The Effects of Dimethyl Phthalate on the Growth of *Pseudomonas aeruginosa*

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INTRODUCTION

The findings of phthalic acid esters (PAEs) in the environment suggests that these compounds may be classified as persistant environmental contaminants (AUTIAN 1973). By comparison to the known persistant organochlorine insecticides, PAEs are produced in much greater quantity and have varied uses in the industrial sector. The PAEs are used mainly as plasticizing agents and represent a group of aromatic compounds which may reach the environment indirectly via waste plastics or directly by industrial manufacturers (GRAHAM 1973). Recent research has been concerned with the degradation of PAEs by microorganisms. Using the Semi-continuous Activated Sludge Biodegradation Test and the River Die-Away Test (SAEGER, et al. 1973) it was found that PAEs and intermediates are rapidly biodegraded by the microbial community present in the environment. However, little work has been done to show the effects of PAEs on specific microorganisms. This study was done to determine what effect the PAE - dimethyl phthalate had on the growth rate of the bacterium Psuedomonas aeruginosa. This species of bacteria was used because of its known ability to utilize various aromatic compounds.

MATERIALS AND METHODS

Determination of the effect of dimethyl phthalate on the growth of Pseudomonas aeruginosa was accomplished by the comparative analysis of growth curves (Dry Weight - mg/ml VS Time). The organisms were grown on "63 Salts" minimal salts medium (MSM) consisting of KH_2PO_4 (13.6g), $(NH_4)_2SO_4$ (2.0g), $MgSO_4$ (0.2g), $FeSO_4 \cdot 7H_2O$ (0.0005g) per liter of distilled water. All cultures included 0.4% glucose as a carbon source. The P. aeruginosa cultures were all grown in 100ml of the liquid medium at 37° C. and at an agitation speed of 100rpm with a 6 inch stroke in an A. O. Model #2156 Water Bath Shaker.

Dimethyl phthalate was incorporated into the experimental media at five definite concentrations: 10ppm, 100ppm, 500ppm, 1000ppm, and 1500ppm. All the experimental media were made seperately, 100ml at a time using the appropriate dilutions from a 1000ppm aqueous stock solution of dimethyl phthalate (DMP).[1] All media containing DMP were autoclaved, stirred continuously for 24 hours,

[1] 1500ppm DMP MSM was made with the direct introduction of DMP.

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then resterilized to insure the maximum amount of DMP in solution.

The innocula for the experimental cultures were taken from an 18 hour culture of P. aeruginosa grown on 0.4% glucose MSM. The innoculum (5ml) was centrifuged for 10 min. in a Clay-Adams tabletop centrifuge and the pellet resuspended in 5ml of the appropriate experimental medium. Cultures were monitered and growth determined every hour for 10 hours and again after 24 hours and 48 hours of incubation by turbidometric measurements (Optical Density @ 450 nm) on a Bausch and Lomb Spectrophotometer (Spectronic 20).

The phthalate concentration of some P. aeruginosa cultures were determined by gas-liquid chromatography both prior to innoculation and following 24 hours of incubation. The aqueous glucose MSM containing DMP was extracted with equal volumes of methylene chloride. The extraction of cultures was done with the medium plus the cells to insure that any phthalate adsorbed by the cell wall of the organisms was also extracted and measured. Measurement of the extracts was effected using a Tracor 550 dual column gas chromatograph equipped with dual flame-ionization detectors. Quantitation was calculated from a standard curve made of DMP.

To obtain a meaningful assay of bacterial growth, Optical Density was correlated to the dry weight of P. aeruginosa (mg/ml). Significance was determined using chi square at the 0.05 level.

RESULTS AND DISCUSSION

The results of this study showed that the bacterium P. aeruginosa did not exhibit any significant inhibition on the first exposure to dimethyl phthalate at concentrations of 10ppm, 100ppm, 500ppm, and 1000ppm. Only slight inhibition was noted at the concentration of 1500ppm.

After 10 hours of incubation the 10ppm DMP culture exhibited a 15.8% growth increase as compared to the control. The 100ppm, 500ppm, and 1000ppm DMP cultures also showed no inhibition at this stage of incubation, growing almost identical to the control. Although the growth enhancement observed in the 10ppm DMP culture is not significant, what is important is the fact that the growth of P. aeruginosa was not inhibited whatsoever at DMP concentrations of 10ppm, 100ppm, 500ppm, and 1000ppm after10 hours of incubation. There was, however, some slight inhibition noted on the 1500ppm DMP culture. (Fig. 1 & 2).

After 24 hours of incubation all but the 1500ppm DMP culture had surpassed the control in cell concentration by the following percentages: 10ppm - 11.1%, 100ppm - 5.5%, 500ppm - 5.5%, 1000ppm - 5.5%. Again, although the growth was not enhanced significantly at these concentrations, the growth of the bacteria had not been inhibited at any of the five concentrations of DMP after 24 hours of incubation. At this stage the slight growth inhibition exhibited by the 1500ppm DMP culture at 10 hours of incubation had been overcome (Fig. 2).

After 48 hours of incubation (death phase) the cell concentration in the 10ppm DMP culture still exceeded the control by 4.9%, together with the 100ppm DMP culture which exceeded the control by 2.9%. The cultures with 1000ppm and 1500ppm DMP lysed more rapidly

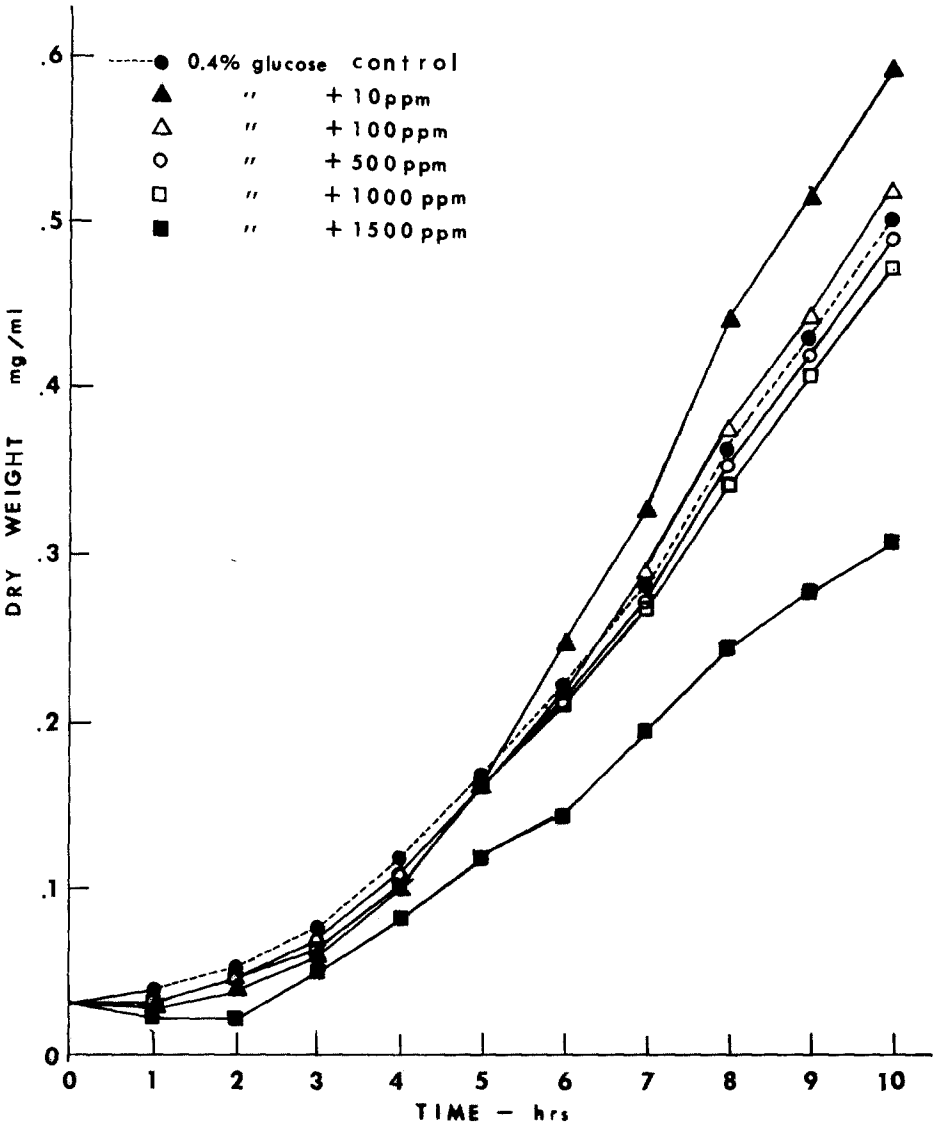

Fig. 1 P. aeruginosa growth curves - 10 hours of incubation.

than the control (Fig. 2).

A gas chromatographic examination of a P. aeruginosa culture growing on 100ppm DMP 0.4% glucose MSM was performed and it was found that the concentration of DMP went from 98ppm prior to innoculation to 88ppm following 24 hours of incubation. To rule out other physical variables which might effect the comcentration of DMP in the media a control flask of uninnoculated 100ppm DMP 0.4% glucose MSM was incubated with the experimental flask and gas chromatograph values of 98ppm prior to innoculation and 97ppm following 24 hours of incubation were obtained.

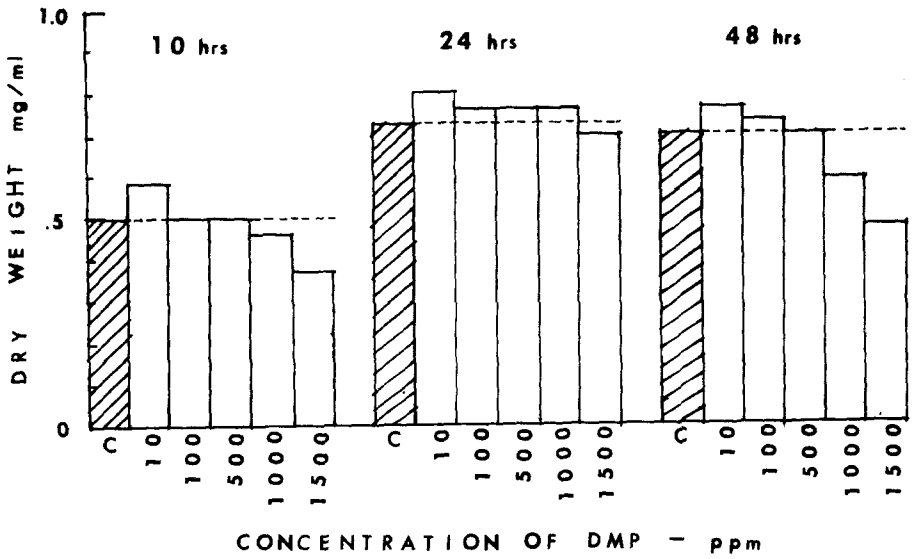

Fig. 2 P. aeruginosa cell concentrations (Dry Weight) at 10hrs., 24hrs., and 48hrs. of incubation.

This fact, coupled with what seemed to be growth enhancement observed at 10ppm, 100ppm, 500ppm, and 1000ppm DMP concentrations, suggests that the bacterium P. aeruginosa may be utilizing DMP as a carbon source. Because of previous studies showing that Psuedomonads, specifically P. aeruginosa, are capable of degrading and utilizing aromatic compounds such as benzene (MARR, et al. 1961), toluene (GIBSON, et al. 1968), catechol (MARR, et al. 1961), and methylcatechols (DAGLEY, et al. 1964) there is reason to speculate that this species may also utilize DMP as a carbon source (Fig. 3).

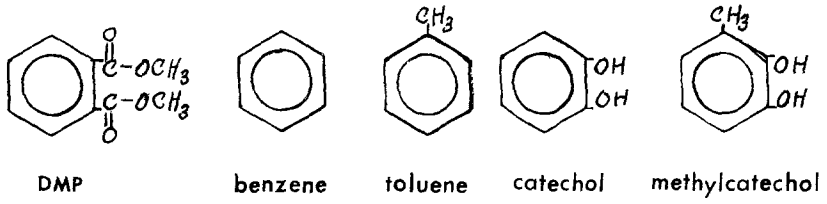

Fig. 3 DMP and other aromatic compounds.

The ecological significance of this study lies in the finding that the growth of the bacterium P. aeruginosa is not inhibited at DMP concentrations of 1000ppm and less with only slight inhibition noted at the 1500ppm concentration. It is interesting to note that it is at these lower concentrations that DMP exists as a pollutant of rivers and streams used as part of industrial waste disposal systems (HITES 1973). Of even greater importance is the

speculation raised that not only is this Pseudomonas species able to grow and metabolize in a DMP polluted aquatic environment, but may in fact also be utilizing this phthalic ester as a carbon source. The gas chromatography results discussed earlier coupled with the lack of inhibition found certainly makes P. aeruginosa a likely candidate for the utilization and degradation of DMP. The findings of this study plus further research now in progress should assist in eventually resolving the problem of the pollutant plasticizers by using our knowledge to improve not only the state of the aquatic environment itself, but also the efficiency of industrial waste disposal systems.

ACKNOWLEDGEMENT

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REFERENCES

AUTIAN, J. Environ. Health Perspec. 4, 3 (1973)
BOOTH, G. H. and J. A. ROBB J. Appl. Chem. 18, 194 (1968)
CARPENTER, E. J. and K. L. SMITH Jr. Science 175, 1240 (1972)
CIFRULAK, D. S. Soil Science 107, 63 (1969)
DAGLEY, S., W. C. EVANS, and D. W. RIBBONS Nature 188, 560 (1960)
DAGLEY, S., P. J. CHAPMAN, D. T. GIBSON, and J. M. WOOD Nature 202, 775 (1964)
GIBSON, D. T. Science 161, 1093 (1968)
GRAHAM, P. R. Environ. Health Perspec. 3, 3 (1973)
HITES, T. A. Environ. Health Perspec. 3, 17 (1973)
MARR, E. K., and R. W. STONE J. Bacteriol. 81, 425 (1961)
MATHUR, S. P. J. Environ. Qual. 3, 189 (1974)
MATHUR, S. P. J. Environ. Qual. 3, 207 (1974)
MILGRAM, J. New Scientist 57, 184 (1973)
MIYOSHI, T., T. HARADA Biochem. Biophys. Acta 222, 684 (1970)
OGNER, G. and M. SCHNITZER Can. J. Chem. 49, 1053 (1971)
SAEGER, V. W., and E. S. TUKER Plastics Engineering Aug. 46 (1973)
SHEA, K. P. Environ. 13, 2 (1971)
STAHL, W. H. and H. PESSEN Appl. Micro. 1, 30 (1953)
WILLIAMS, D. T. J. Agricul. Food Chem. 21, 1128 (1973)