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Final Report - Contract No. N7-ONR 397-4 T.O. IV

between

The Biochemistry Branch, Office of Naval Research

and

The University of Maryland
College Park, Maryland

The primary objective of this research project was to obtain degradation of the lignin molecule through use of either enzyme preparations or by direct attack with microorganisms. Subsequently, the degradation products would be characterized and used for elucidation of the structure of lignin. From a more practical standpoint, since lignin is a major constituent of wood, it might be feasible to design more satisfactory means of wood preservation if knowledge were available as to the mechanism of lignin deterioration. Finally, lignin represents a waste substance encountered in tremendous quantities and might serve as a useful raw material in some microbiological fermentation process if an organism could be discovered capable of attacking this material.

The problem was approached by isolating a type of lignin (native lignin) which according to best authority represents the lignin as it occurs *in situ*. Many obstacles were inherent in this process and one was the extremely small yield. This isolated lignin was used as a substrate and incorporated into media of numerous types which were then inoculated with many kinds of microorganisms. A quantitative method of analysis for the fate of lignin in such culture conditions was formulated. It is believed that the scheme developed contributes a new approach for adequate interpretation of data in this field of investigation.

The limited utilization of lignin obtained under the best cultural conditions did not result in accumulation of any intermediates that could be isolated and the assumption is that utilization was quantitative, CO_2 and mold mycelium being the end products.

Enzyme preparations from various microbial sources appeared to effect some peripheral oxidation of the lignin molecule. Many of the fungi responsible for lignin disappearance in wood, *in situ*, were found to possess enzyme(s) of the phenol-oxidase type. An extensive study has been made of this enzyme system; its qualitative occurrence and quantitative activity characteristics on suspected lignin constituents would indicate the involvement of this enzyme system somewhere in the pathway of lignin degradation.

A species of bacteria was isolated which was capable of degrading the lignin related material, alpha-conidendrin. A polysaccharide was synthesized during this process and its characteristics were determined.

Personnel participating in project:

Investigating staff

Sidney Gottlieb	May 1948 - August 1951
Jerry H. Geller	August 1948 - June 1951
William C. Day	June 1948 - January 1952
Fletcher P. Veitch	September 1948 - April 1950
Michael J. Pelczar	July 1948 - August 1951
Walter A. Konetzka	June 1951 - May 1952
Yolanda Pratt	July 1951 - April 1952
Pamela Ludford	October 1951 - April 1952
Walter H. Martin	May 1952 - January 1953

Stenographical help

T. B. Green	July - September 1948

Student - part time help

W.E. Wolfel	June - July 1948
G.R. Minnick	June - August 1948
C.W. Dulin	July - August 1948
R. Kray	September 1948
A. Brickman	October 1948 - May 1949
B. Baer	July 1948 - March 1952
R. Daneck	October 1949 - August 1950
E. Ruiker	April - June 1950
C. Raabe	June 1951 - April 1952
S. Arella	September - November 1950
E. Angullo	December 1950 - May 1951
J. Tuomo	August 1951 - April 1952
G. Rawlings	April - September 1949

Project reports

Status Report	September 1 - November 1, 1948
Status Report	November 1, 1948 - Jan. 1, 1949
Status Report	January 1 - March 1, 1949
Status Report	May 1 - July 1, 1949
Technical Summary Report	May 1, 1948 - May 1, 1949
Status Report	July 1, September 1949
Status Report	September 1 - November 1, 1949
Annual Research Report	January 1 - December 31, 1950
Semi Annual Research Report	January 1 - June 30, 1951
Annual Research Report	January 1 - December 31, 1951
Semi Annual Progress Report	January 1 - June 30, 1952
Semi Annual Progress Report	June 30 - December 31, 1952
Final Report	June 26, 1953

Publications:

1. The biological degradation of lignin. S. Gottlieb. Monthly Research Report of the Office of Naval Research. May 1, 1949.

2. The biological degradation of lignin. I Utilization of lignin by fungi. W.C. Day, M.J. Pelczar, Jr., and S. Gottlieb. Archives of Biochemistry, 23: 360-369, 1949.

3. Enzymatic decomposition of lignin. S. Gottlieb and J.H. Geller. Science, 110: 18, 1949.

4. Biological degradation of lignin. W.C. Day, M.J. Pelczar, Jr. S. Gottlieb and J.H. Geller. Navy Wood Symposium, 1949 Washington, D.C.

5. Growth of Polyporus versicolor in a medium with lignin as the sole carbon source. M.J. Pelczar, Jr., S. Gottlieb and W.C. Day. Archives of Biochemistry, 25: 449-451, 1950.

6. The biological degradation of lignin. II The adaptation of white-rot fungi to grow on lignin media. S. Gottlieb, W.C. Day and M.J. Pelczar, Jr. Phytopathology, 40: 926-935, 1950.

7. Microbiological aspects of lignin degradation. S. Gottlieb and M.J. Pelczar, Jr. Bact. Rev., 15: 55-76, 1951.

8. Lignin - raw material of the future. M.J. Pelczar, Jr. and S. Gottlieb. Research Reviews (ONR) pp. 4-6, November, 1951.

9. The biological degradation of lignin. III Bacterial degradation of alpha-conidendrin. W.A. Konetzka, M.J. Pelczar, Jr. and S. Gottlieb. J. Bact., 63: 771-779, 1952.

10. The biological degradation of lignin. IV The inability of Polyporus versicolor to metabolize sodium lignosulfonate. W.C. Day, S. Gottlieb and M.J. Pelczar, Jr. Applied Microbiology, 1: 78-81, 1953.

11. The biological degradation of lignin. V Polysaccharide synthesis from ∝-conidendrin. Y.T. Pratt, W.A. Konetzka, M.J. Pelczar, Jr., and W.H. Martin, Jr. Applied Microbiology (in press).

Papers presented:

1. Microbiological degradation of lignin. M.J. Pelczar, Jr. Baltimore Branch of Soc. Amer. Bact. January, 1951.

2. Observations on microbial decomposition of lignin. M.J. Pelczar, Jr.; S. Gottlieb, and W.C. Day. Washington Branch of Soc. Amer. Bact. February, 1951.

3. Quantitative analysis of the medium-mycelium complex following growth of Polyporus versicolor in glucose and sodium lignosulfonate. W.D. Day, M. J. Pelczar, Jr. and S. Gottlieb. Soc. of Amer. Bact. National meeting, Baltimore, May 1951.

4. The degradation of lignin and lignin related compounds. S. Gottlieb. Gordon Research Conference on Microbial Deterioation. July 1951.

5. Studies on the microbiological degradation of lignin and related compounds. S. Gottlieb, M.J. Pelczar, Jr., W.C. Day and W.A. Konetzka. 12th International Congress of Pure and Applied Chemistry. September 1951.

6. Microbial degradation of conidendrin. W.A. Konetzka and M.J. Pelczar, Jr. Washington Branch of Soc. of Amer. Bact. January 1952.

7. Microbial degradation of lignin and conidendrin. W.A. Konetzka M.J. Pelczar, Jr. Maryland Branch, Soc. of Amer. Bact. March 1952.

This research project has resulted in the development of a new experimental design for evaluation of microbial degradation of lignin. Previous claims by others of lignosulfonate utilization were shown to be in error because of inadequate analyses.

It has been established that some microorganisms are capable of unequivocal but limited utilization of native lignin. The partial utilization of any given amount of lignin may be due to one of several factors such as inhomogeneity of isolated native lignin, complete lignin degradation might be dependent upon some simultaneously occurring biochemical transformation in wood, deficiency of some catalytic chemical entity, etc.

The difficulty in isolating appreciable quantities of lignin plus the uncertainty of its chemical identity to the material in situ represent major obstacles in this field of investigation.

Enzymes of the phenol oxidase type found in the wood rotting fungi have been characterized as to spectrum of activity and other chemical and physical charateristics. This information may contribute to elucidation of enzyme types in the phenol oxidase -- laccase group.