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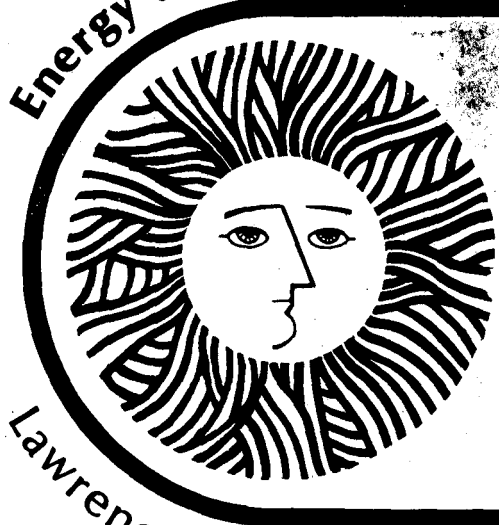
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Physiological Studies Of Lignocellulose
Degradation By The Thermotolerant
Mold Chrysosporium Pruinosum

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PHYSIOLOGICAL STUDIES OF LIGNOCELLULOSE DEGRADATION BY THE
THERMOTOLERANT MOLD CHRYSOSPORIUM PRUINOSUM

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ABSTRACT

Chrysosporium pruinatum degrades significant amounts of lignin and carbohydrate when grown on a moist lignocellulosic substrate, but less carbohydrate and no lignin are lost in submerged shaking cultures. Close hyphal-substrate contact and high concentrations of oxygen appear to be required for lignin degradation to take place under conditions of submerged cultivation. No diffusible lignin-degrading activity was seen in cultures grown in the presence of air, but a small amount was seen in cultures grown with pure oxygen.

INTRODUCTION

Photosynthetically produced lignocellulosic biomass represents a potentially enormous renewable source of chemicals and liquid and gaseous fuels. At the present time, only a small fraction of the total annual yield of this material is used by man chiefly in the form of wood, paper, and grass fed to ruminants. In the case of paper and grass, utilization is incomplete, and the residual lignocellulose may become a waste creating problems of disposal.

The insoluble cellulose and hemicellulose components of lignocellulosic biomass can be converted to soluble sugars by the action of mineral acids or specific enzymes. Once in a soluble form, these sugars may be microbially fermented to a large number of end products including protein, ethanol and methane.

The lignin component of lignocellulose represents a potential source of aromatic chemicals. In addition its partial removal is necessary in order to allow complete hydrolysis of cellulose to soluble sugars by microbial cellulase systems currently being studied (Wilke, 1975, Gaden, et al., 1976, Bailey, et al., 1975).

Lignin can be degraded chemically by a variety of methods, (Sarkanen and Ludwig, 1971), but these are too expensive to be used with present cellulose hydrolysis processes. Moreover, chemically degraded lignin has a low commercial value.

Since some lignin degradation seems to be required to allow extensive enzymatic hydrolysis of cellulose in lignocellulosic materials, and since chemical delignification processes are both expensive and yield modified lignins of low value, biological lignin degrading

systems are being studied in order to develop a better delignification process which will yield not only easily hydrolyzable cellulose, but also useful lignin-derived chemicals (Abson, 1976, Ander and Eriksson, 1977, Kirk, in press). One of the goals of this research is the demonstration of a cell-free lignin-degrading enzyme system. Before this goal can be reached, however, a better understanding of the physiology of lignin-degrading microorganisms is needed.

In an earlier report (Rosenberg, 1978) I noted that the thermotolerant molds Chrysosporium pruinatum and Sporotrichum pulverulentum were able to rapidly degrade small strips of a damp lignocellulosic substrate (newsprint), but this material was not appreciably degraded under conditions of submerged cultivation. Pure cellulose (filter paper) was extensively degraded under both conditions. These organisms have both been characterized as imperfect forms of the basidiomycete Phanerochaete chrysosporium (Burdsall & Eslyn, 1974).

The above observations were qualitative and needed to be quantified. This necessitated the design of a method for cultivating this mold on larger amounts of a moist (non-submerged) lignocellulosic substrate. I also wished to understand why little or no lignocellulose was degraded under conditions of submerged cultivation. Finally, I was interested in determining whether the lignin degrading activity produced by this organism was diffusible, i.e. whether lignin could be degraded in the absence of direct

hyphal-substrate contact.

MATERIALS AND METHODS

Organism

Chrysosporium pruinatum (Gilman et Abbott) Carmichael, ATCC 24782, was used in all experiments to be reported and was obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. Cultures were stored as described previously (Rosenberg, 1975).

Media

The mineral medium described previously (Rosenberg, 1975) was used with the following modifications: 1. Yeast extract was omitted, 2. Where a liquid medium was required, agar was omitted, 3. The pH of the medium was adjusted to 5.0 before autoclaving by adding a few drops of concentrated HCl, 4. An aliquot of a filter-sterilized solution of thiamine HCl (1 mg/ml) was added to the medium after autoclaving to give a final thiamine concentration of 1 ug/ml. Peptone-glucose medium was prepared as described by Fergus (1964).

Preparation of the Lignocellulosic Substrate

Dry feed-lot manure was obtained from Arizona Feeds Company, Casa Grande, Arizona, U.S.A. As received, it contained an appreciable amount of sand and soluble material which might be expected to inhibit lignocellulose degradation. Consequently, the

following washing procedure was developed to produce a substrate that was rich in insoluble lignocellulose (manure fiber) and poor in soluble nutrients and insoluble inorganics.

Two hundred grams of dry manure were added to 2 liters of distilled water in a one-gallon Waring blender (model CB-4, Waring Products Corp., Winsted, Connecticut, U.S.A.) and shredded at low speed for 15 seconds. The mixing was repeated three times with 15 second intervals between mixings. The suspension was transferred to a 30 cm. diam. x 61 cm. high glass jar and diluted 1:1 with distilled water. This suspension was mixed with a variable speed motor connected to a shaft and propeller. The motor speed was set so that all material was suspended, and the suspension was stirred for 1.5 hours. The mixing speed was then reduced for 0.5 hr to allow sand to settle while the lighter fiber particles remained suspended. With the motor running at reduced speed, the suspended particles were siphoned off and caught on a 20-mesh (0.238 mm openings) screen. This material was washed on the screen with distilled water. The stirring, settling and screening process was repeated three times, and the resulting fibers were dried at 65 C for three days. The dried fibers were ground in a meat grinder to redisperse the particles and stored in glass jars. Fiber was dried in a 65 C oven for at least two days prior to use until a constant weight was obtained. Dry manure fiber prepared in this manner contained $14 \pm 1\%$ ash, $37 \pm 2\%$ reducing sugar (cellulose + hemicellulose), $37 \pm 1.5\%$ lignin and $10 \pm 0.5\%$ crude protein (Kjeldahl

nitrogen value x 6.25).

Two hundred mg portions of dry fiber were placed in 16 mm x 125 mm screw-capped test tubes and autoclaved for 40 minutes and dried at 55 C overnight before use. To assure adequate heat transfer during sterilization, the tubes were stacked horizontally with the fiber distributed evenly over the bottom wall.

Preparation of Inoculum

Cultures of C. prunosum were grown at 40 C for 5 days on peptone-glucose slants. Five ml of sterile distilled water were added to a slant, and the surface growth was gently suspended with the pipette. One tenth ml of suspension, which contained mainly conidia and some fragments of vegetative mycelium, was used as the inoculum for the flask and plate cultures. Diffusion cultures received 0.2 ml of inoculum.

Conditions of Growth

All cultures were incubated at 40 C in either air or oxygen.

1) Cultivation with Submerged Lignocellulose.

Two hundred mg of dry, sterile, washed manure fiber were added to 50 ml of sterile mineral medium in 250 ml Erlenmeyer flasks, and the cultures were inoculated. Shaking cultures were incubated with rotary shaking at 200 rpm on a junior orbit shaker (Cole-Parmer Instrument Co., Chicago, Illinois, U.S.A.).

2) Cultivation with Moist Lignocellulose.

Sterile Nuclepore membrane filters (5 um pore size, 47 mm

diameter, Nuclepore Corp., Pleasanton, Calif., U.S.A.) were placed, dull side down, on the surface of 25 mm x 150 mm petri plates containing approximately 200 ml of mineral agar medium. Four filters were used per plate. Two-hundred mg of dry, sterile, washed manure fiber were spread on the surface of each filter. Each pile of fiber was inoculated at the periphery with two drops of conidial suspension. Relative humidity in the incubator was maintained at 80% using water-filled trays.

3) Preparation of Diffusion Cultures

Glass tubes 20-30 mm long, O.D. 46 mm, I.D. 44 mm, were glued to both sides of a 47 mm dia., 0.2 μ m pore size Nuclepore membrane filter using Dow Corning white silicone rubber sealant (Dow Corning Corp., Midland, Mich., U.S.A.). The assemblies were dried overnight, and both ends were plugged with well-washed polyurathane sponge stoppers (Dispo plugs #T1387, Scientific Products, Menlo Park, Calif., U.S.A.). The plugs were washed in detergent, rinsed in distilled water, autoclaved for 20' in distilled water, rinsed again and then dried overnight at 40 C before use to remove potential growth-inhibitors (Keyser, et al. 1977). A piece of 9 mm O.D. glass tubing with an aluminum foil cap was inserted through the top plug. The bottom chamber and plug were covered with foil, and the chambers were autoclaved. The apparatus is shown in Figure 4.

After sterilization, small strips of plastic tape were used to attach the foil tube caps to the 9 mm dia. glass tubes and to attach the top plugs to the top glass cylinders. Two hundred mg of dry, sterile manure fiber were poured into the bottom chamber and distributed evenly over the filter. Distribution must be gentle to avoid

puncturing the membrane. One ml of sterile mineral medium was distributed drop-wise over the fiber, and the sterile plug and foil cover were replaced loosely. The plug was not pushed into the liquid. The inverted chambers were then placed in a 40 C incubator for 3 hours to allow the fiber to absorb the mineral medium. The plugs were then pushed down gently against the hydrated fibers, and the foil covers were tightly crimped. The diffusion chambers were then placed right side up, and 0.2 ml of a spore suspension inoculum was delivered to the top chamber through the glass tube. The cultures were incubated either in air in humidified glass jars or in oxygen-containing plastic bags. At weekly intervals, 0.8 ml aliquotes of sterile mineral medium were added to the top chambers to prevent drying and supply mineral nutrients. Fiber in the bottom chamber was wetted with sterile distilled water and examined microscopically for contamination before harvesting.

4) Growth in an Oxygen Atmosphere

Flask cultures were sealed with rubber stoppers containing holes plugged with rubber serum caps. Cultures were gassed daily for 3 minutes through the serum caps with pure sterile, humidified oxygen. Approximately 20 flask volumes of oxygen were flushed through the cultures during gassing. Agar plate and diffusion cultures were incubated in polyethylene bags containing trays of water for humidification. The bags were closed and flushed and filled 3-4 times with pure oxygen. Because the bags were not completely gas-tight, humidified oxygen was added to them at a rate of approximately one

bags vol/5 hours. In this way the bags remained continuously inflated with a slight positive pressure of oxygen.

Analysis for Carbohydrate and Lignin

Cultures from the agar plates or diffusion chambers containing residual lignocellulose fiber were washed off the membrane filters with distilled water and collected on the surface of 1.0 um pore size, 47 mm dia. Nuclepore filters. Particulate material from the submerged cultures was also collected in this way. Collected material was then transferred to glass vials and dried at 65C. Lignin and carbohydrate were analyzed by modifications of the methods of Moore and Johnson (1967) and Fairbairn (1953), respectively.

Dry samples of fiber or fiber plus mycelium were mixed with 5 ml of 72% (w/w) sulfuric acid and allowed to stand for three hours with mixing every half-hour for a few seconds. These were then diluted to 50 ml with distilled water, allowed to stand overnight and then vacuum-filtered through tared 1.0 um pore size 47 mm diam Nuclepore filter.⁵ Aliquotes of the filtrate were retained for carbohydrate (reducing sugar) assays. In this assay 1.0 ml of suitably diluted sample was mixed with 5.0 ml of a reagent containing 0.75 g anthrone in 1 liter of 72% (v/v) H₂SO₄. The mixture was heated in a boiling water bath for 12 minutes, cooled to room temperature, and the optical density at 620 nm was measured. Glucose was used as a standard. Anthrone reagent was made fresh daily.

The residue on the filter consisting of lignin and insoluble inorganic material was washed with distilled water until the filtrate pH reached 5.0 as measured with pH paper. The filter plus residue

was dried overnight at 65 C and weighed. The filters and samples were then transferred to tared crucibles and ashed overnight at 550 C. Lignin was defined as the fraction of the sample (excluding the filter) which vaporized. The filters contained less than 0.5 mg ash.

RESULTS AND DISCUSSION

Figure 1 (solid lines) shows the rate and extent of lignin and carbohydrate degradation in shaking cultures of *C. prunosum* growing with submerged lignocellulose in the presence of air. Uninoculated controls for all the experiments to be reported typically showed between 5 and 10% loss of both lignin and carbohydrate, so losses of either component of 10% or less in inoculated cultures are not considered significant. The data indicate that lignin is not appreciably degraded in shaking submerged cultures, while carbohydrate degraded reached a maximum of approximate 40% after 20 days of incubation.

Figure 2 (solid line) shows the rate and extent of lignin and carbohydrate degradation occurring in cultures growing with lignocellulose fiber on the surface of mineral agar plates in the presence of air. Lignin loss reaches a maximum of 50% in 12 days, and carbohydrate loss reaches a maximum of approximately 80% at the same time. During the first three days of incubation, the water content of the culture increases from 0 to 80% (w/w) and then stabilizes between 75 and 80% (w/w).

In a separate experiment the culture residue present after 30 days of incubation on the agar surface in the presence of air was

collected, washed with distilled water, dried, sterilized, and used to make fresh agar surface cultures. Only very sparse growth occurred in these cultures, and no further lignin or carbohydrate loss was detected. What growth was seen appeared to be at the expense of impurities in the agar. When the residue was supplemented with cellulose (ball-milled filter paper), mold growth was vigorous, but no lignin degradation was detectable. These results suggest that the incomplete degradation of lignin is due to the chemical or physical nature of the residue rather than to growth inhibiting culture conditions or the absence of a carbohydrate co-substrate (Hiroi & Eriksson, 1976, Kirk, et al., 1976)

Figure 3 (solid lines) shows the rate and extent of lignin and carbohydrate degradation occurring in stationary submerged cultures growing with lignocellulose fiber in the presence of air. Here as in the shaking cultures, lignin degradation is not significant, while carbohydrate loss reaches a maximum of 37% after 30 days.

As growth proceeded in the stationary cultures, a tightly woven mycelial mat developed on the bottom of each flask which encompassed and immobilized all the fiber particles indicating that close contact between the hyphae and the substrate was established. In contrast, the mycelium in the shaking culture was evenly dispersed in the medium, and there was little evidence, either macroscopic or microscopic, of close hyphal-substrate contact.

The fact that carbohydrate degradation was less extensive in submerged cultures than in agar surface cultures may be due to the presence of a largely unattacked lignin residue (Kirk and Moore,

1972, Dekker and Richards, 1975). It appears, then, that in air, under these conditions, while some carbohydrate degradation can be carried out by C. pruinosa, little or no lignin degradation is possible. On agar-surface (non-submerged) substrates both lignin and carbohydrate are extensively degraded.

It was suggested (R. Emerson, personal communication) that submerged cultures may, by their metabolism of carbohydrate, reduce the oxygen tension in the medium to the point where lignin degradation is not possible. To examine this possibility, cultures were constructed as described above but incubated in an atmosphere of pure oxygen instead of air. The results of these experiments are shown in Figures 1-3 (dashed lines).

Data in Figure 1 indicate that a pure oxygen atmosphere has no effect on lignin degradation in shaking submerged cultures. The rate of carbohydrate degradation is increased some, but the extent of carbohydrate degradation is only slightly increased.

Data in Figure 2 indicate that a pure oxygen atmosphere may increase slightly the rate of lignin degradation in agar-surface cultures, but the extent of degradation is unchanged. Both the rate and extent of carbohydrate degradation are increased, however. The increase in carbohydrate degraded could be due to an alteration in the nature of the lignin degradation taking place or to the stimulation of a cellulose-oxidizing activity noted by Eriksson, et al. (1974), or it might represent enhanced autolysis of carbohydrate-containing mycelium in the residue. The dry cell mass of

this organism contains the equivalent of 40% reducing sugar as measured by our assay system.

Data in Figure 3 indicate that pure oxygen does increase the amount of both lignin and carbohydrate degraded in static submerged cultures relative to incubation in air. The findings of Kirk and Moore (1972) would suggest that the increase in carbohydrate degradation is a consequence of the increased lignin loss.

The results of these experiments indicate that both close hyphal-substrate contact and high concentrations of oxygen are necessary for C. pruinosa to effect significant lignocellulose degradation while growing with a submerged particulate substrate. Degradation is still inferior to that found in agar surface cultures, however. This difference may occur because high oxygen concentrations stimulate only one or a few components of the lignin degradation system in submerged cultures leaving many types of chemical bonds in the polymer still unbroken. Alternatively, the oxygen concentration in the cultures may still not be high enough to saturate the system involved. Experiments are being carried out to test this hypothesis.

Demonstrations of lignin degradation by lignocellulose-degrading fungi have usually been conducted with the organism growing on a moist, non-submerged substrate (Ander and Eriksson, 1975, Hiroi and Eriksson, 1976, Kirk and Kelman, 1965). Recently, several authors have reported the occurrence of lignin degradation under submerged conditions of cultivation using as substrates either ^{14}C -labeled synthetic lignins called dehydrogenation polymers (DHP) or ^{14}C -labeled lignins synthesized in vivo using either ^{14}C -labeled

ferulic acid or L-(U- ^{14}C) phenylalanine. The in vivo labeled lignin is part of a natural lignocellulose complex. In both cases degradation is measured by collecting ^{14}C -labeled CO_2 produced during growth. Thus, Haider and Trojanowski (1975) reported 3.8% $^{14}\text{CO}_2$ release from ring- ^{14}C -labeled DHP using Preussia fleischhakkii growing for 10 days in shaking flask cultures and 5.7% $^{14}\text{CO}_2$ released from 3'- ^{14}C -ferulic acid-labeled lignin in lignocellulose using Chaetomium piluliferum growing for 16 days in shaking flask cultures. It is not clear, however, whether the small amounts of lignin degraded in these cases ^{ARE} ~~is~~ representative of the total lignin polymer or a special fraction thereof.

Crawford and Crawford (1976) reported significant degradation of lignin labeled in vivo using ^{14}C -phenylalanine. In this case degradation occurred in submerged shaking cultures using a soil inoculum. Thirty-five percent of the label was recovered as $^{14}\text{CO}_2$ after 25 days of incubation at 42°C. The labeled lignin produced by the in vivo labeling technique may not be chemically or physically identical to the bulk of the plant lignin, however, so the degradation observed may only apply to the labeled fraction.

Using ^{14}C -ring-labeled DHP in stationary submerged cultures, Lundquist, et al. (1977) found 23% labeled CO_2 release by Phanerochaete chrysosporium after 55 days of incubation. Growth took place in a very shallow liquid layer (10 ml of suspension in a 125 ml flask), and this may have allowed sufficient aeration for lignin degradation. My stationary cultures contained a deeper layer of liquid. In all cases involving the use of labeled CO_2 as an indicator of lignin degradation, the values obtained are believed

to represent minimum levels of degradation since some of the metabolized material is presumably incorporated into cell material.

It was also of interest to determine whether the lignin-degrading activity produced by this mold was diffusible and what effect oxygen would have on any diffusible activity. Table 1 shows the results of experiments conducted using diffusion cultures. In this system the mold and the damp lignocellulosic substrate are separated by a bacteriological filter (Fig. 4). Data in the bottom two lines (inoculated fiber) indicate that lignin and carbohydrate can be degraded in this apparatus when the fiber is inoculated directly with the mold. Values for lignin and carbohydrate degradation are lower than seen in agar plate cultures, but they are nevertheless substantial. A pure oxygen atmosphere enhances the extent of lignocellulose degradation.

When the culture is separated from the substrate (inoculated filter), a different pattern appears. A mat of mycelium and spores forms on the surface of the filter in the top chamber, and carbohydrate degradation is evident below. This indicates that cellulase enzymes are being produced and are diffusing down into the damp fiber and that metabolites are diffusing up and supporting mold growth.

The data indicate, however, that in the presence of air, little or no lignin is degraded in this system relative to the uninoculated controls. In the presence of oxygen there appears to be a slight stimulation of diffusible lignin degrading activity, although here

as in the submerged cultures the effect is small and may be restricted to only part of the lignin.

It is interesting to note that the cellulase enzymes produced by this organism can be induced in the absence of close hyphal-substrate contact. This could be due to induction by some small diffusible molecule present in the substrate (Enari & Markkanen, 1977), or it could be brought about by the general condition of starvation (Hulme & Stranks, 1971). Lignin degrading enzymes may require close hyphal-substrate contact for induction, however, and experiments are in progress to test this hypothesis.

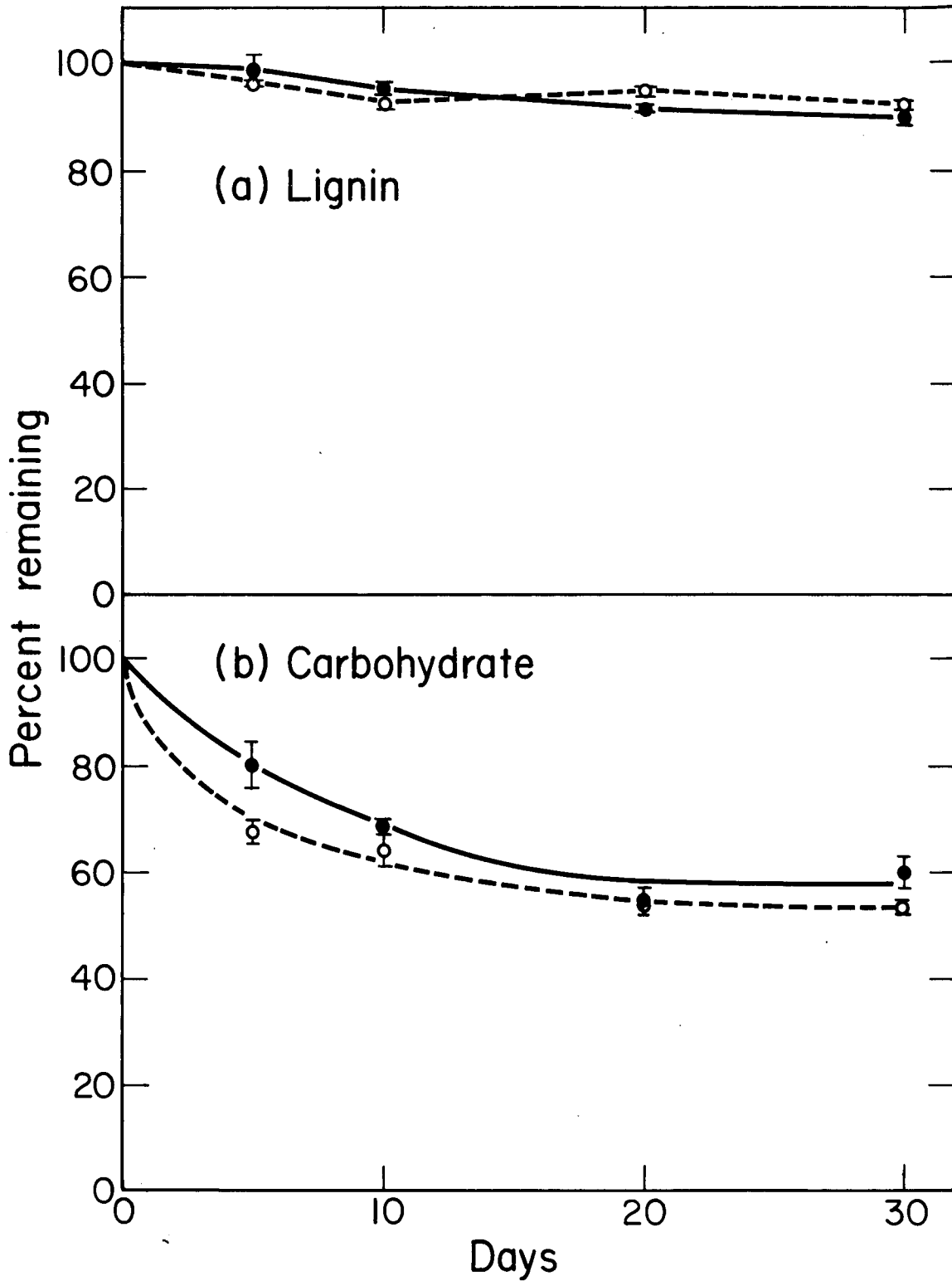
Acknowledgment

I thank Loretta Baier for skillful technical assistance. This work was funded in part by the General Electric Co., Department of Energy, and by NSF/RANN grant No. AER75-23686 to C.R. Wilke. Figure 4 is reprinted with permission from: S.L. Rosenberg and C.R. Wilke, Lignin Biodegradation and the Production of Ethyl Alcohol from Cellulose in T.K. Kirk, ed. Lignin Biodegradation: Microbiology, Chemistry and Applications. in press. Copyright the Chemical Rubber Co., CRC Press, Inc.

Literature Cited

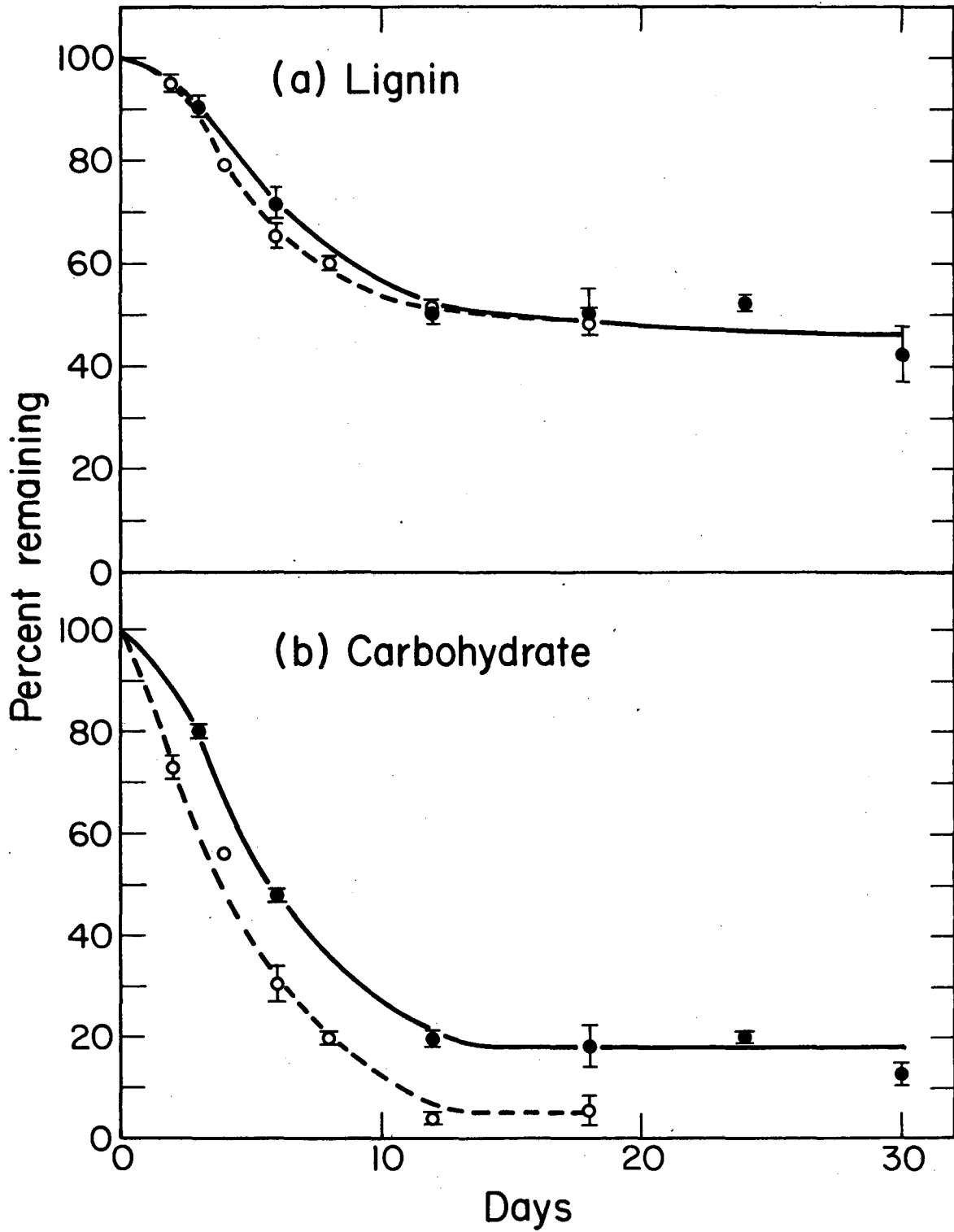
1. Abson, D., ed, 1976. Biological Delignification: Present Status and Future Directions, Weyerhaeuser Symposium, Weyerhaeuser Co., Seattle, Wash., U.S.A. 116 pp.
2. Ander, P. and K.-E. Eriksson, 1975. Influence of Carbohydrates on Lignin Degradation by the White-Rot Fungus Sporotrichum pulverulentum, Svensk Papperstidn. 78:643-652.
3. Ander, P. and K.-E. Eriksson, 1977. Lignin Degradation and Utilization by Microorganisms, in Progress in Industrial Microbiology, 14: M.J. Brill, ed., Elsevier, Amsterdam, in press.
4. Bailey, M., T-M. Enari and M. Linko, eds., 1975. Symposium on Enzymatic Hydrolysis of Cellulose, The Finnish National Fund for Research and Development (SITRA), Helsinki, 525 pp.
5. Burdsall, H.H., Jr., and W.E. Eslyn, 1974. A New Phanerochaete with a Chrysosporium imperfect state. Mycotaxon. 1:123-133.
6. Crawford, D.L. and R.L. Crawford, 1976. Microbial Degradation of Lignocellulose: The Lignin Component. Appl. and Env. Microbiol. 31:714-717.
7. Dekker, R.F.H. and G.N. Richards, 1975. Purification, Properties, and Mode of Action of Hemicellulase II Produced by Ceratocystis paradoxa. Carbohydrate Res. 42:107-123.
8. Enari, T.-M. and P. Markkanen, 1977. Production of Cellulolytic Enzymes by Fungi. Adv. in Biochem. Eng. 5:1-24.
9. Eriksson, K.-E., B. Pettersson and U. Westermark, 1974. Oxidation: An Important Enzyme Reaction in Fungal Degradation of Cellulose. FEBS Letters. 49:282-285.
10. Fairbairn, N.J., 1953. A Modified Anthrone Reagent. Chem. and Ind. (London) No. 4.86.
11. Fergus, C.L., 1964. Thermophilic and Thermotolerant Molds and Actinomycetes of Mushroom Compost During Peak Heating. Mycologia, 56:267-284.
12. Gaden, E.L., M.H. Mandels, E.T. Reese and L.A. Spano, eds. 1976. Enzymatic Conversion of Cellulosic Materials: Technology and Applications. John Wiley & Sons, Inc., New York, 319 pp.
13. Haider K. and J. Trojanowski, 1975. Decomposition of Specifically ¹⁴C-Labelled Phenols and Dehydropolymers of Coniferyl Alcohol as Models for Lignin Degradation by Soft and White-Rot Fungi. Arch. Microbiol. 105:33-41.

14. Hiroi, T. and K.-E. Eriksson, 1976. Microbiological Degradation of Lignin. I. Influence of Cellulose on the Degradation of Lignins by the White-Rot Fungus Pleurotus ostreatus, Svensk Papperstidn. 79:157-161.
15. Hulme, M.A. and D.W. Stranks, 1971. Regulation of Cellulase Production by Myrothecium verrucaria Grown on Non-Cellulosic Substrates. J. Gen. Microbiol. 69:145-155.
16. Keyser, P., L. Lorenz, H.-H. Yang and T.K. Kirk, 1977. Test Tube Plugs, ASM News, 43:297.
17. Kirk, T.K. and A. Kelman, 1965. Lignin Degradation as Related to the Phenoloxidases of Selected Wood-Decaying Basidiomycetes. Phytopathology, 55:739-745.
18. Kirk, T.K. and W.E. Moore, 1972. Removing Lignin from Wood with White-Rot Fungi and Digestibility of Resulting Wood, Wood and Fiber. 4:72-79.
19. Kirk, T.K., W. J. Connors and J.G. Zeikus, 1976. Requirement for a Growth Substrate During Lignin Decomposition by two Wood-Rotting Fungi. Appl. and Environ. Microbiol. 32:192-194.
20. Lundquist, K., T.K. Kirk and W.J. Connors, 1977. Fungal Degradation of Kraft Lignin and Lignin Sulfonates Prepared from Synthetic ¹⁴C-Lignins. Arch. Microbiol. 112:291-296.
21. Moore, W.E. and D.P. Johnson, 1967. Procedures for the Chemical Analysis of Wood and Wood Products. Forest Products Laboratory, U.S. Dept. of Agriculture.
22. Rosenberg, S.L., 1975. Temperature and pH Optima for 21 Species of Thermophilic and Thermotolerant Fungi, Can. J. Microbiol. 21:1535-1540.
23. Rosenberg, S.L., 1978. Cellulose and Lignocellulose Degradation by Thermophilic and Thermotolerant Fungi. Mycologia, 70:1-13.
24. Sarkanen, K.V. and C.H. Ludwig, eds. 1971. Lignins. Occurrence Formation, Structure and Reactions. Wiley-Interscience, New York, 918 pp.
25. Wilke, C.R., ed., 1975. Cellulose as a Chemical and Energy Resource. John Wiley & Sons, Inc. New York, 361 pp.



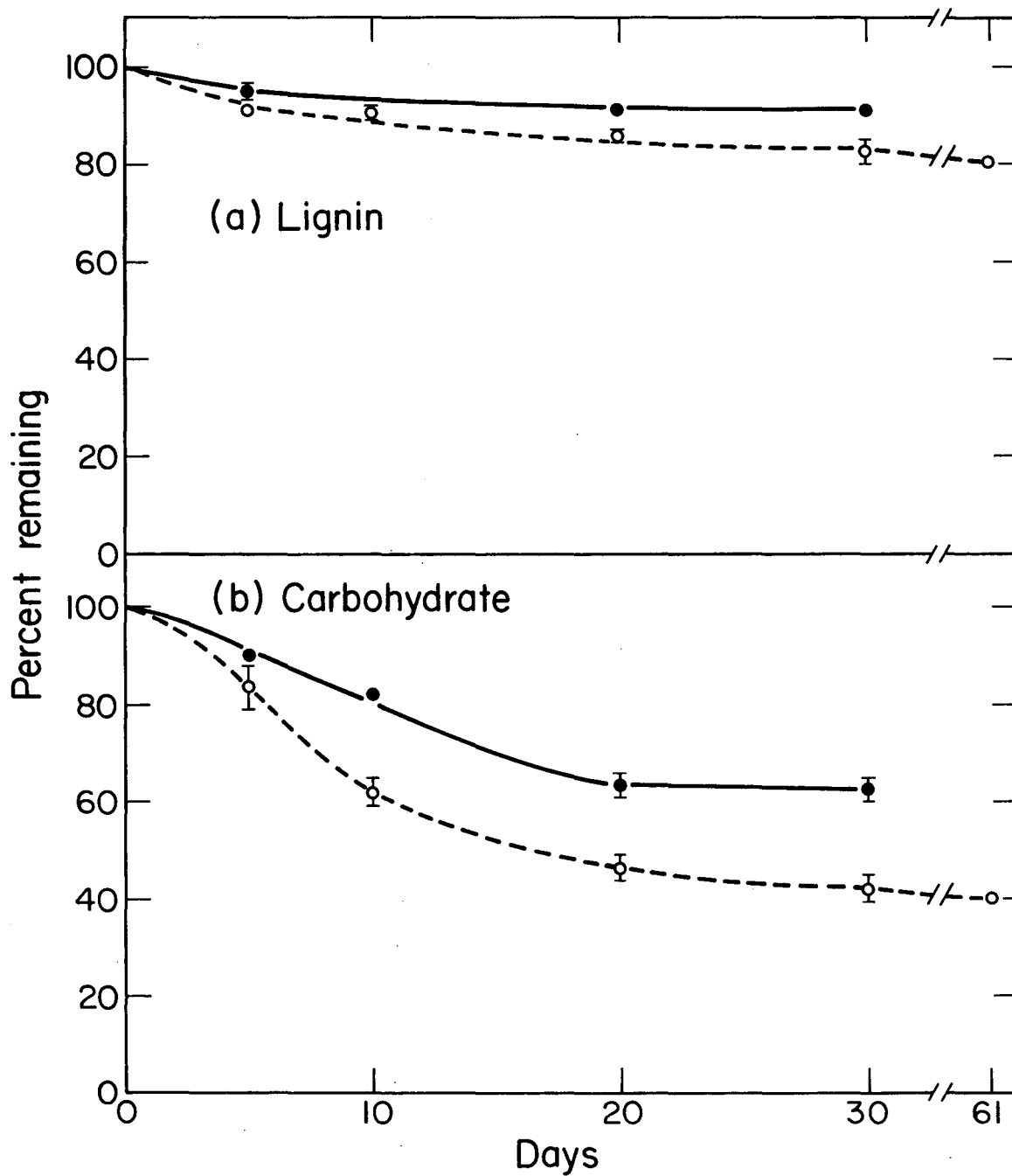
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FIG 1. Lignin and carbohydrate loss in shaking submerged cultures of *C. prunosum* grown in mineral medium plus manure fiber in the presence of air ●—● or oxygen ○---○. Each data point represents the average of two cultures.



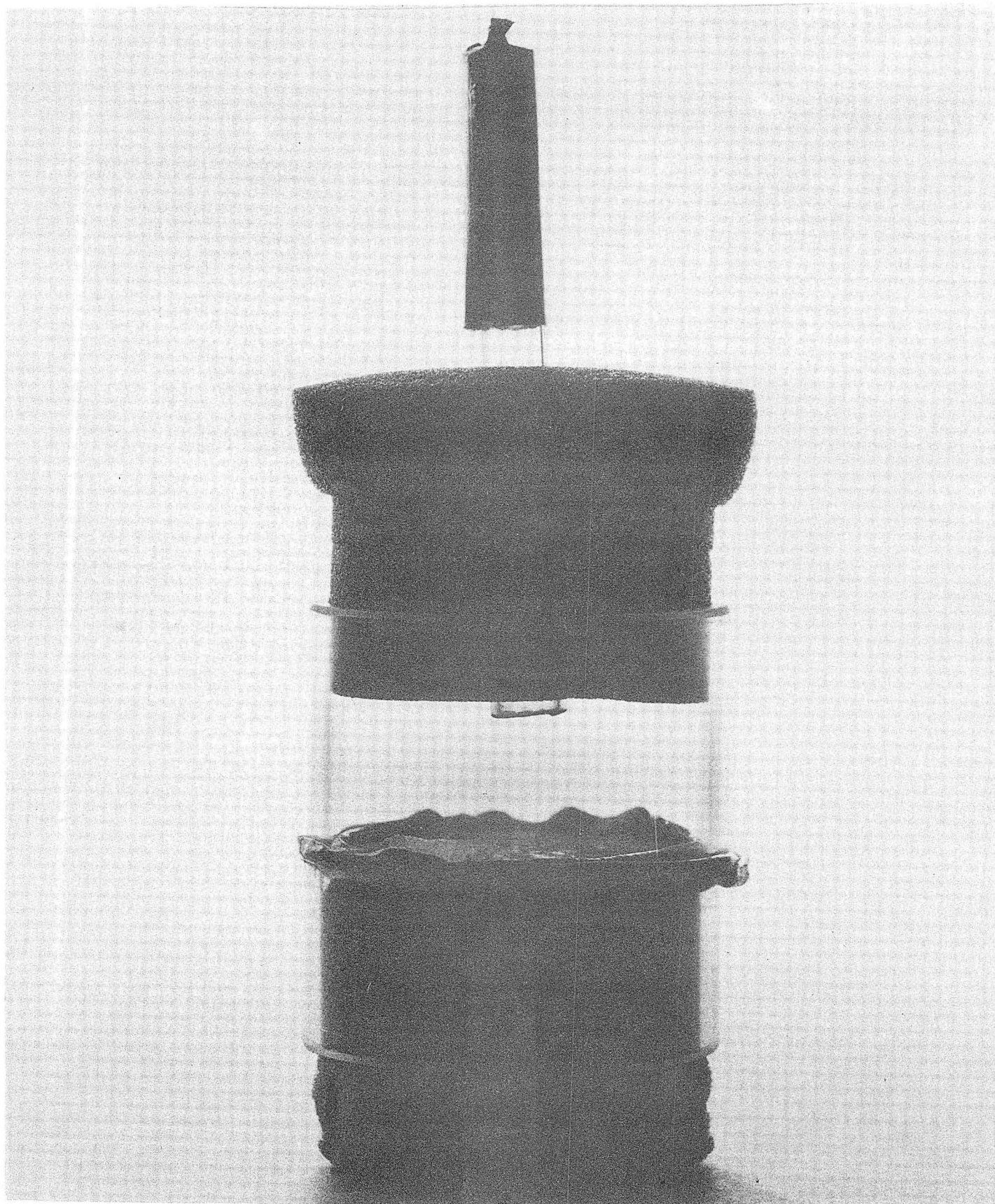
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FIG. 2. Lignin and carbohydrate loss in damp manure fiber cultures of *C. pruinosa* grown on the surface of mineral agar plates in the presence of air ●—● or oxygen o---o. Each data point represents the average of two cultures.



XBL 784-661

FIG. 3 Lignin and carbohydrate loss in stationary submerged cultures of *C. pruinosa* grown in mineral medium plus manure fiber in the presence of air ●—● or oxygen ○----○. Each data point represents the average of two cultures.



CBB 783-2637

FIG. 4. Diffusion Chamber. Top plug has been raised and bottom foil cover has been removed to show details of construction.

Table 1

Residual Lignin and Carbohydrate in Manure Fiber from Diffusion Cultures

SAMPLE	INCUBATION Time-days	Avg. % Lignin Remaining ± Avg. Deviation ^a		Avg. % Carbohydrate Remaining ± Avg. Deviation ^a	
		AIR	OXYGEN	AIR	OXYGEN
Uninoculated Control	0	100.0 ± 0.8	100.0 ± 0	100.0 ± 0.1	100.0 ± 0.8
	14(15) ^c	96.7 ± 0.2	95.2 ± 1.1	94.3 ± 2.8	93.1 ± 0.2
	31(30)	96.5 ± 0	94.3 ± 1.0	89.8 ± 0.4	90.3 ± 0.6
Inoculated Filter	14(15)	94.5 ± 1.0	87.0 ^b	78.1 ± 0.8	52.8 ^b
	31(30)	92.0 ± 2.5	87.1 ± 0.9	54.9 ± 4.2	51.4 ± 4.7
Inoculated Fiber	14(15)	77.5 ± 1.0	62.6 ± 1.5	26.9 ± 7.1	13.8 ± 6.1
	31(30)	76.0 ± 2.8	57.7 ± 2.1	27.4 ± 2.5	16.2 ± 3.1

a) Relative to average value for zero time uninoculated control.

b) One culture analyzed.

c) Parentheses indicate incubation time for +O₂ cultures.

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