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Analysis of Enzymes Activities on Domestic Waste Dump Sites

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Abstract

Introduction: Effects of physico-chemical parameters on microbial dehydrogenases from domestic waste dumpsites were studied.

Methodology: The microorganisms (S. aureus, D2 and EDTA, ethanol and butanol) on the microbial dehydrogenase were determ partially puriýed enzyme with the effectors for 30 min at 4ÁC. Also, the effects of temperature and pH were assessed by varying the temperature and pH ranges from 10ÁC to 60ÁC and 2.0 to 8.0, respectively.

Results: The speciýc activities of the enzyme from the microorganisms were 7.10, 7.73, 6.47, 6.26, 9.66 and 10.58 mg Formazan/mg cell dry wt/h, respectively. Calcium ion, Mg

$^{2+}$, ethanol and butanol signiýcantly increased ($p<0.05$) dehydrogenase activities in all the microorganisms studied while Zn^{2+}, Fe^{2+} and EDTA decreased the activities.

Conclusion: These activators can be harnessed within the recorded optimum temperature and pH ranges to enhance microbial growth; which is essential for the degradation of domestic waste thereby promoting cleaner environment.

Keywords:Dehydrogenase activities; E ectors; Domestic waste

Introduction

e quality of life on the Earth is linked inextricably to the overall quality of the environment. Wastes were traditionally disposed of in land lls in the past. is traditional mode of waste disposal was publically unacceptable due to the increasing conversion of scarce agricultural lands to dumf

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and suspended in the same bu er containing 2 mm EDTA and 1 mm Dithiothreitol (DTT). Cells were ruptured using osmotic shock by Nossal and Heppel [12]. e cells were suspended in 20% sucrose bu er then separated by centrifugation at 4000 rpm for 10 min. e resulting paste was dispersed in acetone at 4°C. Cellular debris and unbroker cells were removed by centrifugation at 4000 rpm for 45 min at 4°C. e supernatant obtained constituted the crude microbial extracts (soluble enzyme fraction) for each microbe.

Puri cation of enzyme

e enzyme was partially puri ed from the crude microbial extracts in four steps: ammonium sulfate precipitation, dialysis, sephadex G200 gel ltration chromatography and DEAE-cellulose column chromatography. All the steps were performed at 4°C.

Ammonium sulphate precipitation

e protein sample was allowed to thaw to determine total volume, and was centrifuged at 3000 rpm for 30 min. is was transferred into a beaker containing a stir bar and was placed on a magnetic stirrer. While the sample was being stirred, solid ammonium sulfate crystals were added to bring the nal concentration to 60% saturation. (e volume of ammonium sulphate used was equal to the volume of the

Ion-exchange chromatography

e enzyme was further puri ed using DEAE-cellulose column chromatography. e method used was as described by Yannis [14]. DEAE-cellulose was suspended in 8 vol. of Tris-bu er containing 50 mM of NaCl and kept overnight for equilibration. e column was carefully packed equilibrated with 8 vol. of same bu er containing 0.25 mM of NaCl. en 3 ml of the partially puri ed enzyme was diluted to 15 ml and loaded into the column and was washed with appropriate 100 ml of the equilibration bu er. e protein was eluted with 0.25M bu er pH 7.2 and NaCl gradient 0.1-1 M, was passed through the exchanger

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C. albican respectively, when compared to the activities in other studied microorganisms.

As shown in Figure 2, the maximum DHA activity was recorded for *F. oxysporium* at pH 6.0 and the lowest was observed in *S. aureus* at pH 2.0. At pH 6.0, 7.0 and 8.0 the DHA activity from *S. aureus* was lower than those from *P. aeruginosa*, *T. mentagraphytes* and *F. oxysporium*.

e maximum activities were recorded at 35°C while minimum activities were recorded at the temperature of 10°C (Figure 3). Among the organisms studied, the highest DHA activity was recorded for *F. oxysporium* and the lowest activity was observed for *P. aeruginosa* at the optimum temperature of 35°C. At 35°C, the DHA activities from *S. aureus, E. coli* and *C. albicans* were lower than those from *P. aeruginosa, T. mentagraphytes, and F. oxysporium*. e result obtained in this study is in contrast with that of Dickinson and Monger [25] who reported that the optimum temperature and pH for microbial DHA were 25°C and pH 7.5, respectively.

Conclusion

e e ects of metal ions and inhibitors on the partially puri ed dehydrogenases from microorganisms were assessed. e optimum pH and temperature were also determined at 6.0 and 35°C, respectively. e activity was severely inhibited by EDTA but butanol and ethanol had marked activating e ects. ere was increase in activity of the enzyme on Ca^{2+} treatment, whereas Mg^{2+} had a moderate increase but activities were decreased by both Zn^{2+} and Fe^{2+}. Hence, in the presence of these activators, optimum temperature and pH, the activity of microbial dehydrogenase can be enhanced for degradation of domestic wastes dump sites and by extension, remediation of the soil for industrial and agricultural purposes.

Declaration of Interest

e authors hereby declare that no con ict of interest exists.

References

1. Vidali M (2001) Bioremediation: An overview. Pure and Applied Chemistry 73: 1163-1172.
2. Hammel E, Cadisch G, Giller K (1997) Fungal degradation of waste. In: Driven by Nature Litter Quality and Decomposition, pp: 33-45.
3. Subhani A, Changyong H, Zhengmiao Y, Min L, El-ghamry A (2001) Impact of Soil Environment and Agronomic Practices on Microbial/Dehydrogenase Enzyme Activity in Soil: A Review. Pakistan Journal of Biological Sciences 4: 333-338.
4. Wolinska J, Stepniewska B (2012) Oxidoreductases extracellularly secreted by microbes. Journal of Molecular Biology 8: 38-58.
5. Zhang SB, Wu ZL (2010) Identiýcation of amino acid residues responsible for increased pH of microbial dehydrogenase. Journal of Bioresources and Technology 102: 2093-2096.
6. Voet S, Donald F (2006) Fundamental of biochemistry life at the molecular level. John Wiley and Sons, New York, USA, pp: 105-114.
7. BrzeziŒska M, Stňpniewski W, Stňpniewska Z, Przywara G (2001) Effect of Oxygen Deýciency on Soil Dehydrogenase Activity in a Pot Experiment with Triticale CV Jago Vegetation. International Agrophysics 15: 145-149.
8. Salazar S, Sanchez L, Alvarez J, Valverde A, Galindo P, et al. (2011) Correlation Among Soil Enzyme Activities Under Different Forest System Management Practices. Ecological Engineering 37: 1123-1131.
9. Watts C, Sun L, Mochly R (2010) Mitochondrial aldehyde dehydrogenase. Biochemistry Journal 45: 227.
10. Sonia S, Saksham G (2014) Heavy metal impact on soil microbial biomass, soil dehydrogenase activity and soil respiration rate. Int J Adv Res Biol Sci 1: 29-34.
11. Pfeiffer J (1954) Enzymes, the physics and chemistry of life. Wiley, New York, pp: 171-173.
12. Nancy GN, Leon AH (1966) The Release of Enzymes by Osmotic Shock from Escherichia coli in Exponential Phase. J Biol Chem 241: 3055-3062.
13. Hassan C, Fredlier A, Neri H, Reed R (2013) Sephadex G200. Method of enzyme puriýcation to increase speciýcity, pp: 98-105.
14. Yannis S (2014) Puriýcation and Characterization of lactate dehydrogenase, An undergraduate biochemistry laboratory experiment. Advances in Biochemistry 2: 14-23.
15. Iheme CI (2013) Enzyme isolation, puriýcation and characterization. In: Laboratory Experiments in Biochemistry (A Students' Manual). In: Ojiako AO (ed.), FUTO Press Ltd., Owerri, Nigeria, pp: 300-333.
16. Nweke CO, Okolo JC, Nwanyanwu CE, Alisi CS (2006) Response of planktonic bacteria of New Calabar River to zinc stress. Afr J Biotechnol. 5: 653-658.
17. Nwogu