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THE ROSSI-CHOLODNY TECHNIC AS AN AID IN THE STUDY OF THE DECOMPOSITION OF LIGNIN [1]

H. H. Mollenhoff, F. B. Smith and P. E. Brown

The study of the soil microflora by direct microscopic examination offers the possibility of observing the activities of the microorganisms in their natural surroundings and the attention of soil microbiologists has been directed along this line from time to time. Rossi (4) and Cholodny (1) working independently proposed similar methods which yield results of a qualitative nature and their methods have come to be known as the Rossi-Cholodny technic. Recently, Conn (2 and 3), Winogradsky (6), and Ziemiecka (7) have contributed to the further development of the method, adapting it to laboratory conditions.

The purpose of the work reported in this paper was to make a study of the organisms that decompose certain organic compounds in the soil, with special reference to lignin, using the direct microscopic examination as a qualitative measure.

Methods of Procedure

Since many organic compounds are formed in the normal decomposition of plant residues eight common forms were used in this study. The substances used may be divided into two classes, namely, nitrogenous and non-nitrogenous. The nitrogenous substances were peptone, and asparagine and the non-nitrogenous substances were phenol, cellulose, starch, lignin (prepared from oat straw by the alkali method), xylan (from oat straw), and glucose. The Dickinson loamy fine sand having a pH of 6.95 was used in this work. The soil was placed in clean glass tumblers and the moisture content was adjusted to 25% by the addition of sterile distilled water. Potassium nitrate was added at the rate of 300 pounds per acre to the soils treated with the non-nitrogenous substances. Cleaned and sterile microscopic slides were then smeared with solutions or suspensions of the substances to be studied and dried at 50°C. A series of four tumblers was used for each compound. The smeared slides were inserted in the soil and kept in a vertical position. The tumblers were placed in the incubator at 30°C. The humidity in the incubator was kept rela-

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Table I. Growth Observed on Slides at Various Intervals of Time

	4 hrs.	6 hrs.	9 hrs.	16 hrs.	24 hrs.	40 hrs.	48 hrs.	72 hrs.
Peptone	Few large rods	Large rods in chains	Heavy growth of rods and actinomycetes	Heavy growth of rods and sporulating actinomycetes	Long chains of sporulating rods, fungi and sporulating actinomycetes	Hyphae in decay attach by pale thin rods, mass sporulating rods and actinomycetes	Same as 40 hrs.	Same as 40 hrs.
Asparagine	None	Large short rods & coccus forms	Moderate growth of short rods in chains and coccus forms	Heavy growth of large rods in chains and few fungi	Mixed growth of rods some sporulating and fungi	Many fungi; small rods; actinomycetes and cocci forms	Same as 40 hrs.	Same as 40 hrs.
Glucose + KNO_3	None	Small clusters of rods and clostridia forms	Same growth as 6 hrs.	Same growth of rods as 6 hrs. and fungi	Heavy growth of very small rods, fungi and clostridia forms	Same growth rods and heavy growth fungi	Same as 40 hrs. and actinomycetes	Same as 40 hrs.
Starch + KNO_3	None	None	None	Moderate growth of large rods fungi and actinomycetes	Heavy growth rods, actinomycetes and sporulating actinomycetes Few fungi	Heavy growth rods, sporulating actinomycetes and few fungi	Same as 40 hrs.	Same as 40 hrs.
Cellulose+ KNO_3	None	None	None	None	Fungi & small rods some in spore stage	Moderate growth fungi and small rods some in spore stage	Heavy growth bacteria, molds & sporulating actinomycetes	Same as 48 hrs.
Xylan + KNO_3	None	None	None	None	Fungi and heavy growth of very minute rods	Moderate growth fungi rods in spore stage	Same as 40 hrs.	Same as 40 hrs.
Lignin + KNO_3	None	None	None	None	Moderate growth rods and fungi	Increase in growth of rods and fungi	Same as 40 hrs.	Same as 40 hrs.
Phenol	None	None	None	None	None	None	None	Few fungi and rods

tively high to prevent excess evaporation of moisture from the soil. The slides were removed from the soil at various intervals and examined, care being taken in removing the slides from the soil to prevent the growth adherent to the surface of the slide from being disturbed. The slides were dried at 50°C., then washed in distilled water to remove the large particles which might interfere with staining and render examination more difficult. The slides were again dried at 50°C. placed over a steaming water bath, and stained with 5% phenolerythrosin for 15 minutes, additional stain being applied as needed to prevent the stain from drying on the slides. The slides were again washed with warm distilled water, allowed to dry, and examined under a magnification of 1000.

Results

The results obtained are presented in Table I and show the growth observed at various intervals of time.

Peptone — This substance seemed to be decomposed most readily and the only one which was attacked after 4 hours. After six hours culture the same large rods were observed as after 4 hours, but they appeared in chains and the growth was much heavier. After 9 hours there was a dense growth of rods and actinomycetes. After 24 hours culture there appeared long chains of sporulating rods, fungi, and sporulating actinomycetes. After 40 hours the hyphae were apparently in decay and attacked by long thin rods. The growth after 48 hours and 72 hours was the same as that observed after 40 hours culture.

Asparagine — This substance was attacked by short, large rods and coccus forms after 6 hours. In the later stages of decomposition rods and fungi predominated.

Glucose — Of the non-nitrogenous substances, glucose and starch were the most readily attacked. Clostridia were observed on the glucose slides after 6 hours. After 9 hours culture fungi were found. Fungi and actinomycetes predominated after 40 hours.

Starch — No growth was observed until after 9 hours. A moderately heavy growth of rods, fungi, and actinomycetes was observed after 16 hours culture. Sporulating actinomycetes were observed after 24 hours growth.

Cellulose — The first growth observed was after 24 hours and consisted of fungi and small rods some of which were in the spore stage. The growth consisted of a mixture of bacteria, molds and sporulating actinomycetes after 48 hours. The same growth was observed after three days culture.

Xylan — The first growth was observed after 24 hours and consisted of fungi and a heavy growth of very small rods. After 40 hours culture the small rods were in the spore stage.

Lignin — After 24 hours culture a moderate growth of small rods and fungi was observed. The growth was more dense after 40 hours.

Phenol — No growth was observed until after 3 days. The growth after 3 days culture consisted of only a few fungi and rods.

DISCUSSION

In this study the Rossi-Cholodny technic was used as a qualitative measure to determine the part played by various organisms in the decomposition of different organic substances. No attempt was made to study the action of the micro-organisms in the undisturbed soil profiles.

It was found that certain micro-organisms were prevalent in the various stages of decomposition. Bacteria were prominent in the early stages of decomposition and when their activity ceased molds continued the process. In the final stages of decomposition actinomycetes replaced the molds. The actinomycetes usually occurred as a secondary growth after the attack by other organisms was completed. This is probably due to the ability of actinomycetes to break down very resistant organic substances and thrive when other organisms fail to find conditions suitable for growth (5).

The same growth of small rods was observed on the lignin slides after repeating this same exepriment several times. Lignin is a very inert plant substance, insoluble in simple solvents and very resistant to the action of plants, animals, and the majority of organisms.

An attempt was made to isolate the organism which was observed in the soil treated with the lignin. A slide treated with lignin was taken from the soil and put in a petri dish containing an inorganic salt solution with prepared lignin as the source of carbon. After 48 hours culture there was an abundant growth of organisms similar to those observed on the lignin slides. Using this as the inoculum, plates were poured on a nitrate-agar with peptone as the source of carbon. Colonies were picked from the plates and an organism similar to those found on the lignin slides was isolated. An inorganic salt solution, containing prepared lignin as the only source of carbon, was inoculated with the organism and there was an abundant growth after 48 hours. Further tests

are being made to determine whether or not the organism decomposes the lignin.

Summary

The Rossi-Cholodny technic was employed in a study of the decomposition of different organic substances and the results obtained indicate that the method may serve as an index to the changes which normally occur in the decomposition of organic matter.

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