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Comparison Of Enzymatic Activity Of Catechol 1,2-Dioxygenase And Catechol 2,3-Dioxygenase In Pseudomonas Spp. Towards Polycyclic Aromatic Hydrocarbons

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Abstract

This study aimed to measure the enzymatic activity of catechol 1,2-dioxygenase (C12O) and catechol 2,3-dioxygenase (C23O) produced by four strains of bacteria include Pseudomonas putida (worod-1and worod-2) Pseudomonas aeruginosa (worod-3 and worod-4) they were submitted to GenBank under the Accession number of MW551536, MW551537, MW551538, MW551539,respectively which isolated from soil contaminated with crude oil and it's derivatives and used for remediation of polycyclic aromatic hydrocarbons.the cleavage of the aromatic ring is the first step in the biodegradation of aromatic hydrocarbons by bacteria. It is catalyzed by C120 and C230 enzymes, the isolates showed the highest activity of C23O than C120 in different concentration of PAHs during one and two weeks of remediation.

INTRODUCTION

polycyclic aromatic hydrocarbons (PAHs) are a group of more than 100 compounds that include two or more fused benzene rings. PAHs are divided into two groups based on the number of rings present: Low Molecular Weight (LMW, containing three or fewer rings) and High Molecular Weight (HMW, containing four or more rings). They are ubiquitous environmental contaminants with higher concentrations in air, water, soil, sediment, and vegetation near urban and industrial areas all over the world (Wilson SC and Jones KC 1993; Manzetti, 2013). PAHs (polycyclic aromatic hydrocarbons) are mutagenic and toxic contaminants, with the Environmental Protection Agency classifying sixteen of them as priority pollutants (EPA) .As a result, environmental pollution studies involving PAHs have risen in recent years (Johnson et al.,2007; Barret et al.,2010 ; Herold et al.,2011). However, bioremediation of PAH-contaminated sites using the biodegradation process is an effective option for reducing these environmental issues. Soil microorganisms have the potential to catalyze the biodegradation of organic compounds; however, PAHs are selective substances that provide a selective source of carbon as energy and can cause toxicity in most microorganisms; As a result, soil can be an important source of PAH biodegradation microorganisms (Alexander ,1999) . The use of microbial enzymes to biodegrade toxic organic compounds like PAH helps to clean up a variety of environments, including water (Guo et al.,2010 ; Zhao et al., 2011) , sludge(Barret et al.,2010) and soil(Moon et al.,2006). The PAHs degradation enzymes are divided into two categories: peripheral and fission (Mishra

et al.,2001). Enzymes play a role in peripheral recognition and convert degradable PAH molecules into fission enzymes, allowing them to participate in microbial cells' traditional energy and carbon pathways (Mishra et al.,2001). Catechol 1,2-dioxygenase has Fe3+ as a prothetic group and is one of the enzymes that perform intradiol cleavage (Harayama, S. and Ann,1992) . Many bacterial species lack alpha-beta Fe3+ subunits, while others have simple polypeptides (alpha-Fe3+), and chloro-catechols have little or no activity. (Harayama, S. and Ann,1992) Catechol 2,3-dioxygenase, on the other hand, is an extradiol cleaving enzyme with four identical 32KDa subunits, each containing a catalytic iron ion (Fe2+).The objective of this study was to measure the kinetic activity of catechol 1,2-dioxygenase and catechol 2,3 dioxygenase in two isolates Pseudomonas putida and two isolates Pseudomonas aeruginosa for further polycyclic aromatic hydrocarbons bioremediation studies .

Materials and Methods

Microorganism, media and growth conditions

An aromatic hydrocarbon degrading bacteria was obtained from soil contaminated with Crude oil and it's derivatives from Al- Najaf refinery /Iraq and an auto-mechanical workshop. An isolates were characterized by VITEK2 system and by the PCR sequence for 16S rRNA gene as Pseudomonas putida (worod-1and worod-2) Pseudomonas aeruginosa (worod-3 and worod-4) they were submitted to GenBank under the Accession number of MW551536, MW551537, MW551538, MW551539,respectively . This isolates were inoculated in Three concentration (5%-20%-40%) of crude oil in flasks contained Nutrient broth with residues of crude oil containing polycyclic aromatic hydrocarbons for each isolate .the nutrient broth comprised of peptone 5g/l ,sodium chloride 5g/l , meat extract B# 1.5g/l and yeast extract 1.5g/l) after being sterilized autoclaving at 15 lbs pressure 121°C for 15 minutes. Flasks were incubated at 37°C for two weeks.

Preparation of cell-free extract

After growth for one week and two weeks , we taken 10ml from each concentration , cells were harvested from nutrient broth by centrifugation(10,000 rpm) for 5 minutes. Supernatants (cell-free extract) were used for enzyme assays as outlined below.

Kinetic activity of C120 and C230

Catechol 1,2 dioxygenase and Catechol 2,3 dioxygenase activity were assayed in a 3 mL reaction mixture as described (Mahiudddin and Fakhruddin ,2012 ; Olaniran et al.,2017).The reaction mixture contained: 10 mM of catechol in 50 mM Sodium phosphate buffer (pH 8.0). The reaction was initiated by adding 100 µL of crude enzyme into the reaction mixture and incubated for 30 min at 30°C. Buffer plus the enzyme only, and buffer plus substrate without the enzyme were used as controls. The initial and final absorbance at 260 nm and 375 nm were measured using UV-Vis Spectrophotometer (UV-1800, Shimadzu), One unit of enzyme activity was defined as the amount of the enzyme that produced 1 µM of either cis, cis-muconic acid at 260 nm (Catechol 1,2-dioxygenase) or 2-hydroxymuconic semialdehyde 366 at 375 nm (Catechol 2,3-dioxygenase) under standard assay conditions. Enzyme activity was calculated using the equation :enzyme activity (µm of product formed/min) = {(E× L/V) (ΔOD /min)}, where : ΔOD: is the optical density at the different wavelengths, E: is the molar extinction coefficient f product ,V: is the reaction volume, and L is the path length (mm). Molar extinction coefficient of 16800 mM−1•cm−1 (muconic acid) and 14700 mM−1•cm−1 (2-hydroxysemialdehyde) were used to determine the activities for catechol 1,2-dioxygenase and catechol 2,3-dioxygenase respectively (Mahiudddin and Fakhruddin ,2012 ; Olaniran et al.,2017).

Statistical analysis

SPSS program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. USA) and Microsoft Excel were used to perform all statistical calculations (2010, Microsoft Corp. USA). All of the data was presented as a mean.

Result

The activity of Catechol 1,2 dioxygenase showed difference value that produced by strains of Pseudomonas putida and Pseudomonas aeruginosa before treatment of polycyclic aromatic hydrocarbons(PAHs) (figure 1),while activity of catechol 2,3 dioxygenase showed the same value in p. putid strain worod-1 and P.aeruginosa strain worod-3 as well as in P.putida strain worod-2 and P.aeruginosa strain worod-4 (figure 2).

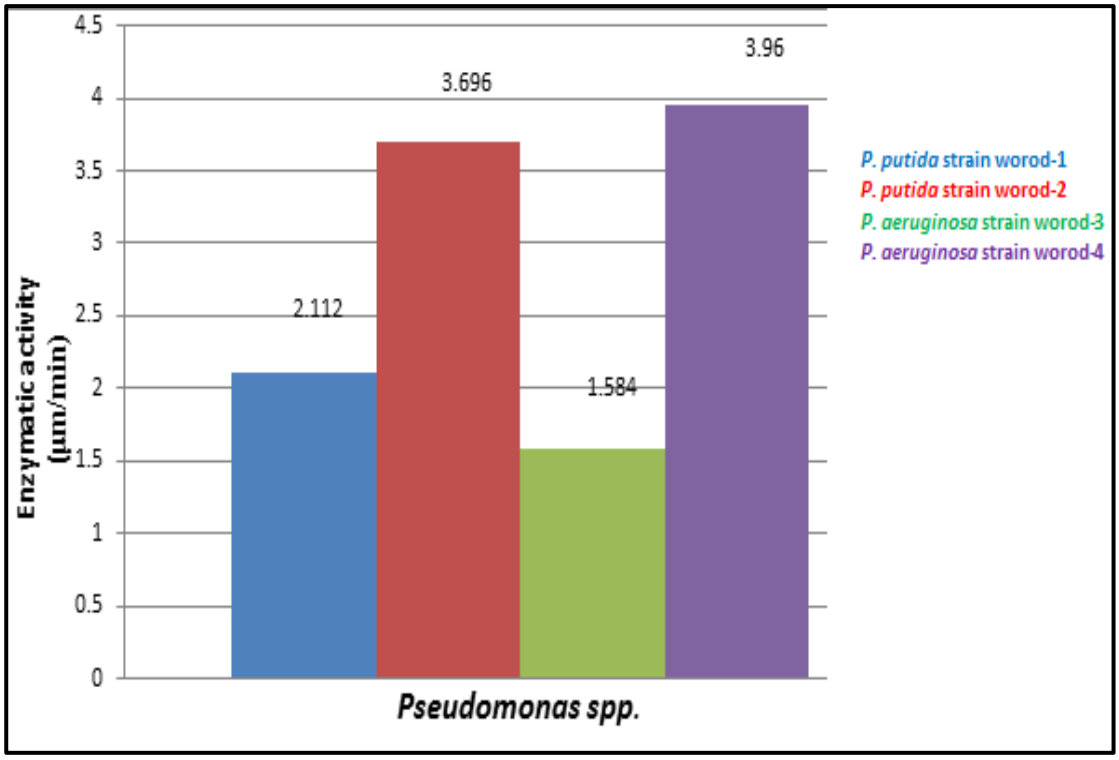

Figure 1: Enzymatic Activity of Catechol 1,2 dioxygenase in strains of Pseudomonas putida and Pseudomonas aeruginosa before treatment of PAHs.

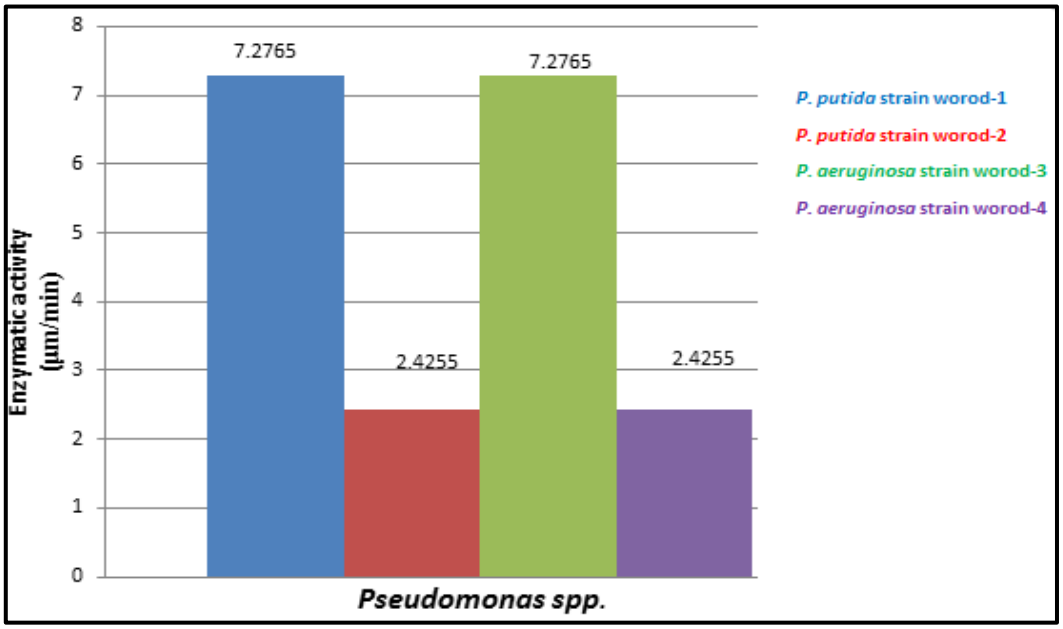

Figure 2: Enzymatic Activity of Catechol 2,3 dioxygenase in strains of Pseudomonas putida and Pseudomonas aeruginosa before treatment of PAHs.

The results in figure 3 showed the effect of polycyclic aromatic hydrocarbons (PAH) concentration level (5% , 20% and 40%) through one week of treatment in the enzymatic activity of C120 produced by Pseudomonas putida strain worod-1 , Pseudomonas putida strain worod-2, Pseudomonas aeruginosa strain worod-3 and Pseudomonas aeruginosa strain worod-4 and indicates to the significant difference ($p< 0.05$) according to LSD value which is 1.685 between concentration of PAHs treatment and enzymatic activity in among pseudomonas strains .there was a significant increasing in content C120 in all strains of Pseudomonas at PAHs concentration (5%,20% and 40%) compared with control (fig.1), the minimum value (2.38µm/min) is recorded in P.aeruginosa strain worod-3 at concentration 40% while the maximum value (7.92 µm/min) is recorded in P.aeruginosa strain worod-4 at concentration 5%.

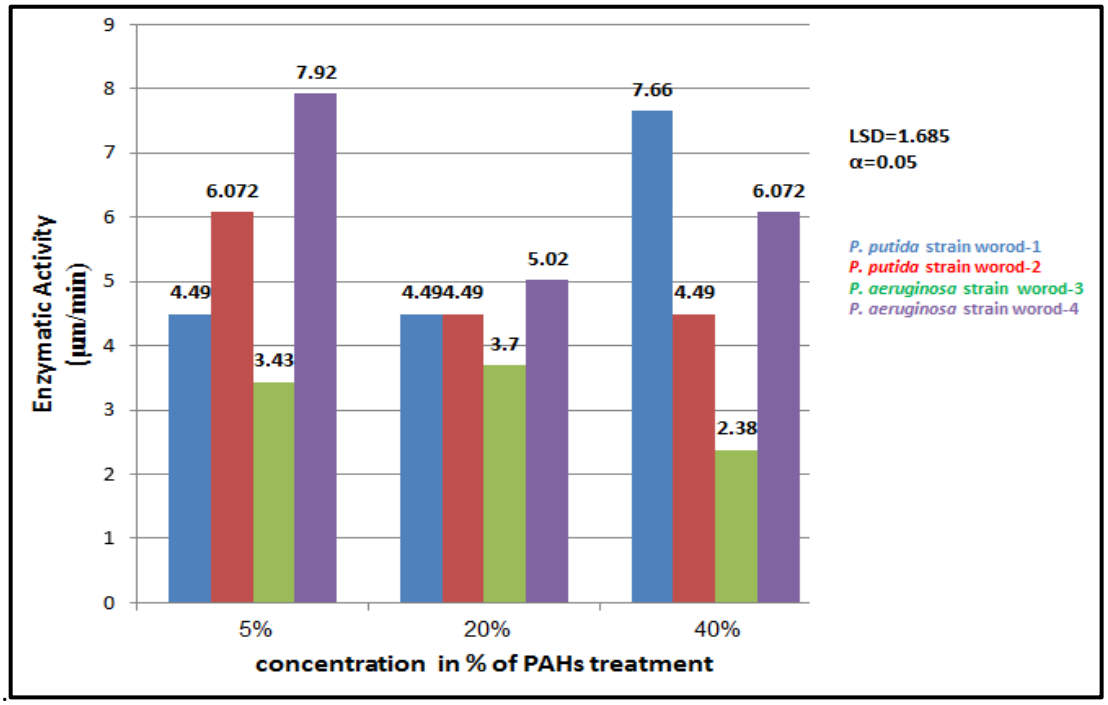

Figure 3: C120 Differential Activity Difference within and among strains of Pseudomonas putida and Pseudomonas aeruginosa after one week of treatment for PAHs compounds.

Figure 4 shows the effect of polycyclic aromatic hydrocarbons (PAH) concentration (5% , 20% and 40%) through two week of remediation in the enzymatic activity of C120 produced by Pseudomonas putida strain worod-1 , Pseudomonas putida strain worod-2, Pseudomonas aeruginosa strain worod-3 and Pseudomonas aeruginosa strain worod-4 and indicates to the significant difference ($p< 0.05$) according LSD value which is 1.255 between concentration of PAHs treatment and enzymatic activity in pseudomonas strains .there was a significant difference in content C120 in all strains of Pseudomonas at PAHs concentration (5% , 20% and 40%) compared with control (fig.1)and enzymatic activity through one week (fig.3) , the minimum value (1.06µm/min) is recorded in P.aeruginosa strain worod-3 at concentration 5% while the maximum value (5.28µm/min) is recorded in P.putida strain worod-4 at concentration 40% .

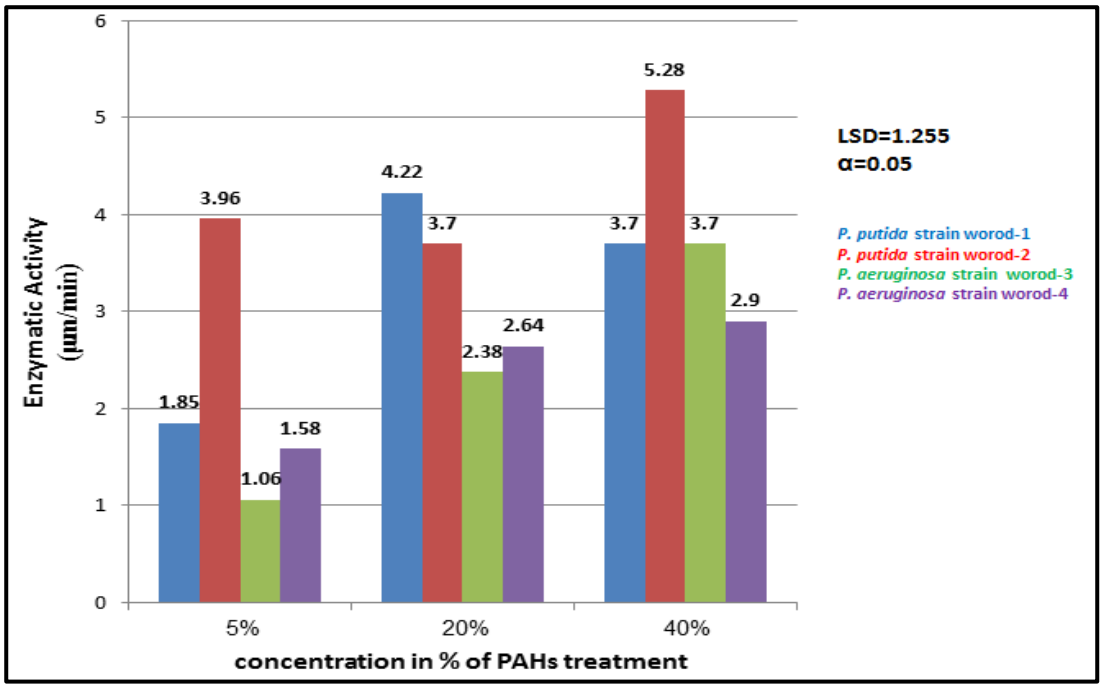

Figure 4: C120 Differential Activity Difference within and among strains of Pseudomonas putida and Pseudomonas aeruginosa after Two weeks of treatment for PAHs compounds .

The results of figure 5 showed the effect of polycyclic aromatic hydrocarbons (PAH) concentration (5% , 20% and 40%) through one week of treatment in the enzymatic activity of C230 produced by Pseudomonas putida strain worod-1 , Pseudomonas putida strain worod-2, Pseudomonas aeruginosa strain worod-3 and Pseudomonas aeruginosa strain worod-4 and indicates to the significant difference ($p< 0.05$) according to LSD value which is 1.094 between concentration of PAHs treatment and enzymatic activity in among pseudomonas strains .there was a significant increasing in content C230 in all strains of Pseudomonas at PAHs concentration (5%,20% and 40%) compared with control (fig.2), the minimum value (4.85µm/min) is recorded in P.putida strain worod-1 at concentration 20% while the maximum value (89.74 µm/min) is recorded in P.putida strain worod-2 at concentration 20% .

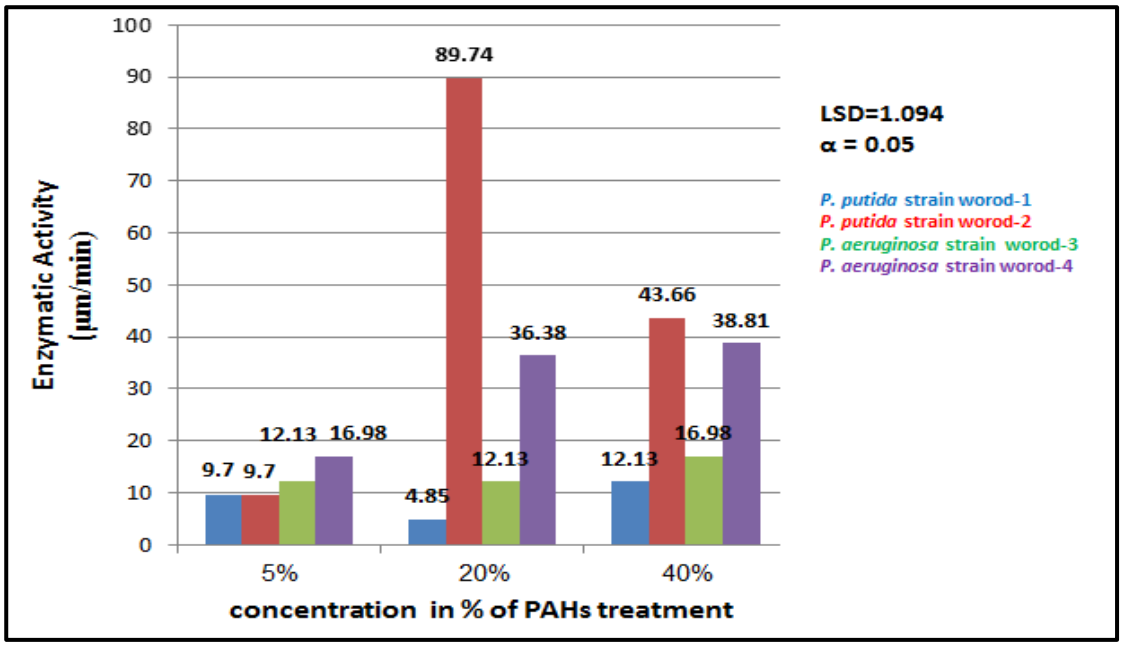

Figure 5: C230 Differential Activity Difference within and among strains of Pseudomonas putida and Pseudomonas aeruginosa after one week of treatment for PAHs compounds.

Figure 6 shows the effect of polycyclic aromatic hydrocarbons (PAH) concentration (5% , 20% and 40%) through two week of treatment in the enzymatic activity of C230 produced by Pseudomonas putida strain worod-1 , Pseudomonas putida strain worod-2, Pseudomonas aeruginosa strain worod-3 and Pseudomonas aeruginosa strain worod-4 and indicates to the significant difference ($p< 0.05$) according to the LSD value which is 1.089 between concentration of PAHs treatment and enzymatic activity in pseudomonas strains .there was a significant difference in content C230 in all strains of Pseudomonas at PAHs concentration (5% , 20% and 40%) compared with control (fig. 2) and enzymatic activity through one week (fig.5) , the minimum value (4.9 µm/min) is recorded in both P.putida strain worod-1and P.putida strain worod-2 at concentration 5% while the maximum value (35.9 µm/min) is recorded in P.aeruginosa strain worod-4 at concentration 20% .

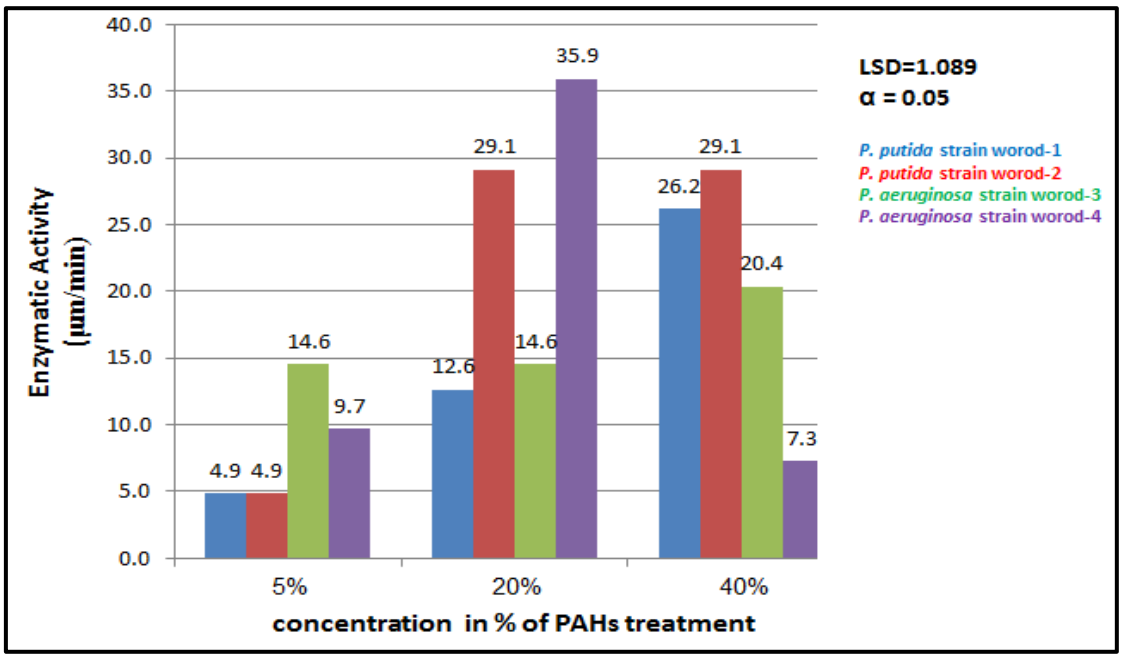

Figure 6: C230 Differential Activity Difference within and among strains in strains of Pseudomonas putida and Pseudomonas aeruginosa after two week of treatment for PAHs compounds.

Discussion

Over the last few decades, bacteria's ability to use individual PAHs as carbon and energy sources has been extensively recorded. (Ahn, Y. et al.,1999).

During the degradation of polycyclic aromatic hydrocarbons (PAHs), bacteria showed a strong induction of ring fission enzymes. Catechol 1,2 dioxygenase (C120) is a metal cleaving enzyme, and Catechol 2,3 dioxygenase (C230) is an ortho cleaving enzyme. The activity of enzymes in aerobic PAHs catabolism primarily includes the incorporation of atoms of molecular oxygen into the aromatic nucleus, resulting in the oxidation of the aromatic ring (Samanta SK.et al.,2002). In this study, C12O and C 230 enzymatic activity was measured in four strains of bacteria including Pseudomonas putida (worod-1and worod-2) and pseudomonas aeruginosa (worod-3 and worod-4). the optimum activity for enzymatic activity was recorded at pH 8 and 35°C Interactions between polycyclic aromatic hydrocarbons (PAHs) and their metabolites have been shown to play a key role in catabolic enzyme induction, and these interactions can be either synergistic or antagonistic (Mohan et al.,2006; Demane`et al.,2004) . In the first case, metabolites generated during the degradation of LMW PAHs in one strain can boost the induction of catabolic enzymes for other LMW or HMW PAHs in other strains (cross-induction and co-metabolism) (Whitman et al.,1998). Due to substrate competition or microbial toxicity, LMW/HMW PAHs or their metabolites can inhibit degradation in the latter case. (Demane`et al.,2004).

In this study We found polycyclic aromatic hydrocarbons(PAH) affect the activity of this enzymes as it led to an increase in enzymatic activity of catechol 1,2 dioxygenase and catechol 2,3dioxygenase in the first week of remediation PAHs compound in three concentration (figure 3and 5) in all four strains of bacteria , while in the second week found enzymatic activity of these enzymes decrease because complete bioremediation of PAHs by Pseudomonas spp.(figure 4 and 6) However ,these results are inconsistent with result of (Nitanshi et al., 2018) were reported the induction of C12O and C230 was slow in Pseudomonas aeruginosa PSA5 , but increased significantly with the incubation period but compatible with (Bin C. et al.,2008) were found both catechol 1,2-dioxygenase and catechol 2,3-dioxygenase enzymatic activities in benzoate-grown P. putida cells, implying that both the ortho- and meta-cleavage pathways are expressed simultaneously in P. putida P8 during the biodegradation of aromatic compounds at high concentrations.. In this study we found The enzyme activity of C230 was higher than that of C120 , This may be due to the bacteria's energy economy, which reduces the output of the enzyme C120, which is part of a more complex degradation pathway (Burlage et al.,1989)

CONCLUSION

The current study dealt with the measure of enzymatic activity of C120 and C230 after one and two weeks of remediation of polycyclic aromatic hydrocarbons by four isolates Pseudomonas putida (worod-1and worod-2) and Pseudomonas aeruginosa (worod-3 and worod-4).the activities of these enzymes increase in one week and decrease with the extension of remediation period due to complete degradation of PAHs compound by bacteria after two weeks.

REFERENCE

1. Ahn, Y., J. Sanseverino, and G. S. Sayler (1999). Analyses of polycyclic aromatic hydrocarbon-degrading bacteria isolated from contaminated soils. Biodegradation 10:149-157.
2. Alexander, M.(1999). Biodegradation and Bioremediation, Academic Press: New York.

3. Barret, M., Carrere, H., Delgadillo, L.; Patureau, D., Water Res. (2010) .44, 3797.
4. Bin Cao, Anli Geng and Kai-Chee Loh,(2008). Induction of ortho- and meta-cleavage pathways in Pseudomonas in biodegradation of high benzoate concentration: MS identification of catabolic enzymes . Appl Microbiol Biotechnol .81:99–107.
5. Burlage, R. S.; Hooper, S. W.; Sayler, G. S. (1989) .Appl. Environ. Microbiol., 55, 1323.
6. Demane che S, Meyer C, Micoud J, Louwagie M, Willison JC and Jouanneau1 Y. (2004) .Identification and functional analysis of two aromatic-ring-hydroxylating dioxygenases from a Sphingomonas strain that degrades various polycyclic aromatic hydrocarbons. Appl Environ Microbial 70: 6714–6725.
7. Guo, C.; Dang, Z.; Wong, Y.; Tam, N. F.(2010). Int. Biodeter. Biodegr. 64, 419.
8. Harayama, S., Ann(1992) . Rev. Microbiol. 46, 565.
9. Herold, M.; Greskowiak, J.; Ptak, T.; Prommer, H.; J.(2011). Contam. Hydrol. 99,119.
10. Johnson, R. B., Onwuegbuzie, A. and Turner, L.(2007) .Toward a definition of mixed methods research. Journ of Mix Meth Res. 1: 112-133.
11. Mahiudddin M, Fakhruddin AN (2012). Degradation of phenol via meta cleavage pathway by Pseudomonas fluorescens PU1. Isrn Microbiology. 2012.
12. Manzetti Sergio(2013). Polycyclic Aromatic Hydrocarbons in the Environment: Environmental Fate and Transformation. Polycyclic Aromatic Compounds 33.311–30.
13. Mishra, V.; Rup, L.; Srinivasan, A.(2001). Crit. Rev. Microbiol. 27, 133.
14. Mohan S, Takuro K, Takeru O, Robert K and Yoshihisa S.(2006). Bioremediation technologies for treatment of PAH contaminated soil and strategies to enhance process efficiency. Rev Environ Sci Biotechnol 5:347–374.
15. Moon, H. S., Kahng, H. Y., Kim, J. Y., Kukor, J. J.; Nam, K.,(2006). Environ. Pollut. 140, 536.
16. Nitanshi Jauhari, Shweta Mishra, Babita Kumari, S. N. Singh, Puneet S. Chauhan & D. K. Upreti (2018): Bacteria Induced Degradation of Anthracene Mediated by Catabolic Enzymes, Polycyclic Aromatic Compounds.
17. Olaniran AO, Singh L, Kumar A, Mokoena P, Pillay B.(2017) Aerobic degradation of 2, 4-dichlorophenoxyacetic acid and other chlorophenols by Pseudomonas strains indigenous to contaminated soil in South Africa: Growth kinetics and degradation pathway. Applied Biochemistry and Microbiology. 1;53(2):209-16.
18. Samanta S.K., Singh O.V. and Jain R.K. (2002). Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol 20:243–248.
19. Whitman BE, Lueking DR and Mihelcic JR (1998). Naphthalene uptake by a Pseudomonas fluorescens isolate. Can J Microbiol 44:1086–1093.
20. Wilson SC and Jones KC (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ Poll 81:229–249
21. Zhao, Z., Selvam, A., Wong, J. W.(2011). Bioresour. Technol. 102, 3999.