

Effects of Temperature and pH on the Activities of Catechol 2,3-dioxygenase Obtained from Crude Oil Contaminated Soil in Ilaje, Ondo State, Nigeria

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Abstract: Enrichment technique was employed for the isolation of the crude oil degrading bacteria. The isolated bacteria were screened for their degradative ability and the best degrading bacteria were selected based on their growth. Specific activities of Catechol-2,3-dioxygenase and effects of temperature and pH and their stabilities on the enzyme relative activities were observed. Bacteria isolated from the soil sample include; *Bacillus cereus*, *B. amyloliquificiens*, *B. firmus*, *Acinetobacter calcoaceticus*, *Pseudomonas* sp. *P. fluorescens*, *P. putida*, *P. aeruginosa*, *Achromobacter xylosoxidans* and *Achromobacter* sp. Screening of the degradative ability of the bacteria revealed *P. aeruginosa*, *Bacillus cereus*, *Acinetobacter calcoaceticus* and *Achromobacter* sp. to be the best degraders. The pH and temperature range with time for the enzyme activity were 6.0-8.0 and 30°C-50°C respectively. The enzyme exhibited activity that was slightly more tolerant to alkaline pH. Therefore, engineering of Catechol 2,3-dioxygenase may be employed for application on bioremediation of polluted sites.

Keywords: Bacteria, catechol 2,3-dioxygenase, crude oil, pH, soil and temperature.

INTRODUCTION

In Nigeria, the growth and activities of petroleum and petroleum associated industries has led to increased oil pollution in our environment, due to problems arising from petroleum exploration, exploitation, transportation and consumption coupled with poor maintenance of oil pipelines resulting in seepages and ruptures [1]. The rising in the incidence of damage and vandalization of pipelines by restive oil communities mostly in the Niger Delta area of the country has resulted into huge problem in these recent years. Ilaje community, located in the Southern part of Ondo State, closer to the Atlantic Ocean, is one of the oil producing communities in the Niger Delta region of Nigeria. Oil explorations in these communities at times result to contamination of lands and water bodies due to spillage [2]. Contamination of soils and aquifers by oil spills has been a continual general pollution problem that resulted into desolation of almost all compartments of the environment and thereby inflicting serious health repercussion and ecological instability [3]. Toxic effects of oil and their products on the soil environment include enhancing hydrophobicity of soils, disturbance of water availability to vegetation, and direct toxicity to plants and microorganisms. The technology commonly used for soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to partial decomposition of contaminants. Bioremediation on the other hand, is an efficient and cost effective approach to reduce or completely remove the

hazardous and toxic organic chemicals from the contaminated environment by microbes which are known for their capabilities of catabolic processes. Microbial remediation of a hydrocarbon-contaminated site is achieved by the help of a diverse group of microorganisms applying enzymes in their metabolism, especially the indigenous soil bacteria. Aerobic biodegradation of aromatic compounds involves their conversion into dihydroxylated intermediates (e.g., catechol or its alkyl- or chloro-substituted derivatives) which are then further metabolized by intradiol (catechol-1,2-dioxygenase) or extradiol (catechol-2,3-dioxygenase) dioxygenases [4]. Intradiol enzymes are known to incorporate two oxygen atoms between vicinal hydroxyl groups, while the extradiol dioxygenases cleave the aromatic ring of the substrate outside the two hydroxyl groups to produce muconic-semialdehyde [5]. Catechol 2,3-dioxygenases are homotetramers and they have been detected from many Gram-negative bacteria (*Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Burkholderia*,) and Gram-positive bacteria (*Nocardia*, *Rhodococcus* and *Bacillus*) strains [6]. The main aim of this study is to assay for the specific activity of Catechol 2,3-dioxygenase from bacterial with the highest degrading activities obtained from crude oil polluted site in Ayetoro, Ondo state. In addition, the study aims to evaluate the environmental conditions for the activity of Catechol 2,3-dioxygenase produced by these bacteria and to determine the optima conditions for pH and temperature of the enzyme activity.

MATERIALS AND METHODOLOGY

Sources and Collection of Samples Soil

Crude oil contaminated and the non-contaminated soils were collected aseptically into a labeled sterile plastic bag at

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Ayetoro, Ilaje Local Government Area of Ondo State. Bonny-light crude oil was obtained from Nigeria National Petroleum Commission (NNPC) Bayelsa State.

Isolation and Selection of Oil Degrading Bacteria

Soil samples (2g) were suspended in 98 ml of Bushnell Haas broth supplemented with 1% v/v crude oil and were incubated at 30°C for 7 days. After which UV spectrophotometer was used to determine the optical density at 600nm at 24 hours interval. After one week, 1 ml of each suspension from the enrichment process was serially diluted and 1ml from 10^{-4} aliquot was seeded into Bushnell Haas agar plates and was incubated at 30°C for 48hrs. Pure colonies of each isolates were obtained by streak plate method on nutrient agar plates and were incubated at 30°C overnight, and were stored on agar slant.

Characterization and Identification of the Isolates

The morphological characteristics (colony colour, size, elevation and shape) of the colonies were observed, Gram staining and biochemical tests (starch hydrolysis, casein hydrolysis, H₂S production, catalase test, oxidase test, methyl red, Voges Proskauer (MR-VP), citrate utilization and sugar fermentation) were performed [7-9], and were identified [10].

Characterisation of the Degradation Potential

Bacterial inoculum (1ml) was transferred into 5ml Bushnell-Hass medium and was supplemented with 1ml of crude oil as the carbon source, incubated at 30°C at 170 rpm in shaker incubator for a period of 7 days. The growth was monitored through culture densities, by taking the O.D readings daily at 600nm against Bushnell Haas medium as blank.

Medium and Culture Conditions

LB medium (5g/l yeast extract, 10g/l casein peptone, 10g/l NaCl,) pH 7.0 was used to grow the isolates. The bacteria inocula (10%) from LB culture were then transferred to 1000ml of freshly prepared mineral salts medium {2.75g/l of K₂HPO₄, 2.225g/l of KH₂PO₄, 1.0g/l of (NH₄)₂SO₄, 0.2g/l of MgCl₂.6H₂O, 0.1g/l of NaCl, 0.02g/l of FeCl₃.6H₂O and 0.01g/l of CaCl₂} pH 7.0, supplemented with 1% v/v crude oil. The medium were incubated at 30°C on a rotary shaker at 200rpm.

Preparation of Crude Cell Extract and Protein Determination

After centrifugation at 5000xg, 10min at 4°C, bacterial supernatant were suspended in the lysis buffer (50mM Tris phosphate pH 8.0, 1% glycerol for the stabilization of the protein and prevention of aggregation, and 0.1% Triton X-100 for the prevention of aggregation of hydrophobic and membrane proteins). Total protein concentrations in cell extracts were determined with BSA (bovine serum albumin) as a standard [11, 12].

Determination of Catechol-2,3-Dioxygenase Activity

Meta-Cleavage dioxygenase activity was assayed by monitoring the increase in absorbance at the corresponding

wavelength of the meta-cleavage product with a DU800 spectrophotometer (Beckman Coulter, Inc., USA) equipped with a thermo-controlled cuvette holder. The pH of the buffer used in this study was adjusted at 30°C. The reaction mixtures contained 2ml of 50mM Tris- HCL buffer (pH 7.5), 6ml distilled water, 0.2ml cell free extract. The contents were mixed by inversion and 0.2ml catechol was added. The increase in absorbance at 375nm caused by the formation of the reaction product 2-hydroxyomuconic semialdehyde was monitored. One unit of the specific activity is defined as the amount of enzyme that converts 1.0µl of meta-cleavage product per minute at 30°C.

pH and Temperature Optima of Catechol 2,3-Dioxygenase

The effect of pH on catechol-2,3-dioxygenase activity was determined by measuring the activity at 30°C over the pH range of 3.0-12 using the following buffers: 50mM sodium acetate (pH 3.0 to 5.0), 50mM phosphate (pH 6.0 and 7.0), 50mM Tris- HCl (pH 8.0 and 9.0) and 50mM carbonate (pH 10 and 12).

The optimum temperature was determined by assaying the enzyme activity at various temperatures (30-80°C) in 50 mM Tris/HCl buffer (pH 8.0). The enzyme and substrate solutions were pre-incubated, mixed and the enzymatic reaction was then carried out.

Determination of Thermal and pH Stability of Catechol-2,3-Dioxygenase

The thermal stability of the enzyme was determined by incubating the enzyme and enzymatic reaction mixtures within the temperature range of 40°C to 60°C for 60 min and measuring the activity at 5 minutes interval of incubation at the same temperatures.

pH stability of catechol-2,3-dioxygenase was determined by dissolving and mixing equal volume of enzymes with equal volume of different buffers and allowing the mixture to stand for the period of five minutes which was followed by the measurement of the enzyme's activity at every one hour interval for the period of five hours.

Statistical Analysis

The statistical analysis was performed using Microsoft office Excel 2007 for calculating mean, standard deviation and standard error.

RESULTS

The optical density at (OD_{600nm}) of the crude oil contaminated soil varied between 0.04 and 2.55 while that of the non-contaminated soil was between 0.01 and 0.52.

Characterization and Identification of the Isolates

The results of the morphological and biochemical analyses of bacterial isolates from the soil sample with bonny light crude oil, revealed ten bacterial strains namely; *Bacillus cereus*, *Bacillus firmus*, *Bacillus amyloliquifaciens*, *Acinetobacter*, *Pseudomonas* sp. *Pseudomonas floresens*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Achromobacter* sp. and *Achromobacter xylosoxidans*.

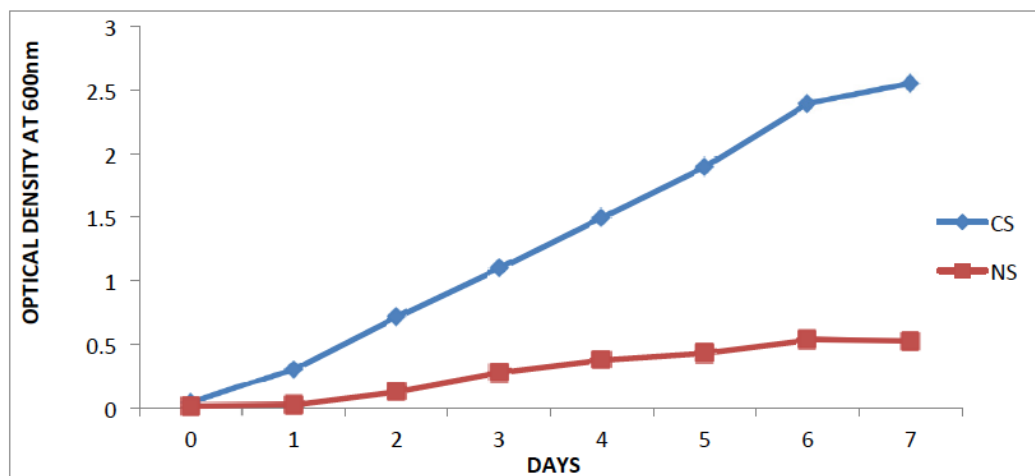


Fig. (1). Isolation and selection of oil degrading bacteria from soil samples, **Legends:** CS- contaminated soil; NS- Non- contaminated soil.

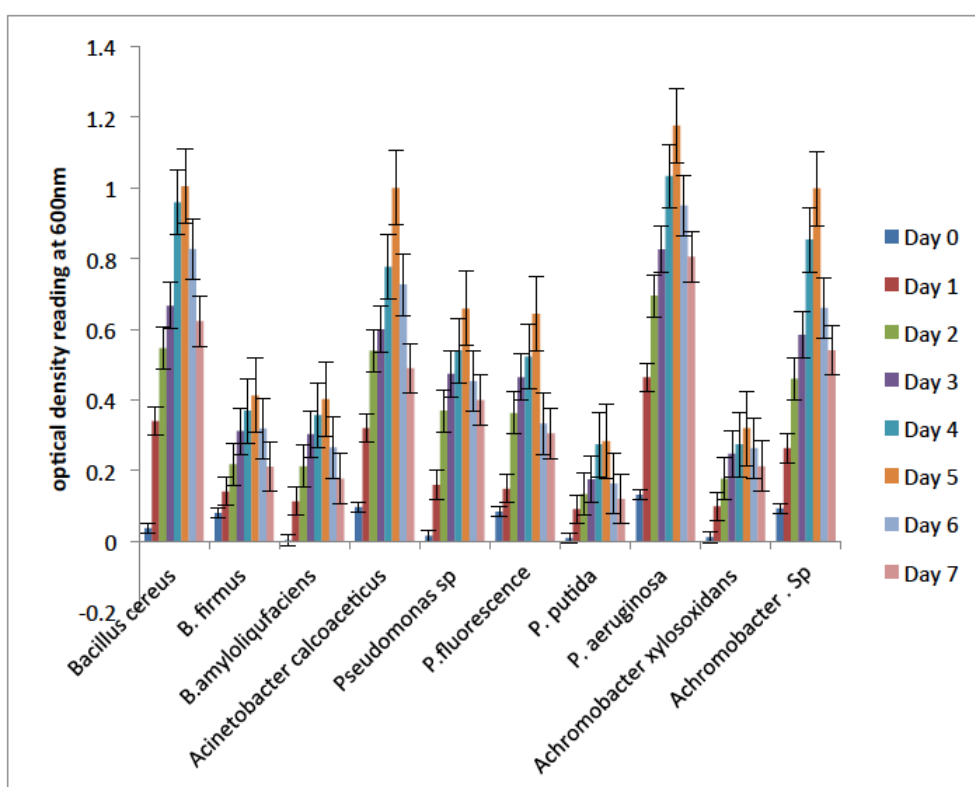


Fig. (2). Degradation potentials of the bacterial isolates on crude oil.

Characterization of the Degradation Potential

Fig. (2) shows the degradation abilities demonstrated by the bacterial isolates during their growth on crude oil as their sole carbon and energy source. The results revealed that *Pseudomonas aeruginosa*, *Bacillus cereus*, *Acinetobacter calcoaceticus* and *Achromobacter* sp. were characterized by rapid initial growth phase within 24 hours and their optimum OD values were observed on the 5th day. The result showed that the four bacterial isolates maximally utilized the hydrocarbon. On the other hand, the other bacterial isolates utilized the hydrocarbon differently with *Pseudomonas putida* and *Achromobacter xylooxidans* having lower growth rate. Hence, the four bacterial isolates with the best degradative

abilities on crude oil based on visual observation for turbidity are the most efficient isolates, and were selected for further study.

Catechol-2,3-Dioxygenase Activity

As shown in Fig. (3), the absorbance at 375nm which corresponds to the enzyme activity of the four isolates. *B.cereus* has the highest activity of $122.57 \mu \text{mg}^{-1} \text{protein}$; *Acinetobacter calcoaceticus* had activity of $105.32 \mu \text{mg}^{-1} \text{protein}$. While the assay of catechol 2,3-dioxygenase (C23O) from *Pseudomonas aeruginosa*, showed the utmost activity of $(126.835 \mu \text{mg}^{-1} \text{protein})$ with *Achromobacter* sp. having the lowest activity of $102.10 \mu \text{mg}^{-1} \text{protein}$.

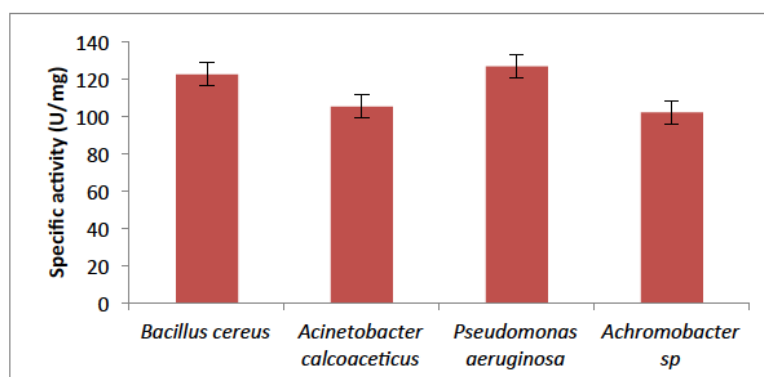


Fig. (3). Activity of Catechol- 2,3-dioxygenase from some bacteria isolates.

Effect of Temperature and Thermal Stability on the Activity of Catechol 2,3-dioxygenase

The temperature optimum of the reaction rate of catechol 2,3-dioxygenase (C23O) from *Pseudomonas aeruginosa* and *Achromobacter sp.* were estimated to be at 40°C (Fig. 4). Catechol 2,3-dioxygenase isolated from *Acinetobacter calcoaceticus* as well as *Bacillus cereus* showed the highest activity at 30°C. Fig. (5) revealed that the enzyme from all the bacteria were thermally stable at 40°C. While, *Pseudomonas aeruginosa* and *Achromobacter sp.* were also partially stable at 50°C. However, their relative activities decreases drastically from 50°C to 80°C with time course during which denaturalization of the enzyme had occurred.

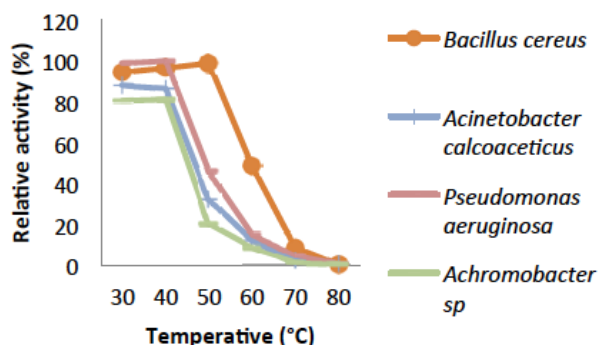


Fig. (4). Effect of Temperature Catechol 2,3-dioxygenase from some bacterial isolates.

Effect of pH and its Stability on the Activity of Catechol-2,3-dioxygenase

The activity of catechol 2,3-dioxygenase for most of the bacteria were dormant at pH 3.0 and 12 (Fig. 6) while they all showed slight activity within the pH range of (4.0 to 5.0) and (10 to 11), On the other hand, *Bacillus cereus* showed slight activity at pH 3.0 and it also had significant activity at pH 4.0 to 5.0. Catechol-2,3-dioxygenase from *Bacillus cereus* exhibited its highest activity of 100% at pH 8.0 which means it is slightly more tolerant to alkaline pH and was still 20% active at pH 9.0 (Fig. 6). Catechol 2,3-dioxygenase obtained from the other three bacterial isolates exhibited activity that is more tolerant to neutral pH and they had lost about 95% of their original relative activity under acidic condition (pH 4.0 to 5.0), and about 55% at pH 9.0 and no activity was observed at pH 12. The pH stability of

Catechol-2,3-dioxygenase from *B. cereus* showed that, it was only partially stable at pH 7.0 while, *Acinetobacter* was also partially stable at pH 6.0 and 7.0 (Fig. 7). However Catechol 2,3-dioxygenase from *Pseudomonas* was partially stable at pH 6.0, but was stable at pH 7.0. The Catechol-2,3-dioxygenase from *Achromobacter sp.* exhibited the highest stability at pH 7.0, while it was partially stable at pH 6.0 and 8.0 with relative activity during the entire time course. The pH stability decreases with incubation period for most of the isolates.

DISCUSSION

The quality of life is being eroded in the affected area due to the ill effect of oil exploitation. The inhabitants of the oil producing areas of Ilaje local Government Area of Ondo State, which depends on the rivers and farmlands for their livelihood, are suddenly ensnared in a desolated environment that could no longer provide them with relief [13].

This study shows that there was increase in hydrocarbon-degrading bacteria obtained from the contaminated soil. According to [14], it was reported that hydrocarbon degraders in unpolluted environments, usually represent less than 0.1% of the microbial community while in crude-oil polluted environments they constitute up to 100% of the viable microbes which corroborates with the result obtained in Fig. (1). The results obtained from the taxonomic characterization of the isolates revealed different groups of bacterial genera: *Pseudomonas*, *Bacillus*, *Acinetobacter* and *Achromobacter*. Members of the above mentioned genera have been reported by many scientists as organisms which utilize hydrocarbons as their source of carbon and energy [2, 15-17]. The extreme optimum crude oil degradative ability of *Pseudomonas aeruginosa* compared to other bacterial isolates from the result is supported by the reports of [17, 18]. In their study, *Pseudomonas sp.* are shown to be the most common aerobic soil bacteria which possess diverse aliphatic and aromatic hydrocarbon degradative genes, most of which have been shown to be plasmid-borne. On the other hand, the degrading ability observed in *Bacillus sp.* in this study has also been reported by [18] as bacteria that have high capacity for crude oil degradation. *Bacillus sp.* was also observed as the predominant isolates of all the crude oil utilizing bacteria from a study on polluted soil samples and it was postulated that *Bacillus* species are more tolerant to high levels of hydrocarbons in soil due to their resistant

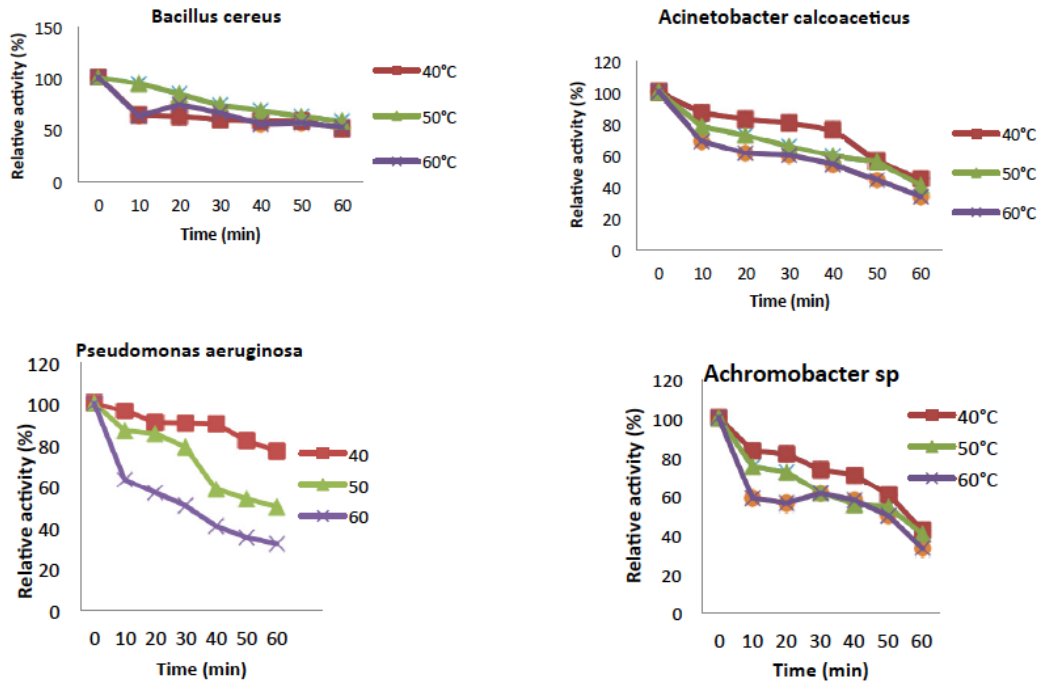


Fig. (5). Thermal stability of Catechol-2,3-dioxygenase activity.

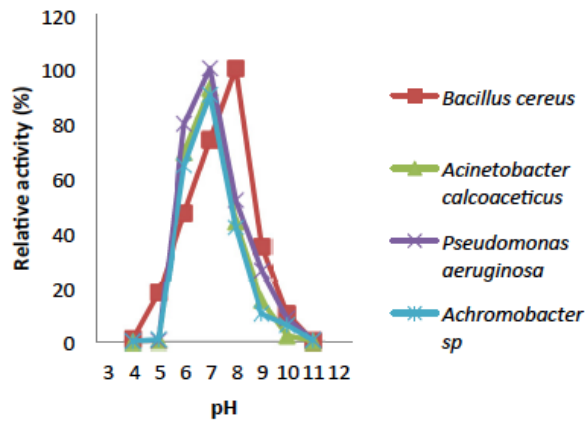


Fig. (6). Effect of pH on activities of Catechol-2,3-dioxygenase.

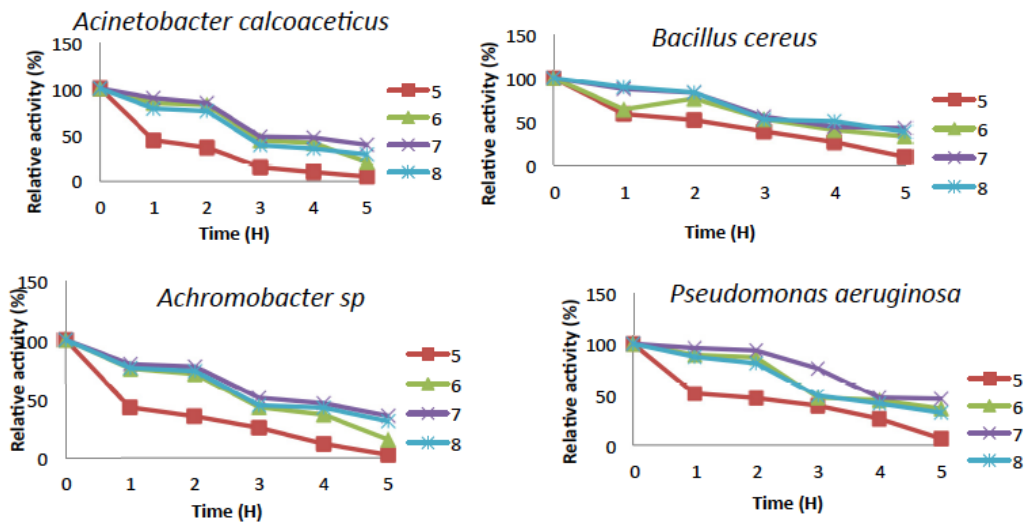


Fig. (7). pH stability of Catechol-2,3-dioxygenase activity.

endospores [19]. It is conceived that the isolated organisms should have catabolic enzymes for definite biodegradation in the presence of diverse Petroleum hydrocarbons. The obtained results (Fig. 3) from activities of catechol 2,3-dioxygenase (C23O) from the isolates revealed *Pseudomonas aeruginosa* with an outstanding activity than the other bacterial isolates. This reflects why the organism shows good growth on crude oil. In this study, the variation found in the result of C23O activity, has also been described by [20, 21] in their findings that enzymatic activity of diverse bacterial strains differ over wide ranges; which might be due to the heterogeneity of enzyme structure. The effects of temperature and pH on the activity of catechol 2,3-dioxygenase were determined in this study as they are important factors influencing the biodegradation of PAH by microorganisms. Enzyme activity depends on temperature [22], and optimum temperature for most enzymes could be linked to the temperature of the environment in which microorganisms were isolated [23]. The optimum temperature observed for catechol 2,3-dioxygenase in this study (Fig. 7) was within the range of 30°C to 50°C, with only *Bacillus cereus* showing its optimum extracellular activity at 50°C. This agrees with the investigation done by [24], who determined the optimum temperature for the activity of the enzyme C23O as 50°C from *Bacillus stearothermophilus*. However, many enzymes exhibit higher activity at a temperature at which they are unstable [25]. Catechol 2,3-dioxygenase isolated from *Pseudomonas aeruginosa* in this study were optimally active at 30°C and 40°C, which coincides with [26] who reported catechol 2,3-dioxygenase isolated from *Pseudomonas strains ZJF08 and S-47* to be optimally active at 40°C and within 30–35°C, respectively. However, catechol 2,3-dioxygenase isolated from most of the isolates in this study had lost most of their activity within the temperature range of 70°C to 80°C and this result agrees with [22] who reported that at low temperatures the movement of the molecules is slow and no activity is required to convert the substrate into a product, while at higher temperatures, the thermal movement of the molecules is too high to ensure enzyme conformation, thus causing their denaturation and activity loss. While the thermal stability of catechol 2,3-dioxygenase was found to be within the range of 30°C to 40°C for most of the isolates (Fig. 6). According to [27], it was postulated that temperature had influence by increasing both microbial activity and PAH solubility, and established the rate of degradation to reduce at temperature above 30°C. This indicates the adverse effect of high temperature on cells. The way that enzymes respond to temperature and pH is important in many areas of biotechnology. Enzyme activity affected in several ways by hydrogen ion concentration [22]. One way to be considered is that catalytic reaction requires by this enzyme, with particular catalytic groups in an ionized or non-ionized state, to react with the substrate without modification in its active conformation and the enzymes stability. Consequently, it does not drastically reduce its activity. The specific activity of pH was highest for all four isolates (Fig. 7) at pH 7.0 except for *Bacillus cereus* that had its optimum relative activity at pH 8.0. This result is in accordance with reports from literature that the pH optimum for this enzyme ranges from 5.5 to 8.2 [28, 29].

CONCLUSION

Petroleum degraders have been reported to be endowed with enzyme systems, nutritional capabilities and metabolic activities which enable them to withstand adverse environmental conditions. It is therefore necessary to confirm the isolates that are able to secrete relevant enzymes with highest activity of degrading hydrocarbons. And the degrading ability demonstrated by the microorganisms is a clear indication that they can be used in hydrocarbon degradation. The selected organism have shown their optimum catechol 2,3-dioxygenase activity within the temperature range of 30°C to 50°C during degradation. Hence, thermophilic nature of these bacteria's enzymes could add further advantage for their use in bioremediation of petroleum contaminated soils during dry season.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

- [1] Okerentugba PO, Ezeronye OU. Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *Afr J Biotechnol* 2003; 2: 288-92.
- [2] Boboye B, Olukunle OF, Adetuyi FC. Degradative activity of bacteria isolated from hydrocarbon-polluted site in Ilaje, Ondo State, Nig. *Afr J Microbiol* 2010; 4(23): 2485-90.
- [3] Okoh AI. Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnol Mol Biol Rev* 2006; 1: 38-50.
- [4] Palaniandavar M, Mayilmurugan R. Mononuclear non-hemiron(III) complex as functional models for catechol dioxygenases. *Comptes Rendus Chimie* 2007; 10: 366-79.
- [5] Viggiani A, Siani L, Notomista E, *et al.* The role of the conserved residues His-246, His-199, and Tyr-255 in the catalysis of catechol 2,3 dioxygenase from *Pseudomonas stutzeri* OX1. *J Biol Chem* 2004; 279: 48630-9.
- [6] Wei J, Zhou Y, Xu T, *et al.* Rational design of catechol 2,3-dioxygenase for improving the enzyme characteristic. *Appl Biochem Biotechnol* 2010; 162: 116-26.
- [7] Fawole MO, Oso BA. Laboratory manual of microbiology. Ibadan, Nigeria: Spectrbooks Limited 2001; p. 127.
- [8] Xiao L, Yang LY, Yin DQ. Environmental Microbiology Experimental Techniques. Beijing: China Environmental Science Press 2004; pp. 19-26, 49-60.
- [9] Cheesbrough M. District Laboratory practice in Tropical Countries; 2nd ed. United Kingdom: Cambridge Unipress 2006: p. 143.
- [10] Holt JG, Krieg NR, Sneath, PHA, *et al.* Bergey's Manual of Determinative Bacteriology. Baltimore, USA: William and Wilkins 1994; pp. 729-59.
- [11] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-54.
- [12] Stoscheck CM. Quantitation of Protein. *Methods Enzymol* 1990; 182: 50-69.
- [13] Celestine A. Hydrocarbon Exploitation, Environmental Degradation and Poverty: The Niger Delta Experience. In: Diffuse Pollution Conference, Dublin 2003.
- [14] Sarma PM, Bhattacharya S, Krishnan, *et al.* Degradation of polycyclic aromatic hydrocarbon by a newly discovered enteric bacterium, *Leclercia adecarboxylata*. *Appl Environ Microbiol* 2004; 7: 3163-6.

- [15] Ollivier B, Magot B. *Petroleum Microbiology*. Washington, DC: American Society for Microbiology (ASM) Press 2005; pp. 154-7.
- [16] Chikere CB. Bacterial diversity and community dynamics during the bioremediation of crude oil-polluted soil. PhD Dissertation. Nigeria: Microbiology Department, University of Port Harcourt, Rivers State 2010.
- [17] Chikere CB, Ughala E. Preliminary screening of hydrocarbon utilizing bacteria harbouring plasmids. *TWOWS Afr Int J Sci Tech* 2011; 2: 26-36.
- [18] Obayori SO, Salam LB. Degradation of polycyclic aromatic hydrocarbons: role of plasmids. *Sci Res Essays* 2010; 5: 4093-106.
- [19] Ijah UJ, Antai SP. Removal of Nigerian light crude oil in soil over a 12 month period. *Int Biodeterior Biodegradation* 2003; 51: 93-9.
- [20] Huang S L, Hsu YC, Wu, *et al.* Thermal effects on the activity and structural conformation of catechol 2,3-dioxygenase from *Pseudomonas putida*. *J Phys Chem Biotechnol* 2010; 14: 987-92.
- [21] Takeo M, Nishimura M, Shirai M, *et al.* Purification and characterization of catechol 2,3-dioxygenase from the aniline degradation pathway of *Acinetobacter* sp. YAA and its mutant enzyme, which resist substrate inhibition. *Biosci Biotechnol Biochem* 2007; 71: 1668-75.
- [22] Whiteley CG, Lee JD. Enzyme technology and biological remediation. *Enzyme Microb Technol* 2006; 38: 291-316.
- [23] Atlas RM, Bartha R. *Microbial Ecology: Fundamentals and applications*. 4th ed. New York: Benjamin and Cummings Science Publishing 1998; pp. 523-30.
- [24] Fernandez-Lafuente R, Guisan JM, Ali S, *et al.* Immobilization of functionally unstable catechol-2,3-dioxygenase greatly improves operational stability. *Enzyme Microb Technol* 2000; 26: 568.
- [25] Eisenthal R, Peterson ME, Daniel RM, *et al.* The thermal behaviour of enzyme activity: implications for biotechnology. *Trends Biotechnol* 2006; 24: 289-29.
- [26] Zou Y, Wei J, Jiang T, *et al.* Characterisation of thermostable catechol 2,3-dioxygenase from phenanthrene degrading *Pseudomonas* sp. strain ZJF08. *Annu Microbiol* 2007; 57: 503-8.
- [27] Jacques RJS, Santos EC, Bento FM, *et al.* Anthracene biodegradation by *Pseudomonas* sp. isolated from a petrochemical sludge landfill site. *Int Biodeterior Biodegradation* 2005; 56: 143-50.
- [28] Ahuatzi-Cacon D, Ordorica-Morales G, Ruiz-Ordaz N, *et al.* Kinetic study of phenol hydroxylase and catechol-1,2-dioxygenase biosynthesis by *Candida tropicalis* cells grown in different phenolic substrates. *World J Microbiol Biotech* 2004; 20: 695-702.
- [29] Viggor S, Heinaru E, Kunnapas A, *et al.* Evaluation of different phenol hydroxylase-possessing phenol-degrading pseudomonads by kinetic parameters. *Biodegradation* 2008; 19: 759-69.

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