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Phosphatase Enzymes in Soils: Nature and Location of the Enzymes and Phosphorus Immobilization and Mineralization.

ADEWALE ADEBAYO
Department of Soil Science,
University of Ife,
Ile-Ife.

Abstract

The nature and location of phosphatase enzymes produced by soil microorganisms under different cropping and ecological conditions were evaluated by incubating the supernatant of a 1:10 soil-distilled water suspension for 40 weeks in microbial P- surplus and P- deficient conditions. At various time intervals, the cell-free, cell-bound, total dissolved P, dissolved inorganic P and turbidity of the cultures were determined. Results indicated that most of the phosphatase enzymes existed in a cell-bound rather than cell-free state irrespective of the P status of the medium during the first 8 weeks of incubation. Total phosphatase activity showed little tendency to decline with continued incubation. However, in all the systems the rate and extent of organic P mineralization varied but was not related to phosphatase activity. Phosphatase activity was also not related to the living biomass in these cultures.

Introduction

The production of phosphatase enzymes has been the subject of extensive investigation in diverse microorganisms: bacteria (Torriani, 1960; Hofsten 1961; Horiuchi *et al.*, 1959), algae (Kuenzler and Perras, 1965; Blum, 1965) and fungi (Dorn and Rivera, 1966; Shieh *et al.*, 1969; Casida, 1959; Ohta *et al.*, 1968). Torriani (1960) surmised that both acid and alkaline phosphatases of *E. coli* were associated closely with the cells whether living or dead, and that neither enzyme was excreted into the growth medium. A similar conclusion that phosphatase enzymes tend to exist cell-bound but in a surface location between the cell-wall and the cytoplasmic membrane was reached by Brandes and Elston (1956) using electron microscopy to study the histochemistry of alkaline phosphatase of *Chlorella vulgaris.*

For the few pure culture studies there is little information on the fate of phosphatase enzymes in culture past the log-phase of growth. Information on whether phosphatase enzymes remain cell-bound or are released into solution as microbial populations pass through the stationary to the death phase is essential for predicting the state in which phosphatase enzymes may exist in soils.

Most soil phosphatase investigations are based on their potential importance in determining the rate and extent of organic P mineralization and soil P fertility status (Cosgrove, 1967). However, most of the data as regards organic-inorganic P dynamics has been of little or no practical value to date: gross relationships do not consistently exist between soil phosphatase activity and the amounts and dynamics of soil organic P (Cosgrove, 1967; Halstead, 1964), nor between soil phosphatases and microbial numbers and activity in soils (Ramirez-Marrinez and McLaren, 1966). The exact role of phosphatases in organic P mineralization in soils and equatic systems has not been clarified.

This paper reports the results of an investigation on the effect of inorganic P status on the nature, amounts and significance of phosphatase enzymes produced by different soil microorganisms and also the relationship between organic-inorganic P dynamics of microbial cultures and the implications with regards to soil phosphorus.

Materials and Methods

The soil samples used in this investigation were fallow and cultivated soils taken from three main ecological zones in Oyo State. The Ife soils representing a forest soil zone were taken from a bush fallow and cowpea plot. The Awe soils representing the forest-savanna transition zone were from grass fallow and a plot planted to cassava while the Ilora soils representing a savanna zone were also from a grass fallow and a maize plot.

The inoculum for the mixed soil microbial population studies was the supernatant of 1:10 distilled water suspensions of the different soils.

The phosphatase substrate was 0.115M distilled water solution of P nitrophenyl phosphate disodium salt (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.).

Aliquots (6ml) of settled soil suspensions were used to inoculate 2 liters of modified Watson's (1965) mineral salts medium: 0.01M Lactate (Merck-85%), 0.1M Tris buffer, 50μg/ml KH_2PO_4 (11.4μgP/ml) for the P-surplus medium or 13.15μg/ml KH_2PO_4 (3μgP/ml) for the P-deficient medium, 1 g NH_4Cl, 0.01 g yeast extract as a growth factor source, H_2O (liter), final pH was adjusted to 7.4. The inoculated flasks were incubated at a temperature of 25 ± 2C with agitation accomplished by continuous magnetic bar stirring from 0 to 8 weeks, and vigorous hand-shaking at weekly intervals thereafter.

After 1, 4, 8 and 40 weeks of incubation, aliquots were removed for determination of pH, turbidity, dissolved inorganic P, total and cell-free phosphatase activity at pH 4.0, 6.5 and 9.0. Cell-bound phosphatase was determined as the difference between total and cell-free phosphatase. Dissolved inorganic P (P_i) was determined on aliquots filtered through 0.45 um Millipore membranes using the Murphy and Riley (1962) single solution colorimetric method for otho P analysis. Initially, total dissolved P (dissolved P_o = Total dissolved P-dissolved P_i) was determined on 0.45 μm Millipore filtered samples by the ammoium persulfate digestion method (Environmental Protection Agency 1979). However, ammonium persulfate digestion caused caramerization of samples obtained from P-surplus (but not from P-deficient) microbial cultures buffered with Tris. This problem could not be satisfactorily resolved by decolorization of the digests wth P-free charcoal. Accordingly, determination of total P in all cultures incubated for 4, 8 and 40 weeks was by perchloric (Mehta *et al.*, 1954) method.

One unit of enzyme is defined as the number of umoles P- nitrophenol released per unit weight or volume per hour.

Preliminary expriments indicated that the pH 9.0 Modified Universal Buffer (M.U.B.) was not strong enough to overcome the buffering capacity of the Tris component of the culture medium, given a final pH 8.8 rather than 9.0. Adjustment of the Tris-buffered microbial systems at pH 9.0 was accomplished using MUB of initial pH 11.0

Results

P-surplus systems: All soil microbial population grown under P surplus conditions showed maximum phosphatase activity at acid, greater than neutral, and greater than alkaline assay pH conditions at all times throughout the 40 weeks incubation period (Figs.

1 - 3) phosphatase level varied markedly between different soil microbial populations but showed no relationship to the type or cropping history of the soil used as the microbial source. Phosphatase levels were not related to biomass. Most of the phosphatase existed in a cell-bound rather than cell-free state for the first 8 and particularly the first 4 weeks. Total phosphatase activity showed little tendency to decline with continued incubation; in fact levels at 40 weeks were frequently higher than those shown at 8 weeks, but an increasing proportion of the phosphatase existed in a cell-free rather than a cell-bound state at 40 weeks. Similar growth trends of a maximum optical density (OD) at 1 week followed by a gradual decline with continued incubation were shown by all the six microbail population grown under this P-sulplus condition, the steepest OD decline occurred from 1 to 4 weeks in the population derived from Awe fallow soil (Fig. 2a).

The initial dissolved inorganic P declined in the first week to amounts ranging from 6.60 μg/ml (Ife fallow) to 7.62 μg/ml (Awe cultivated) with further decreases at 4 weeks. The rate and extent of inorganic P immobilization was not related to biomass differences between soil microbial population. For example, the Ilora fallow and Ife fallow soil populations showed similar inorganic P immobilization at 1 week but differed markedly in OD. From 4 to 8 weeks, organic P mineralization exceeded inorganic P immobilization for all systems. This trend continued over the 40 weeks incubation period. At 40 weeks, except for the Ilora fallow system which contained about 2.60 ug/ml organic P, dissolved inorganic P concentration at 40 weeks were within 1 ug/ml of the initial 11.40 μg/ml. Particulate and dissolved organic P levels at 8 and 40 weeks were not related to phosophatase activity differences shown by the different soil microbial populations at these or earlier incubation times.

P-deficient systems: As found for the P-surplus systems, phosphatase activity levels varied markedly between different soil populations but were not related to microbial biomass or source. Microbial systems which, based on criteria of relatively high biomass and low dissolved inorganic P concentrations above detection limit of 0.005 μg/ml, may not have been P-limited (Ife Cultivated) or may have been only moderately P-limited (Ife fallow and Awe cultivated) showed an acid phosphatase response at 4 weeks (Fig. 4 a, b and 5b). The Ife cultivated soil microbial population maintained a typical

constitutive acid phosphatase response thereafter. In contrast, the Awe cultivated population showed an alkaline rather than acid phosphatase response at 8 weeks and the Ife fallow population showed similar phosphatase activity at pH 9 and pH 4. Both systems reverted to an acid phosphatase response at 40 weeks. The Ilora fallow and cultivated soil microbial population depleted the dissolved inorganic P below the detection limit at 4 weeks and showed alkaline phosphatase activity at this time (Fig. 6). With continued incubation the dissolved inorganic P concentration gradually increased and concommitantly phosphatase activity changed from alkaline to acid.

In general, phosphatase activity over the first 8 weeks of incubation existed mainly in the cell-bound rather than cell-free state although in two systems (Awe and Ilora cultivated) the reverse was true at 1 week. With continued incubation phosphatase activity, particularly at the acid assay pH, showed an increasing tendency to exist in the cell-free rather than cell-bound state. At no time was there appreciable cell-free activity at the alkaline assay pH.

All soil microbial populations grown under this condition reduced the initial 3 μg/ml dissolved inorganic P. The amounts of organic P and biomass at 1 and 4 weeks were not related in any way to form and amounts of phosphatase activity. All systems showed a predominance of organic P mineralization over inorganic P immobilization with continued incubation past 4 weeks, as evidenced by the presence of dissolved inorganic P concentrations ranging from 0.158 μg/ml (Ife fallow) to 0.820 μg/m(Ilora cultivated)at 40 weeks). Neither particulate nor dissolved amounts of organic P in the P-deficient and P-surplus systems showed any relationship to nature and amount of phosphatase at any time during the 40 week incubation period.

Discussion

Nature and Significance of Phosphatases in Soils

Different soil microbial populations not limited by P produce constitutive acid phosphatase. Once these phosphatase are produced, they tend to be maintained cell-bound even over succeeding generations of microorganisms with prolonged incubation (Figs. 1-3). As long as the dissolved inorganic P concentration is maintained at greater than 0.005 μg/ml, the constitutive acid rather than inorganic P-repressible phosphatase will be produced. For most soils the concentration of dissolved inorganic P maintained by particulate P-

dissolved P equilibrium is always greater than 0.005 μg/ml (Barber *et al.*, 1962). Under such circumstances, acid phosphatase production can be expected in most agricultural soils. However, it is possible that in the presence of carbon or high organic amendments, dissolved inorganic P level in the soil solution may fall temporarily to levels lower than 0.005 μg/ml, because of rapid immobilization of inorganic P.

Under P-limiting conditions, production of inorganic P-repressible phosphatase is a common property of most soil microorganisms (Figs. 5-6). Probably in most agricultural soils, the presence of inorganic P-repressible phosphatase is not a common occurrence. However, in high available carbon – low mobile inorganic P systems, it is conceivable that the inorganic P may be depleted and maintained sufficiently low to cause depression of inorganic P-repressible phosphatase production.

It is therefore conceivable that the presence of phosphatase enzymes could serve as an index of microbial biomass in soils. If so, determinations of phosphatase activity could be relied upon to give good indication of the living bomass in soils. However, the levels of activity of both the constitutive acid and inorganic P-repressible phosphatases vary per biomass. Even if the amounts of these enzymes per biomass were relatively constant, the tendency of phosphatase enzymes to remain stable outside the living cell for a prolonged time would lead to an overestimation of the biomass.

Although phosphatase enzyme activity in soils appears to be an unreliable index of biomass of soils, it may be of some value as an indicator of the rate and extent of organic P mineralization in soils. Phosphatase enzymes produced by diverse soil microorganisms show relatively high activity at the indigenous pH of most agricultural soils and are relatively nonspecified (Torriani, 1960; Garen and Levinthal, 1960; Reid 1971) suggesting that organic P compounds susceptible to decomposition by these enzymes would be relatively short-lived in soils.

Inorganic P immobilization and organic P mineralization

Under P-surplus conditions, during the initial week of rapid growth 3.8 to 6.5 μg/ml of the initial 11.40 μg/ml dissolved inorganic P present were converted to microbial P and amounts of microbial P showed little increase up to 4 weeks presumably as the available carbon remained adequate during this period. This is comparable to

adding P-rich residue to soil, the addition of which does not impose any demand on soil P. However, under P-deficient conditions, virtually all the initial added 3 μg/ml inorganic P was immobilized and was kept immobilized for the first 4 weeks. Any mineralized P was presumably quickly immobilized again because of the presumed excess of available carbon in the P deficient systems. This is comparable to the addition of a high C:P residue to soil whose decomposition involves depletion of the soil solution P.

The extent of mineralization at 8 weeks was less for P-deficient than for P-surplus systems; this was presumably related in part to P recycling in the 1 to 4 weeks period for the P-deficient systems thereby extending the phase of inorganic P immobilization - organic P mineralization for the P-deficient systems as compared to the surplus. However, based on the fact that both systems showed organic P mineralization greater than inorganic P immobilization at 8 weeks, the lag in the overall P immobilization-mineralization dynamics for P deficient systems would seem to be less than 4 weeks. If this trend holds in soils, it is conceivable that the addition of inorganic P with high carbon residue will not only effect more rapid decomposition of carbon, but will eventually result in subsequent greater release of microbially-immobilized P than might be predicted on the basis of the length of time for the available carbon to decline to a value at which organic P minerazation exceeds inorganic P immobilization.

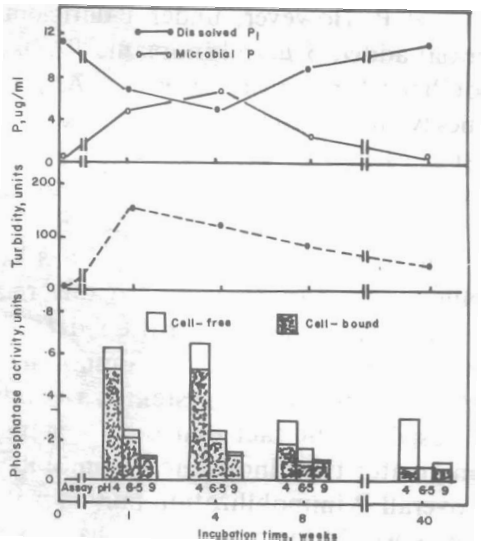

Figure Ia : Phosphatase activity turbidity and microbial P of culture of life fallow soil micro-organism grown in P-surplus system.

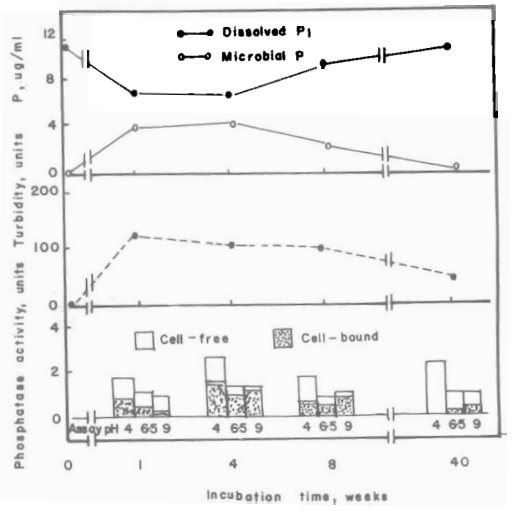

Figure Ib : Phosphatase activity, turbidity and microbial P of culture of life cultivated soil micro-organism in P surplus system

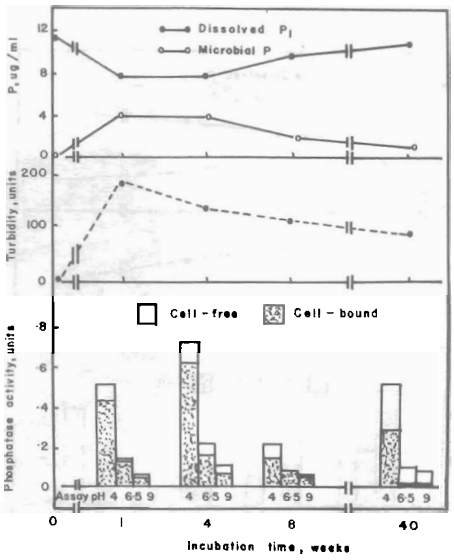

Figure. 2a: Phosphatase activity, turbidity and microbial P of cultures of Awe fallow cultivated soil micro-organism grown in P surplus system.

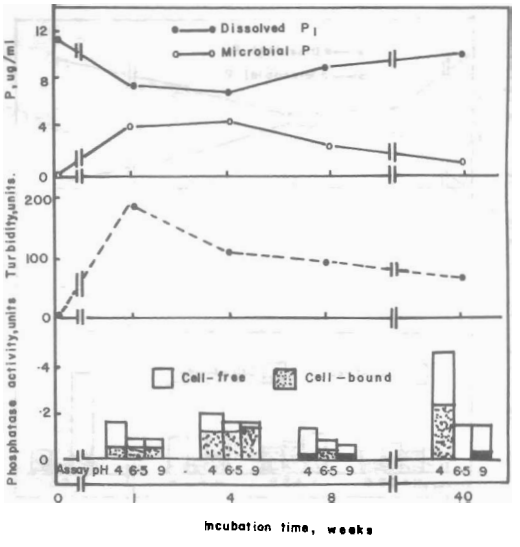

Figure 2b: Phosphatase activity, turbidity and microbial P of cultures of Awe cultivated soil micro-organism grown in P surplus system.

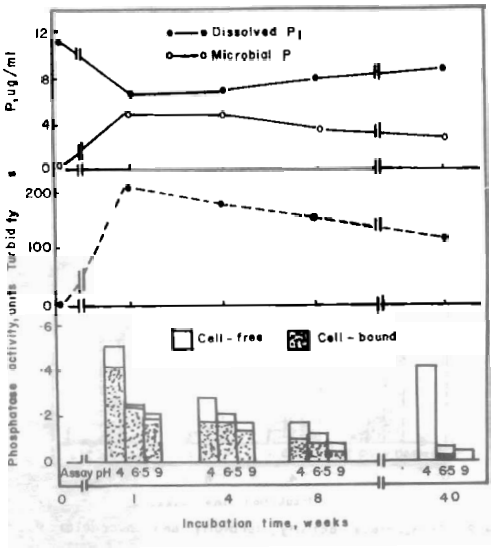

Figure 3a: Phosphatase activity, turbidity and microbial P of cultures of Ilora fallow cultivated soil micro-organism grown in P surplus system.

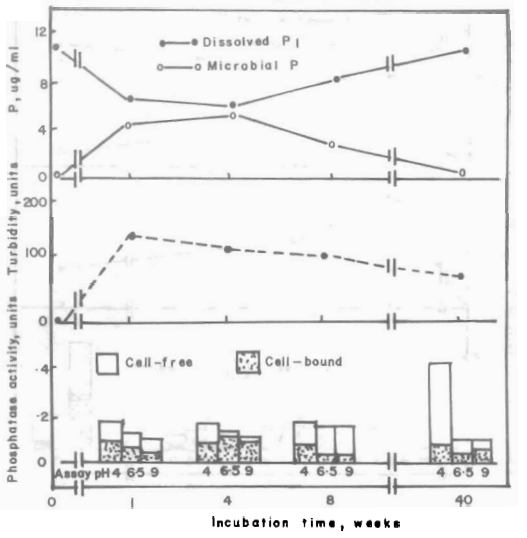

Figure 3b: Phospatase activity, turbidity and microbial P of cultures of Ilora cultivated soil micro-organism grown in P-surplus system.

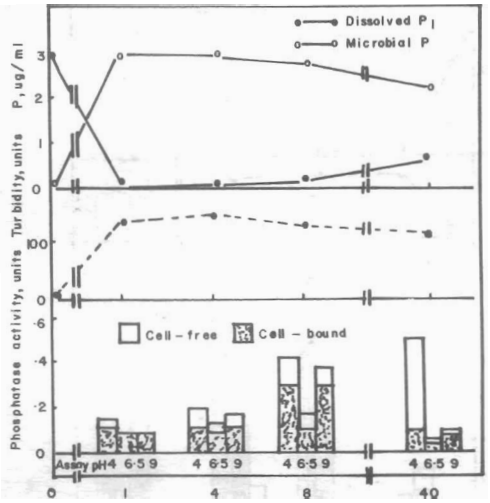

Figure 4a: Phosphatase activity turbidity and microbial P of culture of Ife fallow soil micro-organism grown in P limiting system.

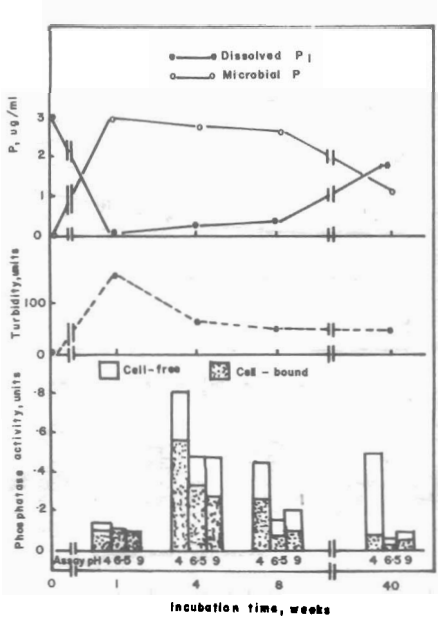

Figure 4b: Phosphatase activity, turbidity and microbial P of cultures of Ife cultivated soil micro-organism

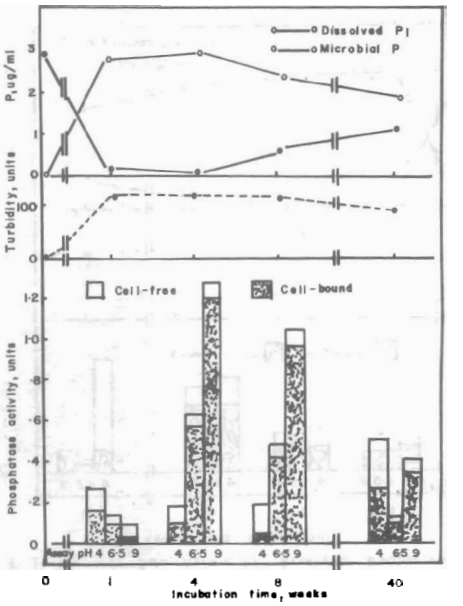

Figure 5a: Phosphatase activity, turbidity and microbial P of cultures of Awe fallow cultivated soil micro-organism grown in P- limiting system.

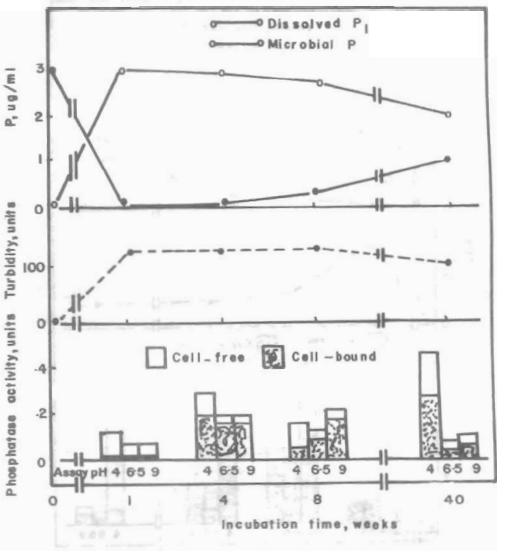

Figure 5b: Phosphatase activity, turbidity and microbial P of cultures of Awe cultivated soil micro-organism grown in P-limiting system.

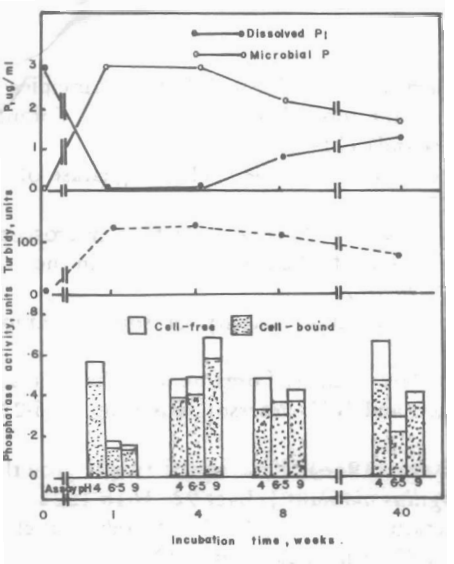

Figure 6a: Phosphatase activity, turbidity and microbial P of cultures of Ilora fallow cultivated soil micro-organism grown in P-limiting system

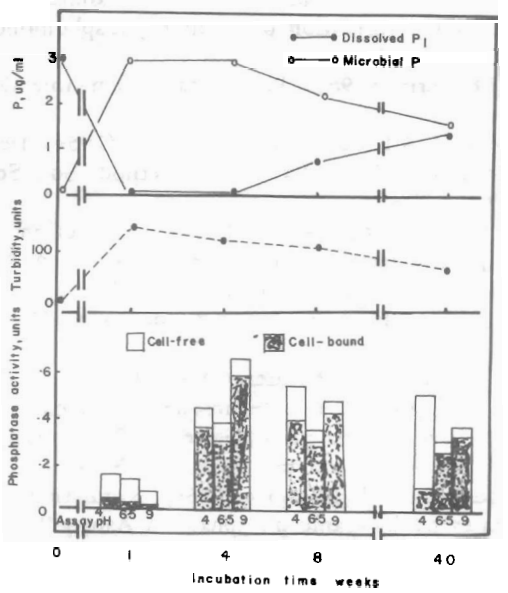

Figure 6b: Phosphate activity, turbidity and microbial P of cultures of Ilora cultivated soil micro-organism grown in Plimiting system.

References

Barber, S.A., J.M. Walker and E.A. Vassey (1962). Principles of ion movement through the soil to plant root. Trans. Joint. Meet. Comm. IV and V. Intl. Soil Sci. Soc. (N. Zealand) 121-124.

Blum, J.J. (1965). Observations on the acid phosphatase of *Euglena gracilis* J. Cell Biol. 24: 223-234.

Brandes, D. and R.N. Elston (1956). An electron microscopical study of the histochemical localization of alkaline phosphatase in the cell wall of *Chlorella vulgaris* Nature 117: 274-275.

Casida, L.E. (1965). Phosphatase activity of some common soil fungi. Soil Science 87: 305-370.

Cosgrove, D.J. (1967). Metabolism of organic phosphates in soils. *In* soil Biochemistry (A.D. McLaen and G.H. Peterson, eds.). pp. 216-228. Marcel Dekker, New York.

Dorn, G. and W. Rivera (1966). Kinetics of fungal growth and phosphatase formation in *Aspergillus nidulans* J. Bact 92: 1618-1622.

Environmental Protection Agency (1979). Methods for chemical Analysis of Water and Wastes. EMSL Cincinnah Ohio 430pp.

Halstead, R.L. (1964). Phosphatase activity of soils as influenced bylline and other treatments. Can J. Soil Sci. 44: 137-143.

Hofsten, B.V. (1961). Acid phosphatase and growth of *E. coli.* Biochem. Biophys. Acta. 48: 171-181.

Horiuchi, T.S., S. Horiuchi and Mizuno (1969). A possible negative feedback phenomenon controlling formation of alkaline phosphomonoesterase in *E. coli.* Nature 4674: 1529-1530.

Kuenzler, E.J. and J.P. Perras (1965). Phosphatases of marine algae. Biol. Bull. 238: 271-384.

Mehta, N.C., J.C. Legg., C.A.I. Goring and C.A. Black (1954). Determination of organic phosphorus in soils. 1. Extraction method. Soil Sci. Soc. Amer. Proc. 18: 443-449.

Murphy, J. and J.P. Riley (1962). A modified single solution method for the determination of phosphates in natural waters. Anal. Chem. Acta. 27: 31-36.

Ohta, Y.K. Ikeda and S. Ueda (1968). Production of phosphatase by *Aspergillus awamori* var. *kawachi* in a low phosphorus medium Appl. Microbiol. 16: 937-980.

Ramirez - Martinez, J.R. and A.D. Mclaren (1966). Determination of soil phosphatase activity by a fluorimetric technique. Enzymologia 30: 243-253.

Reid, J.W. (1971). E. coli alkaline phosphatase. In The Enzymes Vol. IV 3rd Ed. (P. Boyer, ed.). 373-415. Academic Press, New York.

Shieh, T.R., R.J. Wodzinski and J.A. Ware (1969). Regulation of the formation of acid phosphatases by inorganic phosphate in Aspergillus ficcus. J. Bact. 100: 1161-1165.

Torriani, A. (1960). The influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli.* Biochem. Biophys. Acta. 38: 460-479.

Watson, J.D. (1965). Molecular Biology of the Gene. Benjamin Inc. New York. 494pp.