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Achromobacter xylosoxidans bacteria isolated from contaminated Agricultural environment for 2,4-dichlorophenoxyacetic Acid degradation: Experimental study

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Abstract
In the agricultural sector, a class of chemical compounds known as chlorinated phenol and phenoxyacetic acid is employed as an herbicide, wood preservative, and pesticide. Additionally, they are listed as priority pollutants by the USEPA. The other group of chlorinated phenoxyacetic acid is used as herbicides to control broad leaf weeds in both agriculture and domestic application (to control weeds of home gardens). Following systemic dilution of soil sample on mineral salt medium, three bacterial isolates (D1, D2, and D3), were isolated and subjected to lots of screening of which D2 was proven to be the best. Molecular identification includes polymerase chain reaction, gel electrophoresis, sequence alignment and phylogentic analysis which prove the isolate was *Achromobacter sp*. Incubation time, substrate concentration, pH, temperature, and inoculum size in mineral salt medium were all the parameters tested during the characterization work 2,4-dichlorophenoxyacetic acid herbicide was used as the alternative source of carbon. The bacterila isolate grew and degrade 2,4-dichlorophenoxyacetic Acid best at 96 h incubation time, 0.72gL^{-1} substrate concentration, pH of 7.5, 40 ºC temperature, and 400µg/L inoculum size. High performance Liquid Chromatography (HPLC) analysis of the residual 2,4-dichlorophenoxyacetic Acid and the standard solution probe the isolate degrade up to 95.38% of the substrate indicating great potentiality in bioremediation.

Keywords: Bioremediation, *Achromobater*, Herbicide, Agriculture

1.0 INTRODUCTION
In addition to their beneficial effects, pesticides—man-made organic compounds—create detrimental residues that cause a health problem to the environment and crops. In environmental settings, pesticides are used to shield plants from weed diseases and insect damage. These plants typically come into contact with soil, where they experience a range of changes. Depending on the chemical makeup of the pesticide, the soil, and weathering circumstances, they disappear from the soil at different rates. The most important processes that lead to their breakdown are usually the ones that are chemical, photochemical, and microbial. According to Ref. [1], certain pesticides or the byproducts of their breakdown build up in the soil and descend into ground water.

In the agricultural sector, a class of chemical compounds known as chlorinated phenol and phenoxyacetic acid is employed as an herbicide, wood preservative, and pesticide. Additionally,

they are listed as priority pollutants by the US EPA. The other group of chlorinated phenoxyacetic acid is used as herbicides to control broad leaf weeds in both agriculture and domestic application (to control weeds of home gardens) [1]. These xenobiotic compounds of environmental concern can be removed from the environment by biodegradation and biotransformation method [2]. High concentrations of herbicides make them less effective, and organic amendment frequently speeds up biodegradation. Numerous bacteria that break down 2,4-D have been isolated from soil that has been exposed and not. According to Ref. [1], multiple bacterial strains acting alone or in a consortium have been observed to actively degrade 2,4-D. Citation[1], state that this compound serves as a model for examining the breakdown of chlorinated aromatic compounds in soil.

Despite the fact that 2,4-D has a 6.2-day aerobic soil half-life, several factors, including pH, temperature, and moisture, have a significant impact on how quickly it degrades [3]. This increases the possibility of penetration via the soil and into groundwater systems when combined with the high terrestrial mobility of 2,4-D. 2,4-D is regarded as "persistent" to "highly persistent" once present in anoxic environments, such as saturated agricultural land and aquifers, with a half-life of 41–333 days [3]. Because of this, there is worry about 2,4-D's mobility and stability in anoxic environmental systems and its potential for transport to and contamination of off-site areas like surface water and drinking water supplies. Therefore, in order to reduce the risks to human health and the environment that 2,4-D poses, it is necessary to develop cost-effective and dependable technologies for cleaning up contaminated anoxic subsurface environments.

It has been determined that bioremediation is an economical and environmentally benign technology for treating contaminated environments [4]. Furthermore, an increasing amount of research has been done on the anaerobic microbial transformation of 2,4-D in conditions that are sulfate-reducing, iron-reducing, and methanogenic [5]. The extraction of the acetic acid and chloride groups from 2,4-D has been linked to a variety of microorganisms, including those from the genera *Alcaligenes, Bulkoderia, Rhodoferax, Variovorax, Cupriavidus, Achromobacter, Comamonas, Holomonas*, and *Pseudomonas* [6]. Despite the lack of knowledge regarding the enzymatic mechanisms of anaerobic 2,4-D biodegradation, various degradation pathways have been deduced through the identification of multiple metabolic intermediates. It was suggested that 2,4-D functions as a terminal electron acceptor during reductive dechlorination, which removes chloride substitutes from 2,4-D and its metabolites [5, 6]. This study clearly focuses on determining the physical and chemical parameters of the soil used, molecularly and chemically identifying the best bacteria that can degrade the herbicide (2,4-D), as well as characterization. More so, degradation percentage will be determined using High Performance Liquid Chromatography (HPLC).

2.0 Materials and Methods

Fields cultivating rice provided a sample of the soil. Before being used within 72 hours, they were brought to the lab and kept at 4ºC. During sampling, the temperature of the soil was

measured with a portable handled thermometer. The sample was transported in plastic containers to the lab, where it was kept at 4^{o}C. After being dehydrated, the subsamples were run through a sieve with a diameter of less than two millimeters. To create a composite sample from the farm, these subsamples were combined and homogenized. For further analysis, the sample was stored at 4^{o}C.

2.1Determination of physico-chemical characteristics of soil

During field sampling, a portable thermometer was used to record and measure the temperature of the soil. According to Ref. [7], standard method was used to measure the soil pH in a soil water suspension, or a 1:2 ratio of soil to water. 30 milliliters of distilled water were added to a glass beaker containing 30 gram of soil, which was then mixed. For an hour, the sample was kept to stand, with stirring at every ten to fifteen minutes, to allow the soil pH to stabilize. Prior to testing, the sample's temperature was taken into account when calibrating the pH meter. pH4.0,7.0, and 10 buffers were used to standardize the pH meter.

2.2 Isolation and screening of 2,4-D degrading bacteria

Three bacterial strains in total were chosen after additional screening, one from each sampling area. The 2,4-D degrading bacterial isolates were then put through a second test called the tolerance test, which involves double the concentration of the 2,4-D used from 0.72 gL^{-1} to 1.44 gL^{-1}in order to identify the isolates with high substrate resistance and use them for additional analysis. Ref. [8], first reported using 0.72 gL^{-1}.

2.3 Molecular identification of the bacterial isolate

2.3.1 DNA Extraction Protocol (Polymerase Chain Reaction)

DNA isolation was carried out in the Instrumental Laboratory, Bayero University Kano. According to the method used by Ref, [9], the entire microbial DNA was extracted. Proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation were the standard procedures used to isolate high-molecular-weight DNA from bacterial samples. Proteinase K digestion, phenol-chloroform extraction, deparaffination with xylene, and finally ethanol precipitation were the steps used in the DNA extraction process from archival formalin-fixed specimens. A change to the protocol allowed for the purification of DNA from Pap-stained smears. Following xylene incubation, staining, proteinase K treatment (60°C for at least 1 hour), and cell transfer to sterile Eppendorf tubes, this protocol was followed. The protein was subsequently precipitated by adding saturated ammonium acetate. After recovering the DNA supernatant using ethanol, the pellet was dried, cleaned with 70% ethanol, and dissolved in 200 µl of TE-low (10 mM Tris-HCl [pH 7.4], 0.1 mM EDTA).

2.3.2 Phylogenetic analysis

The National Centre for Biotechnology Information (NCBI) BLAST search programme was used to compare the 16S rRNA sequences with the sequences in the public database in order to identify bacterial 16S rRNA gene sequences that are closely related. Next, using ClustawX2 programme, the two 16S rRNA gene sequences were aligned [10]. Then, using MEGA6

programme (http://www.kumarlab.net/publications) and the closely related 16S rRNA gene sequences, a phylogenetic tree was created using the Maximum Likelihood Method [11].

2.4 Characterization of bacteria degrading 2,4-D

2.4.1Effect of incubation period on 2,4-D degrading bacteria

A range of time intervals, from 24 to 120 hours, were used to observe the impact of time of incubation on 2,4-D degradation. For 120 hours, the bacterial isolate was inoculated and kept on an incubator shaker set to 120 rpm [12]. Optical density was measured at 600 nm, and bacterial growth was observed.

2.4.2 Impact of 2,4-D concentrations on bacterial degradation

250 ml Erlenmeyer flasks containing 50 ml of MSM supplemented with varying concentrations of 2,4-D (1.44 gL^{-1}, 0.72 gL^{-1}, and 0.36 gL^{-1}) were inoculated with aliquots (0.72 gL^{-1}) of 24 h initial cultures grown in Luriea broth for their ability to utilize 2,4-D (herbicide). In addition to MSM, 2,4-D was added to maintain a control. Cell number increase was used to track the growth of the bacteria over the course of five days of 24 hour interval incubation. According to Ref. [13, 14], the optical density was measured at 600 nm and bacterial inoculum (100 μgL^{-1}) was extracted from the test and control cultures on a regular basis.

2.4.3 Temperature effect on degradation of 2,4-D

MSM was used to test how temperature affected the growth of bacteria and the breakdown of 2,4-D. According to Ref. [15], every bacterial isolate was inoculated at a different temperature for 120 hours, ranging from 25 to 45ºC at intervals of 5ºC. Using spectrophotometry, the absorbance at 600 nm was used to determine whether bacterial growth had occurred after incubation.

2.4.4 Effect of initial pH on bacterial growth

Bacterial growth on MSM was tested in relation to pH. Using 0.5 intervals, the medium's pH was adjusted between 5.5 and 8.5. Ref. [16], reported that the bacterial isolate was inoculated and then incubated for 120 hours at 35ºC. By measuring the optical density at 600 nm, the growth of bacteria was ascertained.

2.4.5 Effect of inoculum concentration on bacterial degradation

By adding varying concentrations of inoculums (100–500 μgL^{-1}) to the medium at intervals of 100 μgL^{-1}, the impact of inoculum size was examined. For 48 hours, the bacterial isolate was inoculated and kept on an incubator shaker set to 120 rpm. By monitoring the absorbance at 600 nm, bacterial growth was detected [17].

2.5 Preparing samples for 2,4-D HPLC analysis.

The procedure outlined by Ref. [18], was followed when cleaning the samples for HPLC analysis.

2.6 Analysis of statistics

Each experiment was carried out three times. One-way ANOVA with replications ($P < 0.05$) was used to test the physicochemical properties of soil. Additionally, chartsand tables were employed

to display the data. Charts and line graphs were utilized to depict the impact of treatment on the bacterial isolates' growth over the various time intervals.

3.0 Findings and Discussion

3.1 Physical and chemical characteristics of soil from farm

The physical and chemical characteristic of rice cultivated soil obtained from Kura farm was presented in table 1.

Table 1: Physical and chemical characteristics of soil from farm

Farm	Kura
%N	0.34±0.03
P mg kg^{-1}	23.79±0.02
K mg kg^{-1}	2.41±0.01
Ca mg kg^{-1}	0.55±0.02
Mg mg kg^{-1}	20.76±0.04
pH	4.04±0.37
Temp	23.67±0.88
% Moisture	22.35±2.75
%TOC	22.35±2.75
%WHC	76.02±0.34
Texture	Sandy Clay

Water Holding Capacity (WHC), Total Organic Carbon (TOC). Data shows means ± SD, significance difference is at $p < 0.05$,

Because they can be used to draw conclusions about the results of these experiments, soil physicochemical parameters are crucial for biodegradation studies. The activity of the microorganisms that cause the mineralization of the pesticides is primarily dependent on these environmental factors [19]. Additionally, some anomalies in the soil that may be noticed during analysis can be explained by these properties of the soil. The farm had acidic soil, as evidenced by the pH of the soil, which were 5.05. It has been discovered that some pesticides biodegrade slowly at pH values higher than 6 and optimally at pH values lower than 5 [20]. The study's results, which showed that 2,4-D degradation was slow at an acidic pH and reached its maximum at pH 7.5, conflict with this. The compound being broken down and the possible organism that breaks it down determine how the pH affects things, though. The degradation of pesticides is significantly influenced by soil temperatures. It has been observed that most pesticide degradation tends to increase within the range of 10 and 45 ^{o}C in temperature [21]. Since 2,4-D degradation was most effective at 40 ^{o}C, the results of Ref. [21], study were completely consistent with the results of this investigation. The physicochemical characteristics of soil, including temperature, humidity, and moisture content, have an impact on how quickly herbicides break down in the soil [22]. The moisture content of the soil is very important to the degradation process, according to Ref. [23]. Water controls the pesticides' availability to

microorganisms and serves as their solvent. In contrast to wet soil, dry soil typically has slower biodegradation. Anaerobic degradation has been observed in water-logged soil, as opposed to aerobic degradation because of the restriction of oxygen entry into the soil. However, depending on the pesticide in question, too much moisture content may speed up or slow down the utilization. On the other hand, prolonged pesticide use may negatively impact certain physical and chemical components of the soil. As per Ref. [24], the use of certain pesticides can result in modifications to the nitrogen (N2) fixing organisms, including Rhizobium, Azotobacter, and Azospirillum. Additionally, cellulolytic and phosphate-solubilizing microbes may be impacted [25].

3.2 Isolation and screening of 2, 4-D resistance bacteria from soil

According to early research using 2,4-D as the only source of energy, three bacterial isolates coded (D1, D2, and D3) in all were able to grow during the screening process when 2,4-D was present, while best one was chosen through the means of tolerant test by increasing the normal concentration of 2,4-D used from 0.72 gL^{-1} to 1.44 gL^{-1} to find the most resisting strain based on its ability to tolerate high concentration of the substrate as the only carbon and energy source.Out of the three bacterial strain D2 shows higher optical density measurement of (0.272) and was therefore, chosen and identified as the best candidate for bioremediation of 2,4-D as shown in Table 2.

Table: 2 Tolerance test

S/N	Isolate	Optical density (OD600nm)
1	D1	0.208
2	D2	0.272*
3	D3	0.206

Keys: D= Danhassan, * indicate isolate with high optical density

3.3Molecular identification

3.3.1 Sequencing Alignment and Phylogenetic result

The phylogenetic analysis of D2-BUK-BCH also clustered with *Achromobacter xylosoxidans* with 100% similarity with those deposited in the gene bank as showed in Figure 1.

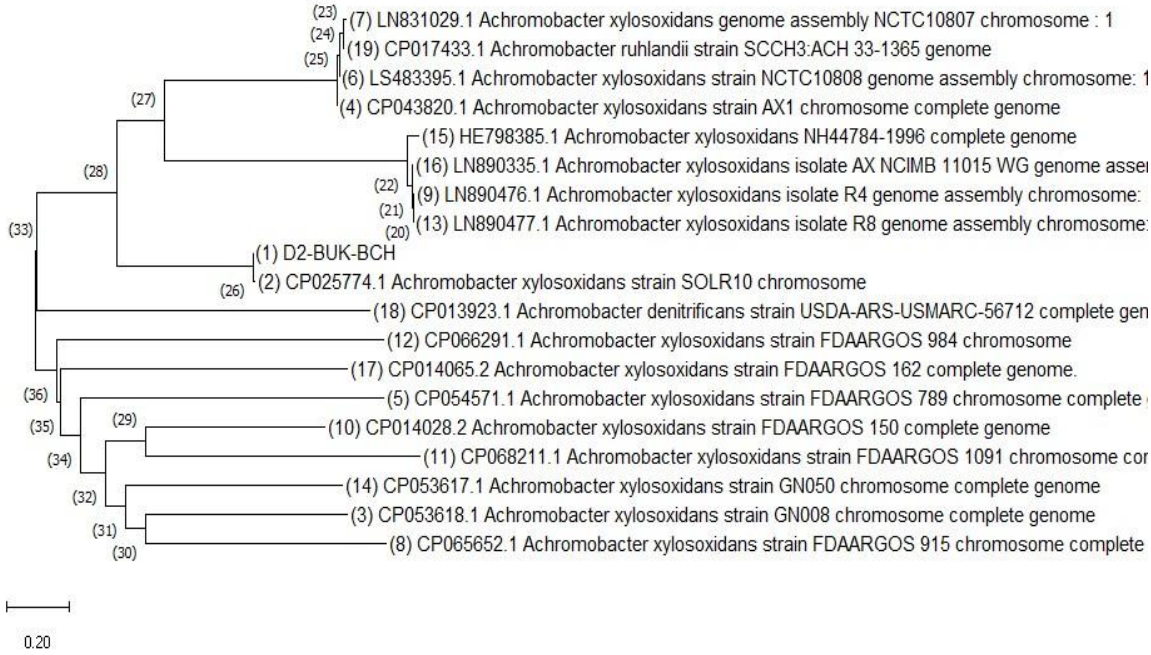

Figure 1: D2-BUK-BCH phylogenetic analysis Display the correspondence sequences' outcome.

3.4 Characterization activities

3.4.1 Substrate concentration effect on bacterial degradation

The selected bacterial isolate was tested at three (3) different 2,4-D concentrations to find the best condition for their growth for enhanced degradation ranging from 0.36 gL^{-1} , 0.72 gL^{-1} and 1.44 gL^{-1}. The results shows that bacterial growth was best at 0.72 gL^{-1} of 2,4-D and therefore, used for the subsequent studies (Figure 2).

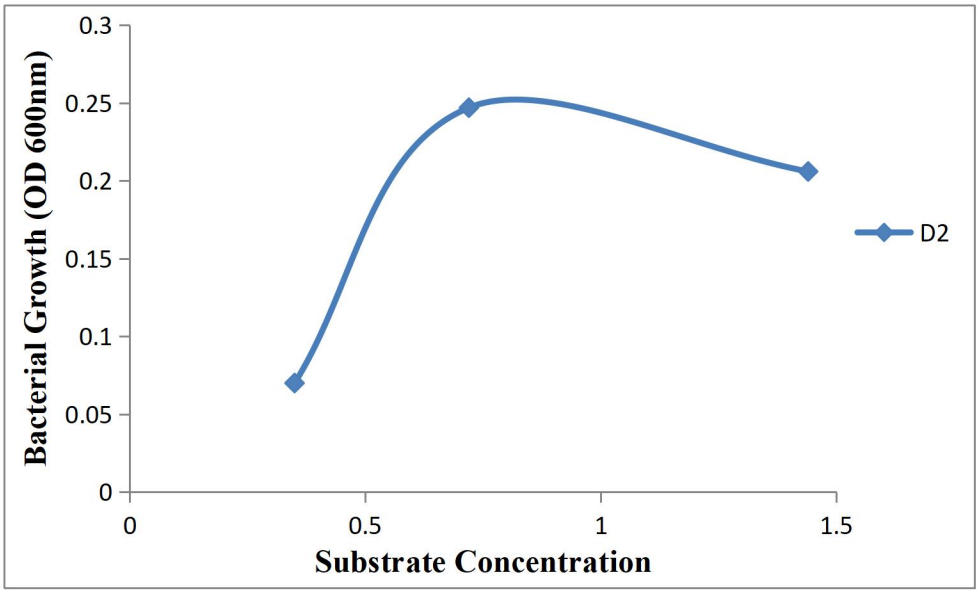

Figure 2: Effect of 2,4-D concentration on bacterial degrading

3.4.2 Temperatures effect on 2,4-D resistance bacteria

Over the temperature range of 30 to 45°C, the impact of temperature on the growth of 2,4-D resistant bacteria was demonstrated. Since growth peaked at 40°C and then began to decline, as Figure 3 illustrates, this temperature was maintained for the duration of the experiment. The optimal temperature for the growth of the isolate was 40°C, after which the growth drastically decreased with increasing temperature Figure 3. A study by Ref. [26], reported that the cell extracts of strain *Cupriavidus* showed 2,4-D degradation activity at temperature range of 10–40°C, with an optimal temperature of approximately 30°C. However, *Cupriavidus* showed lower activity (almost 80%) at 10°C. The rate at which pesticides break down is significantly influenced by temperature. It has been observed that most pesticide degradation tends to increase within 10 and 45ºC in temperature [21, 26]. The rate at which herbicides break down is stimulated by physical and chemical characteristics, including temperature, moisture content, and humidity[22, 27]. In order to degrade 2,4-D, Ref. [27], operated the anaerobic SBR at a lower mesophilic range (30±2°C) and the aerobic SBR at an ambient temperature of 22±2°C. Sludge was used as a seed. According to their report, at feed concentrations of up to 500 mgL-1, the aerobic reactor achieved complete utilisation of 2,4-D. However, 120 mgL-1 of 2,4-D, or 40% of the maximum feed concentration used, could not be broken down by the anaerobic SBR. The strain CY1 demonstrated the highest 2,4-D degradation at a temperature of about 30°C, which is in line with the results obtained in this study and the results of Ref. [27]. Using the bacteria Arthrobacter D9, Pseudomonas H1, and Bacillus H9 at pH 6 and temperature of 28°C, Ref. [28], also carried out studies on 2,4-D degradation.

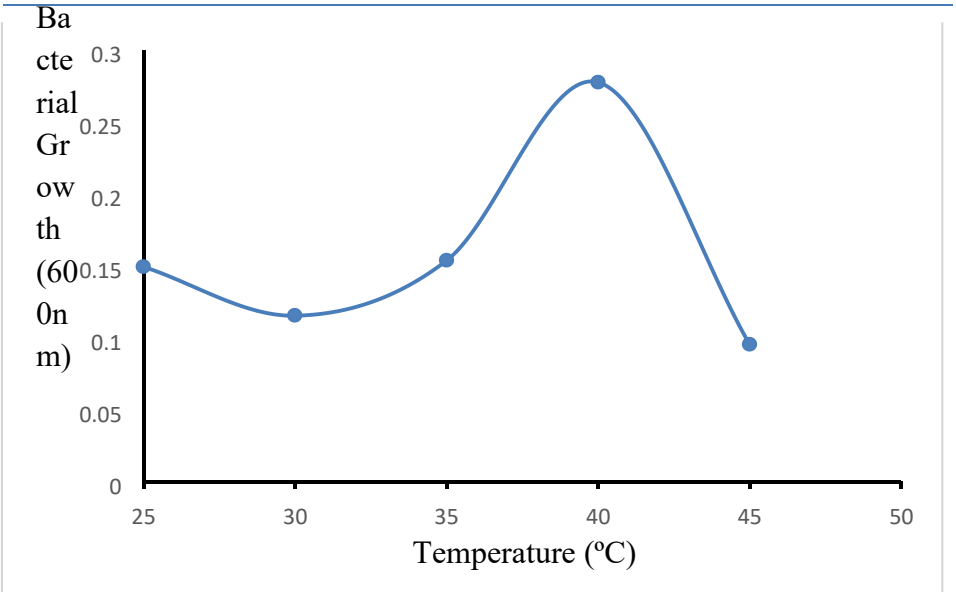

Figure 3: Effect of temperature on 2,4-D degrading bacterium

3.4.3 pH effect on 2,4-D degrading bacteria

In a study conducted to assess how pH affected the growth of a bacterial isolate. In Figure 4, it is demonstrated that the isolate could withstand 2,4-D over a broad pH range (5.5-8), growing at its maximum at pH 7.5. As a result, pH 7.5 was chosen as the ideal pH for degradation activity. A higher pH caused the growth pattern to decline. Based on Figure 3, the isolate exhibits maximum activity at pH 8 and greater activity between pH 7 and 8. The farm had an acidic soil, as indicated by the pH range of 4.16 to 5.05. According to Ref. [29], the biodegradation of certain pesticides is slow at pH values greater 6 and optimal at pH values less than 5. The pH impact, however, varies depending on the compound being degraded and the possible organism doing so. This result was in contrast to the study's findings, which showed that all three isolates exhibit higher activity at pH values of 7-8, with a maximum at 8. This discrepancy may be caused by changes in the soil types, locations, and chemicals to which the soil is exposed.According to Ref. [26], Pseudomonas cepacia degrades 2,4-D slowly above pH 7 and does not break down the compound at alkaline (pH 8.1) or acidic (pH 3.3) pH values. Our results were consistent with the fact that 2,4-D's CY-1 degradation was greater at neutral pH (pH 7.5). Ref. [26], found similar results to ours, with the exception of unbiased conditions, where CY-1 degraded more quickly.

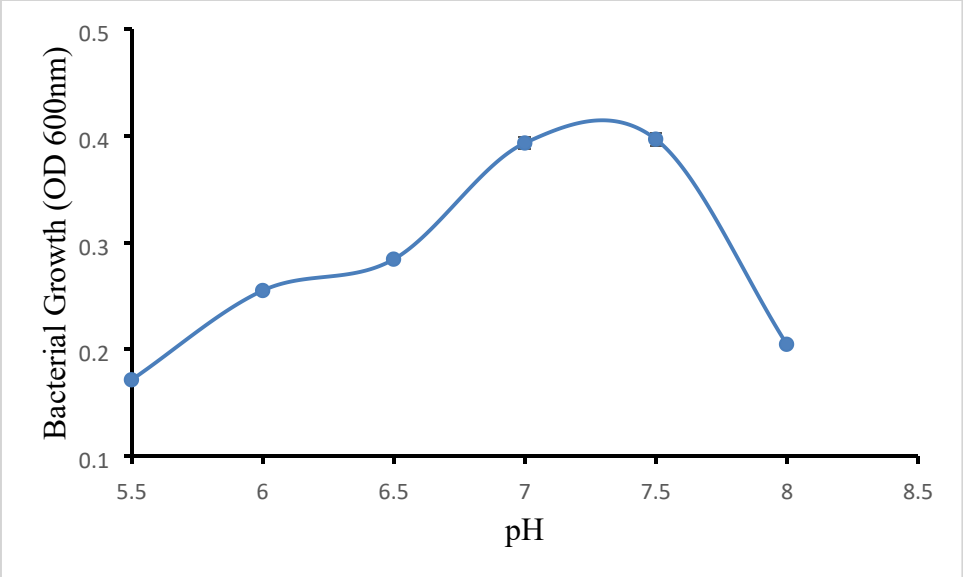

Figure 4: Effect of initial pH on bacterial degradation of 2,4-D

3.4.4 Incubation time effect on 2,4-D degradation

Incubation time was found to be a key factorto consider for the growth of 2,4-D by bacterial strain D2-BUK-BCH. It was observed how the culture of 2,4-D degrading bacteria was growing. As seen in Figure 5, the medium's turbidity peaked over the course of the duration at 96 hours. There have also been reports of 2,4-D resistance linked to growth [12, 14, 27].

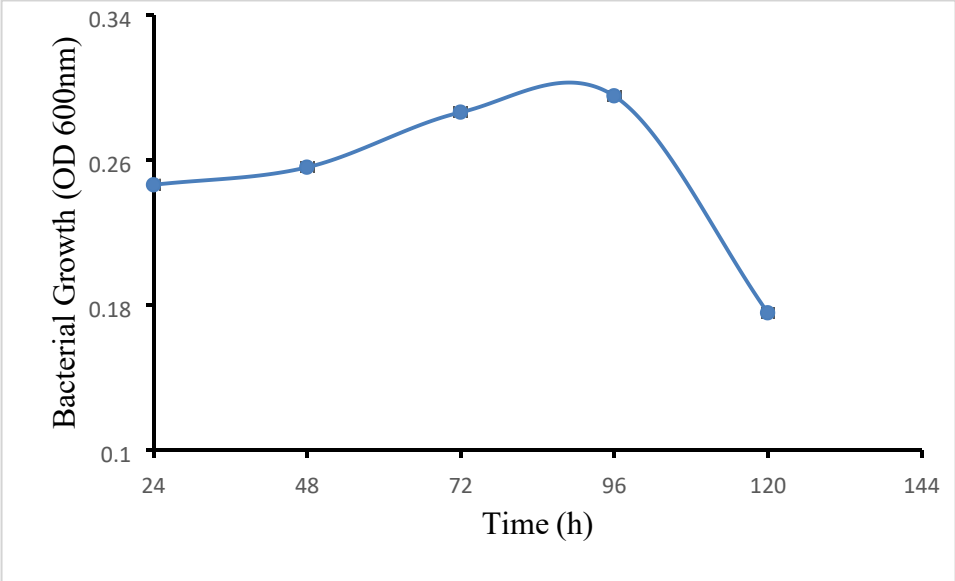

Figure 5: Incubation Time effect on bacterial degradation of 2,4-D

3.4.5 Impact of inoculum concentration on 2,4-D degrading bacteria

The concentration of the inoculum was a crucial factor in the development of bacteria resistant to 2,4-D. At various inoculum concentrations between 100 and 500 μgL^{-1}, the 2,4-D utilizing bacterium culture pattern was observed as in Figure 6. The isolate grew more at 400 μgL^{-1} than it did at 100 μgL^{-1}, 200 μgL^{-1}, 300 μgL^{-1}, and 500 μgL^{-1}, in that order. Figure 6 illustrates how the isolates' physiologies differ from one another, which could account for these variations.

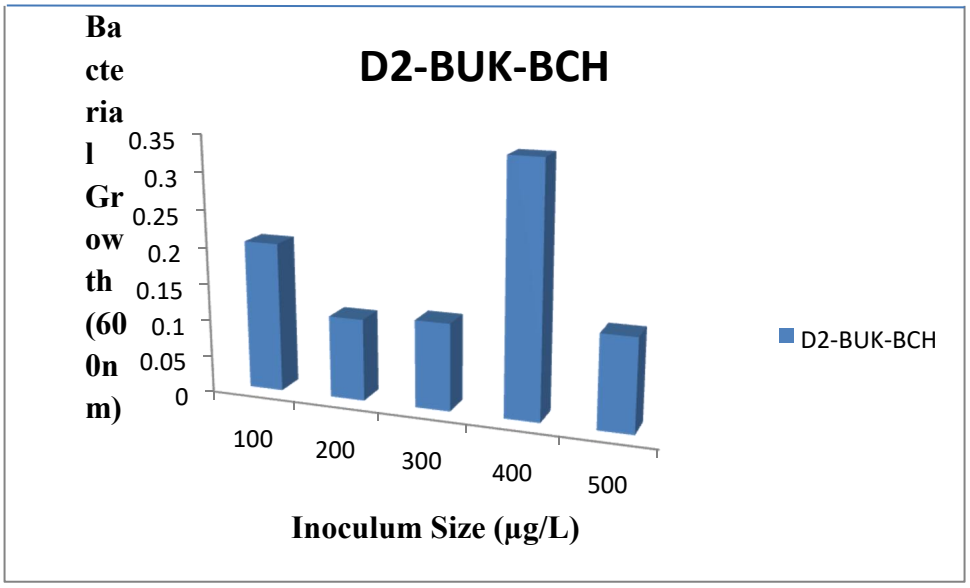

Figure 6: Effect of inoculum Size on bacterial degradation of 2,4-D

3.5HPLC result of 2,4-D degradation

HPLC analysis was performed on the sample and control solutions to ascertain the degradation percentage (%) by the isolate. In this method, there was only one retention time peak produced by the bacterial degradation of 2,4-D. Because of this, the 2,4-D degradationpercentage was determined using the 2,4-D peak level. By utilising the 2,4-D peak area values acquired for both control and sample, the strain's degradation value is D2-BUK-BCH 95.38%.

3.5.1 Percentage Degradation

The level of degradation was calculated using the residual 2,4-D concentration in the sample.
Amount of 2,4 – Dichlorophenoxyacetic acid residues in samples (0.0332) and the initial sample used (0.72) all in gL^{-1}

$$Percentage\ Degradation\ (\%) \quad = \frac{0.72}{0.72} - \frac{0.0332}{\qquad} \times 100\% = 95.38\%$$

CONCLUSION

The bacterium was isolated from agriculture contaminated soil sample with history of herbicide application. The isolated strains were screened to select the isolate with higher potential for 2,4-D degradation. The bacterium showed good growth and degraded 2,4-D by 95.38% respectively at 0.72 gL^{-1} concentration within 76 h. Phylogenetic analysis of this isolate identified this isolate as *Achromobacterxylosoxidans*. It showed *Achromobacterxylosoxidans* work better at the temperature of 40ºC and pHof 7.0, and inoculum size 400 μgL^{-1} , respectively. From the study it can be concluded that this isolate could be effective for bioremediation of 2,4-D contaminated site.

References

1. Nisar, A.K., Ameer, A.A., Ghulam, S.M., Tatheer, N., (2014). Degradation of 2,4-dichlorophenoxy acetic acid in soil by bacterial consortium. ***Int. J. Biol. Biotech.,* 10 (3): 395-400, 2013.**

2. Haggblom, M. (1990). Mechanism of bacterial degradation and transformation of chlorinated monoaromatic compounds. *J. Basic Microbiol*., 30: 115 – 141.

3. Gunther B., Andrea A. V., Zacchariah R., Peng P.,Siavash At., Hauke S., Alette L., Nora B. (2021). 2,4-Dichlorophenoxyacetic acid degradation in methanogenic mixed cultures obtained from Brazilian Amazonian soil samples. Biodegradation (2021) 32:419–433

4. Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J MicrobiolBiotechnol 32(11):180

5. Yang Z, Xu X, Dai M, Wang L, Shi X, Guo R (2017) Rapid degradation of 2, 4-dichlorophenoxyacetic acid facilitated by acetate under methanogenic condition. BioresourTechnol 232:146–151

6 Zharikova NV, Iasakov TR, Zhurenko EY, Korobov VV, Markusheva TV (2018). Bacterial genes of 2,4- Dichlorophenoxyacetic acid degradation encoding a-ketoglutarate- dependent dioxygenase activity. Biol Bull Rev 8:155–167

7. Geotechnical Engineering Bureau (2007). Test method for the determination of pH value of water or soil by pH meter, Department of transportation, New York.

8. Okpala G. N., (2010), Studies on biodegradation of glyphosate by bacterial species isolated from rice field. Department of Microbiology, (Masters dissertation), *University of Nigeria, Nsukka*

9. Jahun, B.M., Ibrahim, S., Muhammad A.B., and Shehu, D., (2022). Isolation and Characterization of 2,4-Dichlorophenoxyacetic Acid Degrading Bacteria from Rice Cultivated Soil in Kura Local Government Area of Kano State. Nigeria. KIU Journal of Science, Engineering, and Technology. Vol. 01 No. 2 Pp 32-39.

10. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin,F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *25*:4876-4882.

11. Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725-2729.

12. Sultan S, &Hasnain S., (2007). Reduction of toxic hexavalent chromium by *Ochrobactrumintermedium*strain SDCr-5 stimulated by heavy metals. *Bioresour. Technol.* 98(2): 340-344.

13. Shobana, N., (2017). Characterization of chromium bioremediation by *Stenotrophomonasmaltophilia*SRS 05 isolated from tannery effluent. *International Research Journal of Engineering and Technology*. **4,** 406-429.

14. Jahun, B.M., Dayyabu, S., Shehu, U., Saifullahi, M. D., Muhammad, Y. G., Ahmad, H. J., (2023). Biodegradation potential of 2, 4 dichlorophenoxyacetic acid by Cupriaviduscampinensis isolated from rice farm cultivated soil, Case Studies in Chemical and Environmental Engineering (2023), 100434.

15. Damaris L., Juan F., Víctor M. M., & Ismael A. R. (2013). Hexavalent chromate reductase activity in cell free extracts of *Penicillium*sp. *HindawiPublis. Corp. Bioinorganic Chemistry and Applications*.1-6.

16. Wang, Y. J., & Xia, K. (2010). Characterization of phenol degradation by high-efficiency binary mixed culture.*Environmental Science and Pollution Research*, *17*(5), 1035-1044.

17. Bidlas, E., Du, T., & Lambert, R. J. (2008). An explanation for the effect of inoculum size on MIC and the growth/no growth interface.*International journal of food microbiology*, *126*(1-2), 140-152.

18. Bicalho, S.T., Langenbach, T., Rodrigues, R. R., Correia, F. V., Hagler, A. N., Matallo, M. B., &Luchini, L. C. (2010). Herbicide distribution in soils of a Riparian forest and neighboring sugar cane field.*Geoderma*, *158*(3):392-397.

19. Njue, R. M. (2018). *Assessment and molecular characterization of bacterial degraders of hexazinone and 2, 4-d herbicides from sugarcane cultivated soils in Nzoia company nucleus estates, Kenya* (Doctoral dissertation, Egerton University).

20. Schoenholtz, S. H., Van Miegroet, H., & Burger, J. A. (2000). A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. *Forest Ecology and Management*, 138 (1), 335-356.

21. Rani, S., &Sud, D. (2015). Effect of temperature on adsorption-desorption behaviour of triazophos in Indian soils. *Plant Soil Environ*, *61*(1), 36-42.

22. Govedarica, M. M., Marinković, B., Milošević, N. A., Đukić, D. A., &Mandić, L. G. (2000). The number and enzymic activity of microorganisms in the soil under wheat grown after sugar beet.ActaagriculturaeSerbica, 5(10), 55-63.

23. Shahgholi, H., &Ahangar, A. G. (2014). Factors controlling degradation of pesticides in the soil environment: A Review. *Agriculture Science Developments*, *3*(8), 273-278.

24. Omakor, J. E., Onyido, I., &Buncel, E., (2001). Mechanisms of abiotic degradation and soil–water interactions of pesticides and other hydrophobic organic compounds.Part 3.Nucleophilic displacement at the phosphorus centre of the pesticide fenitrothion [O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] by oxygen nucleophiles in aqueous solution: α-effect and mechanism. *Journal of the Chemical Society, Perkin Transactions 2*(3), 324-330.

25. Gulhane, P. A., Gomashe, A. V., &Sunderkar, K. M. (2015). Influence of pesticides on nitrogen fixing bacteria. *International Journal of Technical Research*, *3*(4), 157-160.0.0

26. Greer G. W, Hawari J, & Samson R. (1990). Influence of environmental factors on 2,4-D degradation by *Pseudomonas cepacia*isolated from peat. *Arch Microbiol*. 154: 317–22.

27. Celis E., Elefsiniotis P., &Singhal N. (2008). Biodegradation of agricultural herbicides in sequencing batch reactors under aerobic or anaerobic conditions.*Water Resources Journal*. 42:3218–24.

28. Roane T. M., Josephson K. L., & Pepper I. L. (2001). Dual-bioaugmentation strategy to enhance remediation of cocontaminated soil.Appl Environ Microbiol. 67:3208–15.

29. Schoenholtz, S. H., Van Miegroet, H., & Burger, J. A. (2000). A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. *Forest Ecology and Management*, 138 (1), 335-356.