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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 [illegible]

Results: [illegible] 10.58 mg Formazan/mg cell dry wt/h, respectively. Calcium ion, Mg [illegible] (p<0.05) dehydrogenase activities in all the microorganisms studied while Zn^{2+}, Fe^{2+} and EDTA decreased the activities.

Conclusion: [illegible] 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 unbroken 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 sample. Adding the ammonium sulphate very slowly ensured that local concentration around the site of addition did not exceed the desired salt concentration). Once total volume of ammonium sulphate was added, the beaker was kept at 4°C for 6 hours. e solution was transferred to centrifuge tube and the precipitate centrifuged at 10,000 rpm for 10 min. e supernatant was carefully concentrated to 80% using ammonium sulphate, and was then stirred. A er 60 min, it was centrifuged at 12,000 rpm. e supernatant was discarded and the pellet or precipitate collected and re-suspended in the minimal volume of 0.002 mM phosphate bu er pH 7.2. e DHA at this level was checked by TTC reduction. e protein was further puri ed using dialysis.

Dialysis

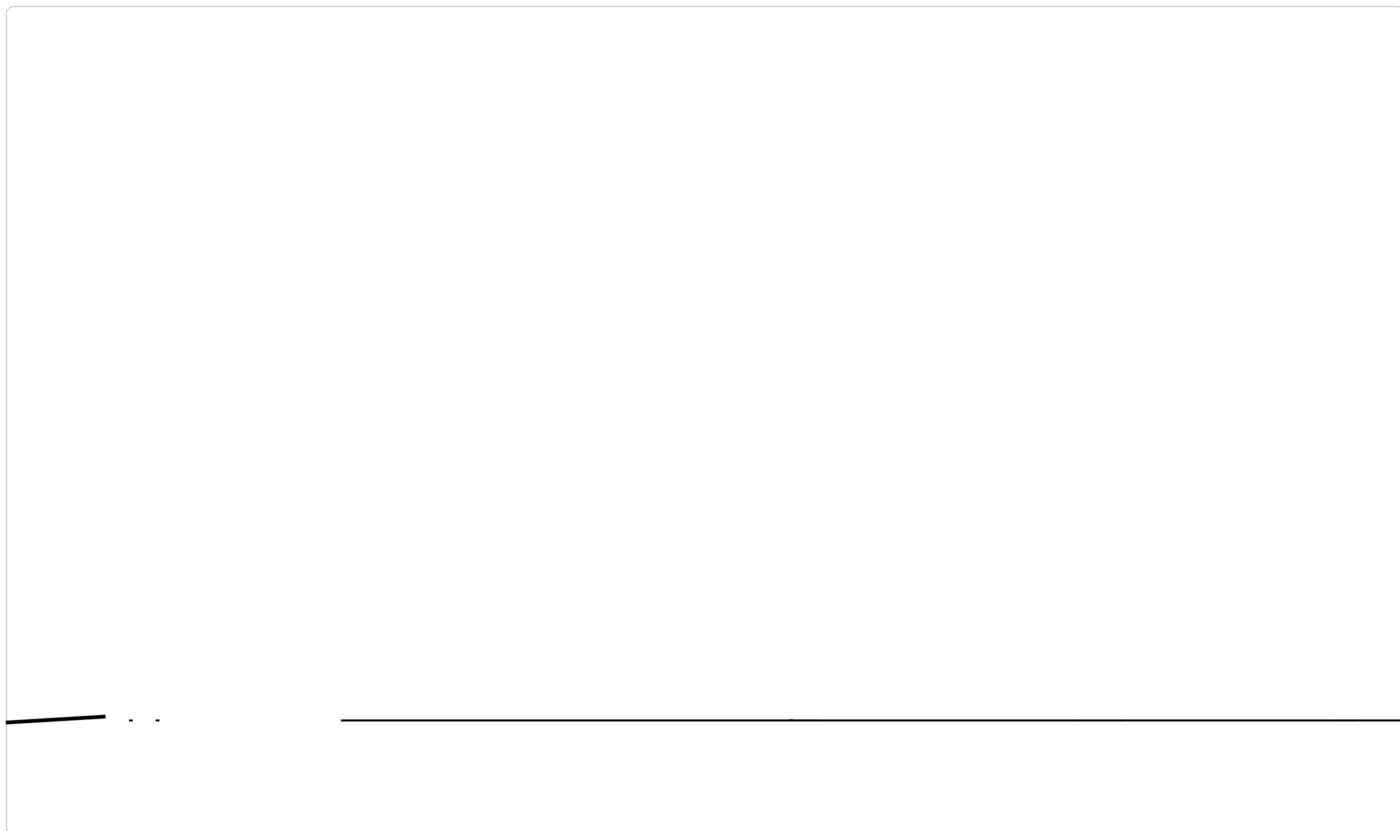
e membrane was placed in 1% sucrose solution for 30 min and the cell free crude extract that was re-suspended in minimum volume of 0.02 M phosphate bu er solution (pH 7.2) was introduced into the dialysis bag. e solution was dialyzed against 500 ml of the same bu er for 12 hours at 4°C in a refrigerator to remove the excess salt with one change a er every 4 hours. us, an enzyme preparation concentrated against sucrose was obtained. is was followed by centrifugation of the resulting solution at 12,000 rpm at 4°C and the supernatant was tested for DHA.

Sephadex G-200 method

is technique was used to further purify the enzyme extract. is was carried out using the method of Hasan e[13]. e enzyme extract was equilibrated with 0.01 M sodium phosphate bu er (pH 6.5) and the slurry was allowed to swell overnight at room temperature. Sodium azide (0.02%) was added to prevent microbial growth. is was then loaded into a 1.6 × 40 cm column, equilibrated with 0.25 M sodium phosphate bu er and fractionated through the Sephadex G-200 column. Five milliliters (5 ml) of the enzyme preparation was applied carefully to the top of the gel and allowed to elute through the gel in the column. e bu er was added without disturbing the gel surface and the reservoir. Elution was carried out

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.

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