Enzyme Activity in Terrestrial Soil in Relation to Exploration of the Martian Surface.

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From

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Our purposes were to study enzyme activities in soil, to devise sensitive tests for soil enzymes where plausible, and to study enzyme action at solid-liquid water interfaces and at low humidity.

The most sensitive procedure devised was to utilize ¹⁴C-Urea as a substrate for soil urease. It was found that urease can be active at a relative humidity as low as 60%, but this humidity is orders of magnitude greater than on Mars. With this technique, soils as old as 10,000 years were found to possess urease activity. A less sensitive assay was that for soil phosphatase; it was based on ultraviolet radiation fluorescence of a product of phosphatase hydrolysis, namely napathol. Irradiation sterilized soils were sometimes used to rule out artifacts resulting from microbial growth.

We found a way of extracting humus from soil by a method so mild as to result in preservation of its enzyme activity. The humus-enzyme complex with urease activity was resistent to hydrolysis when mixed with proteolytic enzymes, which may explain the persistence of urease activity in soll.

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(NASA-CR-138587) ENZYME ACTIVITY IN TERBESTRIAL SOIL IN RELATION TO EXFLORATION OF THE MARTIAN SURFACE Final Report (California Univ.) 5 p HC Unclas S4.00 Soluble chitinase was isolated from a streptomyces culture and characterized. This enzyme was adsorbed to chitin and kinetics of hydrolysis of chitin was examined. The rates do not obey Michaelis Kinetics and a new Kinetics scheme for soluble enzyme-insoluble substrate was devised.

Finally, as a problem of consecutive reaction in a soil profile, a kinetic scheme, based on growth of microorganisms and oxidation of ammonium to nitrite and then to nitrite, was devised and tested. It was found possible to determine rates per organism per unit flow rate with considerable precision and the mathematical model was vindicated.

During the course of the work it was found by NASA that there is a very low humidity on Mars and a paucity of nitrogen. 'Hence we do not feel that investigation along the lines we have followed should be continued. There seems to be a low probability of detecting extracellular enzyme activity in Martian soil.

There follows a list of our publications which have just been summarized.

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Articles

1965

1966

- Skujins, J. J. ¹⁴CO₂ detection chamber for studies in soil metabolism. Biologie du Sol, N.S., No. 4, p. 14-17, 1965.
- McLaren, A. D. The biochemistry of terrestrial soils; <u>In</u>: Biology and the Exploration of Mars, C. S. Pittendrigh, W. Vishniac, and J. P. T. Pearman, Eds., Washington, D. C., Nat. Acad. Sci., - Nat. Res. Counc., p. 147-163, 1966.
- Ramirez-Martinez, J. R. and McLaren, A. D. Determination of soil phosphatase activity by a fluorimetric technique. Enzymologia <u>30</u>:243-253, 1966.
- Ramirez-Martinez, J. R. and McLaren, A. D. Some factors influencing the determination of phosphatase activity in native soils and in soils sterilized by irradiation. Enzymologia <u>31</u>:23-38, 1966.
- McLaren, A. D. and Peterson, G. H. Introduction to the Biochemistry of terrestrial soils; <u>In</u>: Soil Biochemistry, A. D. McLaren and G. H. Peterson, Eds., Marcel Dekker, New York, p. 1-15, 1967.
- Skujins, J. J. Enzymes in soil; <u>In</u>: Soil Biochemistry, A. D. McLaren and G. H. Peterson, Eds., Marcel Dekker, New York, p. 371-414, 1967.
- McLaren, A. D. and Skujins, J. J. Physical environment of microorganisms in soil; <u>In</u>; Ecology of Soil Bacteria, T. R. G. Gray and D. Parkinson, Eds., Liverpool University Press, p. 3-24, 1967.
- Skujins, J. J. and McLaren, A. D. Enzyme reaction rates at limited water activities. Science 158:1569-1570, 1967.

-3 -

1968

- Skujins, J. J. and McLaren, A. D. Persistence of enzymatic activities in stored and geologically preserved soils. Enzymologia <u>34</u>: 213-225. 1968.
- 1969
 - 10. Skujins, J. J. and McLaren, A. D. Assay of urease activity using ¹⁴C-urea in stored, geological preserves, and in irradiated soils. Soil Biol. Biochem. <u>1</u>:80-00, 1969.
 - McLaren, A. D. Steady state studies of nitrification in soil.
 Soil Sci. Soc. Amer. Proc. <u>33</u>:273-276, 1969.
 - McLaren, A. D. Nitrification in soil: Systems approaching a steady state. Soil Sci. Soc. Amer. Proc. <u>33</u>:551-556, 1969.

1970

- Skujins, J. J., Pukite, A. and McLaren, A. D. Chitinase of <u>Streptomyces</u> sp: Purification and properties. Enzymologia <u>39</u>: 353-370, 1970.
- 14. Skujins, J. J. and Pukite, A. Extraction and determination of N-acetyglucosamine from soil. Soil Biol. Biochem. <u>2</u>:141-143, 1970. 1971
 - 15. Skujins, J. J., Pukite, A. and McLaren, A. D. Application of Scanning and transmitting electron micrography in species differentiation of soil <u>Streptomyces</u>. Soil Biology and Biochemistry, <u>3</u>:181-186, 1971.
 - 16. Skujins, J. J. and McLaren, A. D. Urease reaction rates at low water activity. Space Life Sciences, <u>33</u>:3-11, 1971.
 - 17. McLaren, A. D. Kinetics of nitrification in soil: Growth of the nitrifiers. Soil Sci. Soc. Amer. Proc. <u>35</u>:91-95, 1971.

4

- 18. Burns, R. G., El-Sayed, M. H. and McLaren, A. D. Extraction
 of an urease-active organo-complex from soil. Soil Biol. Biochem.
 4: 107-108, 1972.
- 19. Burns, R. G., Pukite, A. and McLaren, A. D. Concerning the location and persistence of soil urease. Soil Sci. Soc. Amer. Proc. <u>36</u>: 308-311, 1972.
- 20. Mc Laren, A. D. Consecutive biochemical reactions in soil with particular reference to the nitrogen cycle. Consiglio Nazionale Delle Recherche, Pisa, Conferenza - 1, 1972, pp 1-18.
 - 21. Skujins, J. J., Pukite, A. and McLaren, A. D. Adsorption and reactions of chitinase and lysozyme on chitin. Molecular and Cellular Biochemistry <u>2</u>:271-230, 1973.

1974

22. Skujins, J. J., Pukite, A. and McLaren, A. D. Adsorption and activity of chitinase on kaolinite. Soil Biol and Biochemistry, in press.

-5-