloride (Cl) ions in higher concentrations (mg/g) revealed the level of pollution of the soil with crude oil as well as the impact of other anthropogenic activities going on at the Onne oil jetty site Eleme, such as farming, fishing, trading, truck loading, accidents involving tankers and oil storage facilities, etc. The result showed a slight difference from the unpolluted soil sample which showed a relatively lower concentration of K, Mg, and Cl ions at 7.42, 4.27, and 393 mg/g, respectively. Moreover, calcium and phosphorus (P) were higher in the unpolluted soil compared to the polluted soil. Chikere *et al*. [20], in their study at Eleme petrochemical jetty port site, reported a similar result of ion concentrations in the contaminated Eleme port soil. They revealed a higher concentration of potassium, nitrate, magnesium, and chloride ions in the following order of 2.28, 1.84, 5.22, and 1,789.22 mg/g, respectively. Analysis of heavy metal ions which included iron (Fe), cadmium (Cd), mercury (Hg), arsenic (As), copper (Cu), and lead (Pb) of contaminated soil in this study showed a greater proportion of Fe in the soil with a concentration of 38.74 mg/g, followed by Cu with a concentration of 12.59 mg/g and Pb with 9.69 mg/g, which were significantly higher than that of the unpolluted soil ($p<0.05$). As was found in relatively lower concentrations of 0.45 mg/g in the contaminated sample while it was found below the detectable limit in the control sample (unpolluted soil). Cd and Hg were below the detectable limit in both contaminated and uncontaminated samples. This finding corroborates with findings of Oparaji *et al*. [21], on the bioaccumulation of heavy metals in aquatic faunas and sediments at Eleme River, Port-Harcourt. Their results showed a higher proportion of Fe in all the tested species of aquatic fauna in the contaminated Eleme River while they reported Hg to be below the detectable limit in all the tested faunas and surrounding sediments.

The quantity of Total Petroleum Hydrocarbon (TPH), Total Organic Carbon (TOC), and Total Organic Matter (TOM) in the contaminated soil were 1,672, 10.64, and 7.23 mg/g respectively. This showed a strongly significant difference from the uncontaminated soil (control sample) which showed TPH, TOC, and TOM levels of 342, 2.85, and 2.78 mg/g, respectively ($p<0.05$). Mbachu *et al*. [22] reported a similar finding in their research on microbial diversities in a spent engine oil-polluted site at Mgbuka, Onitsha Anambra State which showed a high level of TOC (4.06 mg/g) and TOM (5.21) contents in the soil. The elevated level of both TPH and TOC of the contaminated soil in comparison to the control in this study is as a direct result of soil contamination with crude-oil deposits via related activities of the oil jetty. The increase in hydrocarbon content in the contaminated sample could be attributed to the metabolic activities of microorganisms that used organic

Table 1. The physiochemical constituents of the petroleum contaminated and uncontaminated soil.

Physiochemical parameters	Polluted Soil	Unpolluted Soil	*p*-value
pH	5.02	7.2	
Temperature (°C)	33.0	24.5	
Soil Conductivity (µS/cm)	723	398	
Soil Moisture Content	12.43±0.1	15.40±0.12	0.022
Soil Acidity (SO_4)	11.2±0.01	1.06±0.1	<0.001
Chloride ion (mg/g)	1151.614±0.36	393±0.18	0.001
Phosphate (mg/g)	1.53±0.02	1.86±0.02	0.04
Magnesium (mg/g)	11.27±0.21	4.27±0.09	0.012
Potassium (mg/g)	12.52±0.45	7.42±0.22	0.036
Calcium (mg/g)	14.23±0.17	22.34±0.17	0.045
Iron (mg/g)	38.74±0.14	6.55±0.02	0.015
Cadmium (mg/g)	BDL	BDL	
Mercury (mg/g)	BDL	BDL	
Arsenic (mg/g)	0.45±0.04	BDL	
Lead (mg/g)	9.69±0.04	0.23 ± 0.01	0.0016
Copper (mg/g)	12.59±0.13	4.28±0.03	0.037
Total petroleum hydrocarbon (TPH) (mg/g)	1672±0.13	342.44±0.01	0.021
Total Organic Carbon (TOC)	10.64±0.12	2.85±0.02	0.038
Total Organic Matter (TOM)	7.23±0.1	2.78±0.01	0.041

Note: BDL-Below Detectable Limit

matter up for energy generation thereby converting them to hydrocarbons.

Microbial isolation and identification

The results obtained during the microbial isolation and identification showed that the contaminated soil had 6.9 x 10^8 and 1.15 x 10^7 colony-forming unit per gram (CFU/g) of total heterotrophic and hydrocarbon utilizing fungi while the uncontaminated soil had 2.58 x 10^8 and 5.5 x 10^2 of total heterotrophic and hydrocarbon utilizing fungi, respectively [10] (Figure 1). This study concurs with that of Chikere and Azubuike [23] which showed a higher level of heterotrophic fungi than hydrocarbon utilizing fungi in crude oil soil confirming that the site was chronically polluted with hydrocarbons. Four fungi isolates which included *Saccharomyces*, *Penicillium*, *Rhizopus*, and *Aspergillus* species were isolated from the polluted soil as reported in our previous study [10].

Fungi are currently optimized active agents in petroleum degradation, and they work as primary degraders of spilled oil in the environment [24, 25]. This result concurs with the finding of Adieze *et al*. [26] on microbial diversities during bioremediation of crude oil-polluted sites using the culture-dependent technique. Also, this finding is in line with the results obtained from the study of Balaji *et al*. [27].

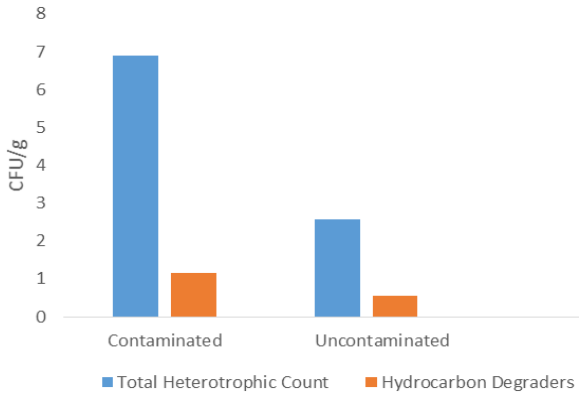

Figure 1. Soil microbial counts (CFU/g) for total heterotrophic and hydrocarbon-degrading organisms from the contaminated and uncontaminated soil.

Emulsification screening assay on the identified isolates

Table 2. Emulsification screening assay result (Drop collapse and Oil spread plate).

Organisms	Drop collapse assay	Oil spread plate (mm)
Rhizopus sp.	++	++
Saccharomyces sp.	+	++
Aspergillus sp.	+	+
Penicillium sp.	-	+

Notes: Drop collapse assay: No collapse (-), slow drop collapse (+), vigorous drop collapse (++). Oil spreading plate: no clear zone diameter (-), clear zone diameter >1 and <3 (mm), clear zone diameter >3 and <6 (mm) (++).

Table 3. The emulsification screening assay (% E24).

Organisms	Diesel + microbial suspensions (%)	Kerosene + microbial suspensions (%)	Crude oil + microbial suspensions (%)
Rhizopus sp.	50	51.2	56
Saccharomyces sp.	46	54	42
Aspergillus sp.	39	36	10
Penicillium sp.	28	39	3

Three emulsification assays carried out which included drop collapse (Table 2), oil spread plate (Table 2), and emulsification index (%E24) (Table 3) assays on the isolates using the crude oil as the sole carbon source without other supplements. The results showed that two fungi isolates (*Rhizopus* sp. and *Saccharomyces* sp.) had higher emulsification potentials. The drop collapse assay and the oil displacement assay using the crude bonny light oil showed that two (*Rhizopus* sp. and *Saccharomyces* sp.) out of the four isolates scored positive results. Moreover, drops of cell-free culture from *Penicillium* sp. remained intact on a glass slide coated with the oil after one hour and could not displace oil too, and therefore, *Penicillium* sp was considered a non-surfactant producer. Also, *Aspergillus* showed a weak collapsing and displacement of the oil at the oil liquid interphase in both the emulsification test conducted as they may have utilized the hydrocarbon for the production of other metabolites. Thus, the spp. of *Aspergillus* and *Penicillium* generally showed less bio-surfactant potentials.

For the oil spread plate assay, the same trend for bio-surfactant activity was observed for the isolated fungi. *Rhizopus* sp. and *Saccharomyces* sp. showed the highest strength in oil displacement in the presence of the crude oil while *Penicillium* sp. showed no positive reaction upon immersion on the oil-water interface. *Aspergillus* showed a weak oil displacement potential for the oil spread plate assay just as in the drop collapse test. Therefore, the spp. of *Aspergillus* and *Penicillium* were eliminated from further studies. This was following the study of Mbachu *et al*. [22] which reported that filamentous fungi have much ability to produce surface-active compounds than the non-filamentous group. Emulsification activity of the culture isolates showed that *Rhizopus* sp. has the best emulsification activity with 51.2% and 56% index in the presence of kerosene and crude oil, respectively. It was followed by *Saccharomyces* sp. with 54% and 48% emulsification activity for crude oil and kerosene. Thus, *Rhizopus* and *Saccharomyces* spp. were used for peroxidase production.

Hydrocarbon biodegradation studies under optimized parameters using peroxidase from di-cultures of *Rhizopus* and *Saccharomyces* spp.

Peroxidase from the fungi di-culture was efficient in degrading crude oil hydrocarbons as the biodegradation analysis under optimized condition. pH (Figure 2), incubation days (Figure 3), varying concentrations of crude oil (Figure 4),

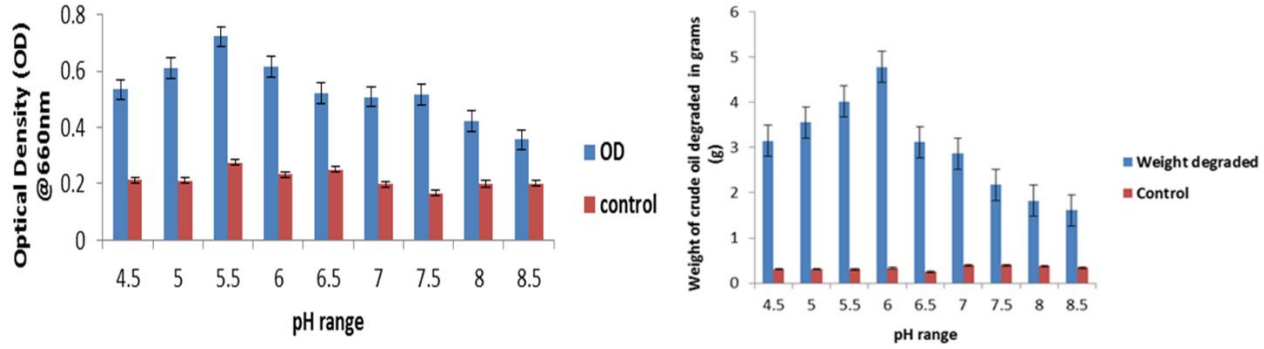

Figure 2. Optical density effect of pH and effect of pH on weight loss of crude oil biodegradation at 10% (v/v) concentration of the peroxidase ($p<0.05$).

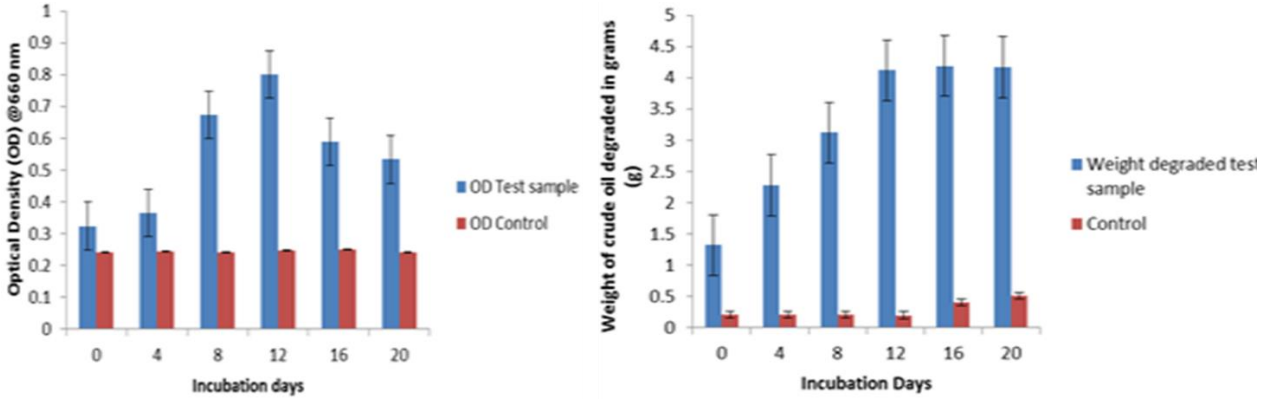

Figure 3. Optical density effect of incubation days and the effect of weight loss of crude oil biodegradation at 10% (v/v) of the peroxidase incubated at pH 5.5 ($p<0.05$).

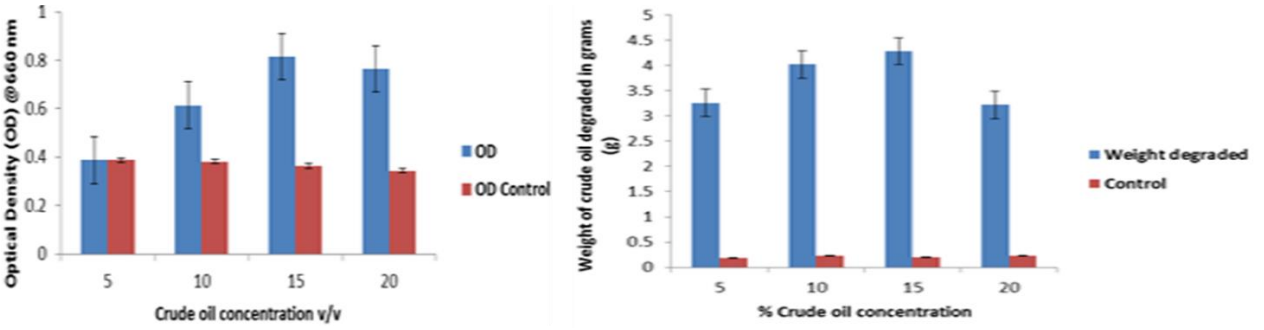

Figure 4. Optical density effect and effect of weight loss of varying concentrations of the crude oil biodegradation at 10% (v/v) peroxidase incubated for 20 days at pH 5.5 ($p<0.05$).

and at varying concentrations of peroxidase (Figure 5) showed the peroxidase to significantly degrade hydrocarbon compared to that of the control ($p<0.05$). The optimum pH for hydrocarbon degradation was 5.5 and the peak degradation was observed on the day 12. The highest weight of oil degraded was 4.02 g. Effective degradation was achieved as the incubation day progresses. An increase in the concentration of peroxidase was proportional to

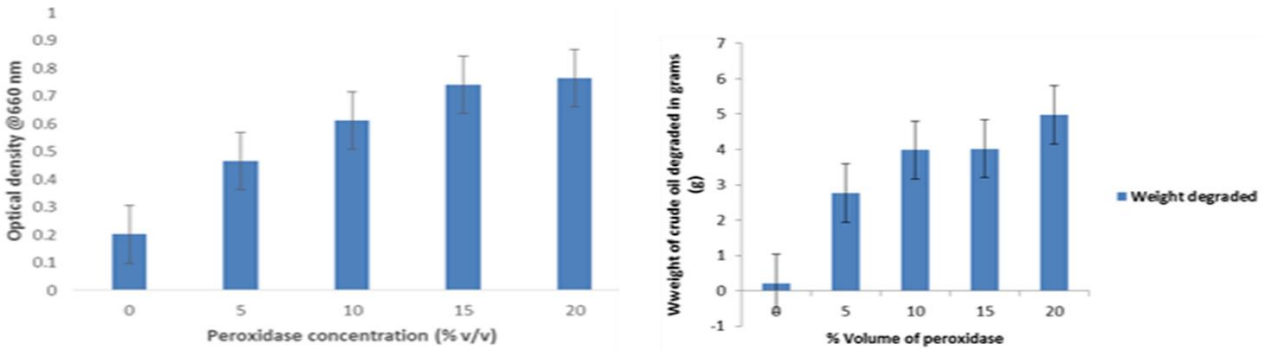

Figure 5. Optical density effect and effect of varying concentrations of the peroxidase produced from the fungal di-cultures on the weight loss during the biodegradation of 10% (v/v) crude oil incubated for 20 days at pH 5.5 ($p<0.05$).

the increase in degradation strength (Figure 5). Overall, approximately 80% of degradation was achieved in all optimized conditions. Fariba [28] evaluated the efficiency of peroxidase isolated from *Streptomyces albus* for crude oil bioremediation. The peroxidase showed 93% removal of benzopyrene from petroleum polluted soil. Lateef *et al*. [29] also reported more than 90% degradation of Bonny light crude oil by two *Pseudomonas* isolates for 21 days and 84.89 % for 5% crude oil concentration. In this study, an increase in crude oil concentration between 5% and 15% (v/v) led to an increase in the efficiency of degradation while an increase of 20% (v/v) of crude oil led to a reduction of the degradation efficiency as shown in Figure 4. This was because higher crude oil concentration saturated the peroxidase enzyme thereby inhibiting its activity. The increase in peroxidase activity also increased the efficiency of crude oil degradation as shown in Figure 5. This finding agrees with the report of Ehiosun and Usman [7] who suggested that total peroxidase was actively involved in the degradation of crude oil.

Peroxidases have proven to be useful in the biodegradation of recalcitrant pollutants. A research carried out by Dubrovskaya *et al*. [30] on the use of peroxidase from alfalfa roots showed that peroxidase possesses catalytic properties and participate in the degradation of polycyclic aromatic hydrocarbons. They can transform various types of recalcitrant aromatic compounds.

Conclusion

The results from this study showed that peroxidase produced from *Rhizopus* and *Saccharomyces* spp. di-culture was efficient in the degradation of hydrocarbon contents of crude oil-polluted soil. Peroxidases among other vital biotechnological applications are useful tools in environmental monitoring. They have very promising biodegradation ability and they are relatively cheap to produce and do not require much augmentation and bio-stimulation in the presence of recalcitrant. However, this is a preliminary study that was limited on a partially purified peroxidase for its biodegradation potential of petroleum hydrocarbons. Moreover, assessments were done *in vitro* in the laboratory, and therefore, further studies are necessary to better purify the peroxidase and ascertain its effectiveness in the field.

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