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Research Article

Co-Kinetic Assessment of Tri-Catalytic Activity of Peroxidase, Urease and Lipase Produced from Hydrocarbonoclastic Bacteria

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Abstract

Biological catalysts foster less adiabatic the transformations of substrates into products and in thereof providing suitable physiologic condition that lower the activation energy. Prevailing environmental physiologic conditions impounds on the microbial physiology necessitating the stability of their respective producing proteins. In the present study, three stress marker enzymes were produced from a hydrocarbonoclastic bacteria isolated from a crude polluted soil using standard microbiology and biochemical techniques respectively. Petroleum hydrocarbon polluted soil from Atali Rivers state from which the bacteria strain was produced showed the following physicochemical properties: pH of 5.44, Soil from Atalia showed electrical conductance of 190(uS/cm) respectively while the control experiment showed conductance of 145(uS/cm). Dissolved mineral contents were found in the following order: SO_4>NO_3>P>N while heavy metals of Hg, As and Cd were found at below detectable limit range (BDL) in both the contaminated and non contaminated soils. Strains of *Pseudomonas* were isolated using standard microbiology and biochemical techniques respectively. Submerged fermentation technique was employed for the respective hydrolytic enzymes production. Lipase, urease and peroxidase production showed optimum production day(s) on day 7, 14 and 7of the 21 days incubation study respectively. Peak activity of the enzymes (Lipase, urease and peroxidase respectively) were recorded at 72, 75.67 and 69.32 µmole/min. in the presence of p-NPP, O-dianisidine and urea respectively. Extracellular enzymes: lipase, urease and peroxidase from *Pseudomonas* sp. showed optimum production at pH 7.5, 7.5 and 4.0 respectively. The study has shown with empirical the kinetic cycles of three catalytic quality marker enzymes from crude oil contaminated soil and their wide range catalytic stability suitable for an ecological remediation.

Keywords

Petroleum Hydrocarbon, Biological Catalyst, Atali, Rivers

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1. Background of the Study

Petroleum, the main hydrocarbon classes found in crude oil are the normal alkanes (rapidly degraded), branched alkanes, and cycloalkanes (difficult to identify), isoprenoids (very resistant to biodegradation), the aromatics(fairly identified and much more soluble than other hydrocarbons), and finally the polar ones containing primarily sulfur, oxygen, and/or nitrogen compounds [11, 38]. Exploration of these mineral composites has left the nation vulnerable in a special way [39]. Human aggression, marginalization, and major health issues brought on by environmental pollution are among these vulnerabilities [32-35, 41].

The use of biological materials in phytoremediation and bioremediation offer promising technique for sustainable waste management (Gerhardt *et al.*, 2009). These biological processes are important and effective over physicochemical processes because their end products are non-toxic and ecofriendly [42]. As stated by [32, 33] generally, they are very integral as cost-efficient and environmentally sensitive biological adduct(s) for chemical processes in the industries which include: biotechnological and other alike.

The synthesis of 3-D activity proteins in organisms is vulnerable to the presence of catabolite inducers that need the translation of polycystronic mRNA [43]. Housekeeping enzymes, acylhydrolyases, and nitrification enzymes are primarily examples of these signaling protein reactions within an ecosystem known as stress marker proteins. Peroxidases are widely dispersed in plants, animals, and microbes. They protect essential cells and organelles from the deadly effects of oxidative stress, which is caused by the release of H_2O_2 [1-5]. Recently, there are aroused noticeable concerns in organismal peroxidases; these interests are because of their numerous catalytic advantages including: their ability to display a high level of biocompatibility, biodegradation, biodigestibilty, less technicality in preparation and stability over a wide range of physicochemical condition(s): temperature, pH and salt concentration when compared to peroxidases from other sources [13-15]. Acylhydrolases, or lipases, are widely distributed proteins with proven catalytic and commercial potential. At the oil-water interface, they catalyze the hydrolysis of triacylglycerols [30-32], releasing glycerol and free fatty acids (Abolemonaem, Hatem & Jeannette, 2011). Lipase has a lot of promise in waste treatment systems, according to ecology impact assessment studies, in addition to other biotechnological applications [27, 32]. The nitrogen cycle of food chains is maintained by ureases, which are in-situ inductive enzymes that act on nitrogenous materials found in organic matter by releasing ammonia. The present study was conducted within the heavy wet season of the sub Saharan around the ecological niche of petroleum hydrocarbon polluted soil where there are heterotrophic presence of hydrocarbonoclastic bacteria capable of producing thesestress marker enzymes.

2. Materials and Methods

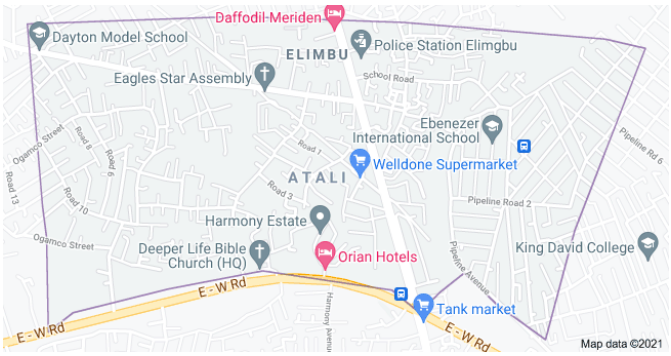
2.1. Materials

Materials including those of equipments, apparatus and reagents utilized during the course of the present study were of products of designated companies like BDh, Bristol, May and Bakers; the equipments were calibrated at each use and in good working condition. The reagents were analytical graded and properly stored.

2.2. Method

Soil Sample Collection

Soil samples were collected from petroleum drilling sites located at Atali (Figure 1) (LONG.7°03”,25°.2”E; LAT.4 °51”,53°.03”N). Atali was known with illegal pipeline bunkering activities by oil hooligans. The samples were collected as described by Eze et al. [25] from the stated areas at 6:00am in the morning using a spade. The collections were done at the four perimeters of the marked soil with average of 9m apart from each other.



Source: UNEP, (2104).

Figure 1. *Map of Atali oil bunkering site in Rivers state, Nigeria.*

2.2.1. Determination of Physicochemical Properties of the Soil

Physicochemical properties of the petroleum spilled soil were determined in the presence of the reference as described in the [4-6].

2.2.2. Microbial Isolation from the Polluted Soil

Microbial ecology of the polluted soil was determined according to the method described by Ezemonye and Ezemonye [26]. From the isolates, strains of the bacteria were identified using standard microbiology and biochemical techniques.

2.2.3. Bacteria Standardization

Standard Macfarland solution ($BaCl_2$ and sulphuric acid (2N)) were prepared in 100 ml solution using distilled water. The mixture was used to monitor bacteria growth exponentially using spectrophotometer at 660nm.

2.2.4. Hydrocarbon Efficiency Properties of the Organisms

Bacteria hydrocarbon clastic potentials were determined as described by Ezemonye and Ezemonye [26] using the Bush-Nell Has media. The prepared medium was suspended with 1%v/w of the crude oil asceptically and incubated accordingly.

2.2.5. Enzyme Production

Submerged fermentation system will be employed for the enzyme production as described by Ezeonu et al. [28]. The conical flasks (250 ml) were supplemented with nutrient media necessary for protein production whereas optimized conditions for peak production were employed during the fermentation process. The whole setups were sterilized at 121 ℃/ 15psi for 20 minutes using the electronic autoclave.

2.2.6. Determination of Enzyme Activity

Activity of peroxidase was monitored using o-dianisidine and hydrogen peroxide at spectrohotometric wavelength of 460 nm as described by Evanko. and Dzombak, [23].

Lipase activity was determined using the colorimetric method as described Ejedegba et al.; Eze et al. [19, 25] using p-nitrophenylpalmitate (p-NPP) as the standard substrate.

Urease activity in the soil will be determined in the presence of standard urea as described by Douglas and Bremner, Chukwu and Adams, [16, 18].

Corresponding total protein was determined in each enzyme assay as described by [36].

2.3. Statistical Analysis

Data obtained are expressed as mean ± SD and tests of statistical significance were carried out using two-way analysis of variance (ANOVA). Mean values with $p < 0.05$ i.e 95% confidence interval were considered as significant.

3. Results and Discussion

Physicochemical properties of the soil samples.

Physicochemical properties of the soil samples are presented in Table 1. The result indicate that' site with oil spillage history such as Atalia show similar physicochemical contents such as pH, conductivity, soil minerals relative to the control experiment.

Table 1. *Physicochemical Properties of the Soil from the three Different Points of Collections in Rivers state, Nigeria.*

Physicochemical parameters	Control	Soil sample1Atalia
pH	6.79 ± 0.02^{c}	5.44 ± 0.012^{c}
Moisture contents (%)	11.09 ± 0.02^{c}	12.34 ± 0.015^{c}
Electrical conductance(μS/cm)	145 ± 0.018^{c}	190 ± 0.014^{b}
Total organic carbon (%)	1.02 ± 0.014^{c}	0.72 ± 0.018^{a}
Total organic matter content (%)	13.00 ± 0.03^{c}	11.41 ± 0.05^{c}
Total Sulphate(mg/kg)	1.12 ± 0.016^{c}	9.622 ± 0.03^{c}
Total Nitrate(mg/kg)	4.212 ± 0.02^{c}	3.12 ± 0.025^{c}
Total Phosphate(mg/kg)	6.121 ± 0.014^{bc}	7.02 ± 0.039^{c}

N=3mean ±SD. Mean values with the same superscript letters are not statistical significant at p≤0.05.Mg/kg=milli gram per kilogram; %=percentage; μS/cm=micro resistance per centimeter.

In the presence of the control experiment, which maintained a pH of 6.79 throughout the experiment, the physiological

characteristics of the hydrocarbon-polluted soil from which the enzyme-producing bacteria were recovered showed a pH of 5.44. According to the proceedings of the [4, 5, 20-24], this might be ascribed to the type of the pollutant in the soil, such as oil and other acidic substances (oleic, benzoic acids). The corresponding polluted soils' electrical conductance gradually increased. The electrical conductivity of the Atalia soil was 190 (uS/cm), whereas the conductance of the control experiment was 145 (uS/cm). Their respective moisture contents (%) were: 12.34 while the control had moisture content of 11.09. It will be recalled from [7-13, 39, 40] that certain friable soil is characterized with low water retention ability and as such it conductivity of ions is seen very minimal.

In both the contaminated and uncontaminated soil, the dissolved mineral concentrations were detected in the following order: SO4>NO3>P>N, and the heavy metals Hg, As, and Cd were found at levels below the detectable limit range (BDL).

With the exception of organic nitrogen and phosphorus, the Atalia soil sample's mineral concentrations were comparatively lower than those of the control trial.

Petroleum effluents contain a variety of minerals that accumulate in any receiving body and improve the body's physicochemical characteristics [29, 36, 37, 39].

The progressive decrease in the concentration of these ions in the respective contaminated soils can be attributed by the availability of masking elements such as oxygen, sulphur and other ligands which competitively bind to afore enlisted minerals (elements) and diminishes their bioavailability.

Microbial Isolation and Biochemical Characterization

Heterotrophic counts of the total viable cells and coliform numbers of the bacteria and hydrocarbon utilizing bacteria as shown in Table 2.

Pure colonies of *Pseudomonas* sp. were obtained using differential nutrient culture medium from the polluted soil. From the cultured plate, distinct colonies of the organisms were observed with total heterotrophic number of 3.9 x 10^8 CFU/ml (Figure 2).

The micrograph of the bacteria (*Pseudomonas* sp) suspensions under an objective magnification of x40, showed clusters of rod shaped, non-sporulating cream coloured non-motile bacteria cells in a lactophenol blue stained background (Figure 3).

Table 2. *Hetertrophic Counts (CFU/ml) of the bacteria.*

Coliformcounts	Control	Atali	H-C Bacteria
Total Viable Counts (10^{-2})	2.7 x 10^5	4.5 x 10^7	2.65 x 10^3
Total Coliform Counts (10^{-2})	0.6 x 10^2	1.2 x 10^3	

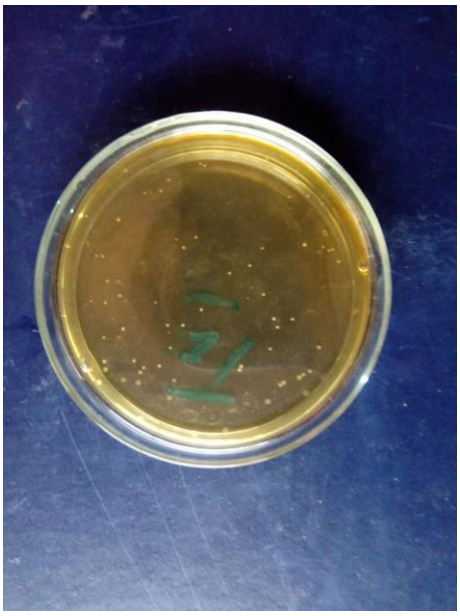


Figure 2. *Pure colonies of strains of Pseudomonas sp.on nutrient media plate.*

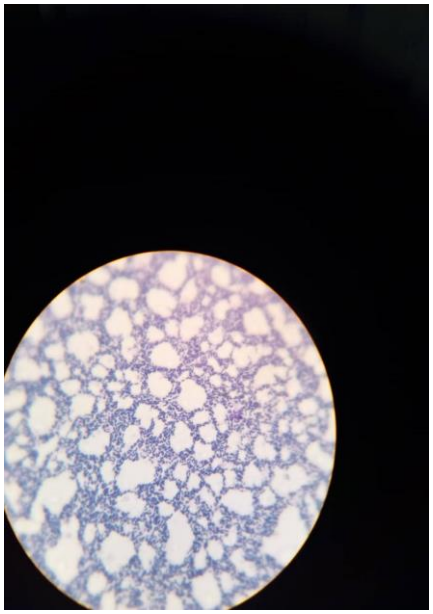


Figure 3. *THe micrograph of the bacteria (Pseudomonas sp) suspensions under an objective magnification of x40.*

High levels of heterotrophic microbial diversity and activity were found in Rivers State's crude oil-polluted soil.[14] found that an effluent site undergoing in situ cleanup had more eutrophication of microorganisms from a wide kingdom. They went on to say that a favorable and, more importantly, nutrient-rich environment for microbial growth and proliferations was the cause of the rise in microbial cells. The kingdom of bacteria had the most variety among the microorganisms isolated from the soil contaminated by crude oil. The total heterotrophic counts of the bacterium isolate were 4.5 x 107 CFU/g on nutrient medium plates and 2.65 x 103 CFU/g on

differential media of Bush Nell Haas Agar, respectively, plated from dilution factors of 10-2 folds. These finding showed a relatively higher correlation with that of Corral *et al.* (2006) on ecological relevance in whey surge polluted water. They reported a heterotrophic microbial population of 3.5 x 10^6 CFU/g bacteria on nutrient media plate and that of 2.0 x 10^3 CFU/g on differential media of Bush Nell Haas Agar.

The isolate was identified as Pseudomonas using basic morphological and biochemical screening. Basic morphological fea-tures of the bacteria showed that strains of Pseudomonas under an objective magnification of x40, showed clusters of rod shaped, non-sporulating cream coloured non-motile bacteria cells in a lactophenol blue stained background bacteria; biochemically, they are obligate gram-negative, non-starch hydrolyzing, catalase (+), oxidase (+) and lactic acid forming organisms with optimum growth at temperature of 25-40oC.

These results are consistent with Ezeonu et al. [28] basic manual for organism isolations and identifications. The pure isolates of Pseudomonas were identified using a molecular test (16S rDNA).

Enzyme Production

Effect of incubation days on lipase, urease and peroxidase production showed the optimum activity of the enzyme on day 7, 14 and 7 of the 21 days incubation study respectively. Peak activity of the enzyme was recorded at 72 and 75.67 and 69.32 µmole/min (Figure 1). Corresponding protein concentration was seen on day 4 of the 7 days incubation study.

Effect of pH on production of lipase, urease and peroxidase from *Pseudomonas* sp. showed optimum production of the enzyme at pH 7.5, 7.5 and 4.0 respectively (Figure 2).

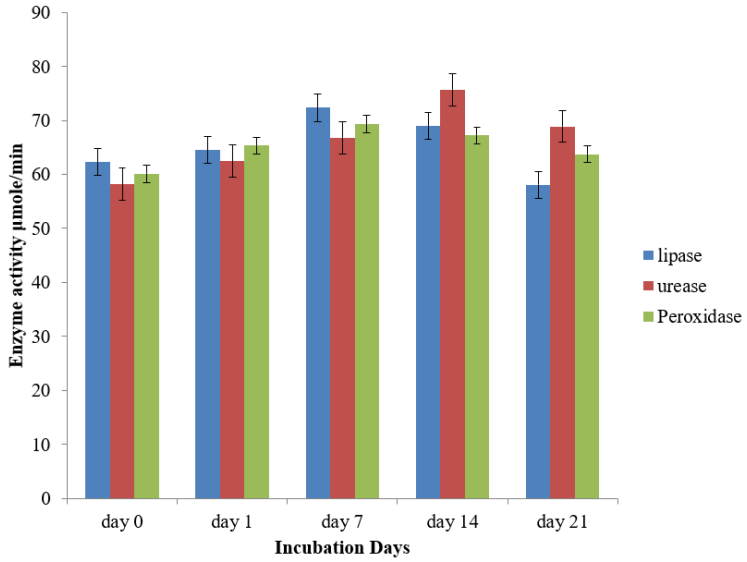


Figure 4. *EFfect of incubation days on stress responsive enzyme production from strain of Pseudomonas sp.*

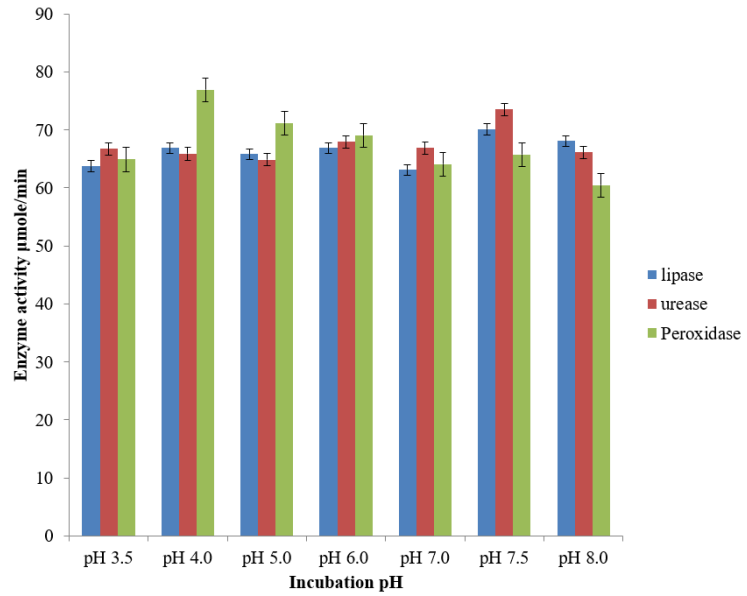


Figure 5. *Ffect of incubation pH on stress responsive enzyme production from strain of Pseudomonas sp.*

Figure 1 illustrates the impact of incubation days on the production of lipase, urease, and peroxidase. The enzymes were produced on days 7, 14, and 7 of the 21-day incubation research, respectively. The enzyme's peak activity was measured at 72, 75.67, and 69.32 µmole/min. On day four of the seven-day incubation trial, the corresponding protein concentration was observed. The extracellular synthesis of lipase, urease, and peroxidase on these days is indicative of catabolite induction of the substrate in the fermentation media to the organisms for increased protein production [14, 39]. Peak lipase and urease activity was observed in the last phase of the tri-phasic curve, which makes this clear in the outcome. Closeness in the optimal enzyme activity and protein concentration is unique to most extracellular protein especially those no sporulating bacteria. [14] reported day 8 as the peak production day of lipase activity produced from *Bacillus licheniformis* ATCC-12759 in submerged fermentation system while [11, 12] reported day 5 for peroxidase production from *Nonomuraeagerenzanensis* growing on alkali lignin. [44-46] day 5 for urease production from *diculture of E.coli and Bacillus* sp.

The optimal synthesis of lipase, urease, and peroxidase from Pseudomonas sp. was found at pH 7.5, 7.5, and 4.0, respectively. [4] found that peroxidases preferentially need a low pH medium for their maximum synthesis in their study on the characteristics of peroxidase from white and red cultivars of kolanut. Since they identified pH 4.5 as their peroxidase's peak production pH, their conclusions closely match the data of the current investigation. On day five, however, [17] found that yeast from extra virgin oil produced the most lipase. He continued by saying that the ideal pH for lipase synthesis was 8.0. However, Immanuel *et al*. (2018) reported that urease from seeds are mostly produced within the peak time of the seeds germination which are usually 3-4 days after seed imbibitions and usually at seeping basic buffer of 7.5-9.0.

4. Conclusion

Environmental sustainability and ecotoxicological revolution still anchors on availability of essential bioprocessing indicators and their stability for efficient utilization. Quality marker proteins are multi-purpose enzymes that have found its way in many signaling pathways of ecological reclamations, management and toxicity profiling. The present study has shown the basic enzymology and kinetic properties of three quality marker enzymes of peroxidase, lipase and urease produced from strains of *Pseudomonas* from a crude oil polluted soilthrough the significance of their large range of catalytic peak conditions and stability.

Abbreviations

BDL	Belwo Detectable Limit
mM	Mili Mole
µmol/min	Micro Mole Per Min
CFU/ml	Coliform Unit Per MiliLitre
Km	Michealis Menten
Vmax	Constant Velocity Maximal

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AuthorContributions

Azubuike Umeh Aninachukwu Chukwudi: Conceptualization, Project administration

Ezenwelu Chijioke Obinna: Data curation, Investigation, Resources, Writing – original draft

Chigbo Malachy Chidiebere: Project administration, Validation

Udemezue Onyeka Izuchukwu: Methodology, Software

Ilechukwu Cyril Chijioke: Resources, Validation

Oparaji Emeka Henry: Methodology, Supervision, Writing – review & editing

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Conflicts of Interest

The authors declare NO conflict of interest as regards to the empirical of the present research work.

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