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Microbial Decomposition of Wood in Streams: Distribution of Microflora and Factors Affecting [^{14}C]Lignocellulose Mineralization†

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The distribution and lignocellulolytic activity of the microbial community was determined on a large log of Douglas fir (*Pseudotsuga menziesii*) in a Pacific Northwest stream. Scanning electron microscopy, plate counts, and degradation of [^{14}C]lignocelluloses prepared from Douglas fir and incubated with samples of wood taken from the surface and within the log revealed that most of the microbial colonization and lignocellulose-degrading activity occurred on the surface. Labeled lignocellulose and surface wood samples were incubated in vitro with nutrient supplements to determine potential limiting factors of [^{14}C]lignocellulose degradation. Incubations carried out in a nitrogenless mineral salts and trace elements solution were no more favorable to degradation than those carried out in distilled water alone. Incubations supplemented with either $(NH_4)_2SO_4$ or organic nitrogen sources showed large increases in the rates of mineralization over incubations with mineral salts and trace elements alone, with the greatest effect being observed from an addition of $(NH_4)_2SO_4$. Subsequent incubations with $(NH_4)_2SO_4$, KNO_3, and NH_4NO_3 revealed that KNO_3 was the most favorable for lignin degradation, whereas all three supplements were equally favorable for cellulose degradation. Supplementation with glucose repressed both lignin and cellulose mineralization. The results reported in this study indicate that nitrogen limitation of wood decomposition may exist in streams of the Pacific Northwest. The radiotracer technique was shown to be a sensitive and useful tool for assessing relative patterns of lignocellulose decay and microbial activity in wood, along with the importance of thoroughly characterizing the experimental system before its general acceptance.

The large biomass of old-growth coniferous forests of the Pacific Northwest can result in substantial accumulations of logs on the forest floor and in the streams which drain the surrounding watershed (17). Standing crops of wood in Cascade Mountain stream channels can exceed 40 kg m^{-2} (21). The determination of the role large woody debris plays in ecosystems is essential, especially in view of current management strategies for logging operations which may significantly decrease the amounts of debris available for habitat formation and biological processing (36; J. D. Hall and C. O. Baker, 1975, A workshop on logging debris in streams, Oregon State University, Corvallis, and F. J. Swanson and G. W. Lienkaemper, 1978, U.S. Department of Agriculture Forest Service General Technical Report PNW-69).

A major component of this woody debris is lignocellulose, which represents more than one-half of the total carbon present in wood. Recently developed radiotracer methodologies that specifically label the lignocellulose fraction of plant tissue have led to ever increasing amounts of information on the microorganisms, biochemical processes, and environmental factors which affect the degradation of lignocellulose (9).

In recent years, much emphasis has been placed on studies involving specific microorganisms in pure culture (4–6, 11, 19, 22, 23, 30). Although this is of undeniable importance, such information cannot be extrapolated easily to natural systems. Research that uses radiotracer methods with environmental samples has been limited to soil and sediment systems (6–8, 14, 18, 25). Large woody debris differs from soils and sediments in that the substrate has a very small surface area-to-volume ratio, is structurally recalcitrant, and sometimes occurs in flowing-

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water environments which are frequently low in nutrients.

This paper describes the development and use of a radiotracer assay to determine the characteristics of lignocellulose degradation in wood. The assay was used to determine patterns of microbial decomposition in large logs of Douglas fir (*Pseudotsuga menziesii*) which have been lying for known periods of time in a stream channel of an old-growth forest.

MATERIALS AND METHODS

Preparation and characterization of [^{14}C]lignocelluloses. Douglas fir lignocelluloses labeled specifically in either the lignin or the cellulose fraction were prepared by the method of Crawford (9). Freshly cut ends of Douglas fir branches, each ca. 1 m in length, were immersed in aqueous solutions of either 50 μCi of L-[*U*-^{14}C]phenylalanine (10 mCi mmol^{-1}) or 50 μCi of D-[*U*-^{14}C]glucose (3 mCi mmol^{-1}) to initiate the labeling of lignin and cellulose, respectively. Radiochemicals were obtained from Amersham Corp., Arlington Heights, Ill. Just before uptake of the radioactive solution was completed, additional water was added as needed to keep the cut ends immersed, and the stems were allowed to metabolize the radiolabels under constant illumination until wilting began to occur (ca. 1 week). The cambial tissue was stripped from the main stem and dried at 50°C for 2 weeks. The labeled tissue was ground to pass a (no. 40) mesh screen and subjected to a sequence of hot water, ethanol-benzene, and ethanol extractions to remove undesired labeled plant constituents, as previously described in detail by Crawford (9). The extractive-free tissue was dried at 50°C and stored in a desiccator at room temperature.

The specific activities of the extractive-free [^{14}C-lignin]- and [^{14}C-cellulose]lignocelluloses were determined by combustion of 10-mg samples in a model 306 Packard Tri-Carb oxidizer (Packard Instrument Co., Inc., Rockville, Md.), and the resulting $^{14}CO_2$ was trapped and counted by standard liquid scintillation techniques.

Distribution of ^{14}C within the labeled material was determined by the modified Klason procedure of Effland (13). Triplicate 200-mg portions of each labeled substrate were digested for 1 h with 72% (wt/wt) H_2SO_4. The digest was diluted (1:28), heated at 120°C and at a pressure of 103 kPa for 1 h, and then filtered through tared, fritted glass crucibles. The residue was dried overnight at 105°C and weighed to determine the initial lignin content of the woody tissue. The specific radioactivity was determined with the Packard oxidizer as described above. The Klason filtrate was adjusted to pH 4.5 with $CaCO_3$ and stored at 4°C for subsequent carbohydrate determinations. A portion of the filtrate was used for determination of its radioactivity by pipetting 0.1-ml samples onto 5.5-cm-diameter Whatman no. 1 filter papers which were combusted, and the $^{14}CO_2$ was quantified as described above.

The distribution of radiolabel in wood sugars was determined by TAPPI method T250 pm-75 (The Technical Association of the Pulp and Paper Industry, Atlanta, Ga.), with the following modifications. Portions (100 ml) of the Klason filtrates were lyophilized to concentrate the filtrate and then rehydrated with 5 ml of distilled water. Incorporation of ^{14}C into wood sugars was determined by descending paper chromatography with a butanol-pyridine-water (10:3:3, vol/vol) solvent system. Solutions of glucose, mannose, and xylose were used as standard markers. The sections of the chromatograms corresponding to glucose, mannose, and xylose were removed and analyzed for ^{14}C activity by oxidation.

The total nitrogen remaining in the radiolabeled lignocelluloses was estimated by micro-Kjeldahl analysis. Radioactivity associated with the protein fraction was estimated by incubating 10-mg samples of the labeled substrates with 6 U of Type XIV bacterial protease (Sigma Chemical Co., St. Louis, Mo.) in 0.05 M phosphate buffer, pH 7.5, at 37°C for 2 h. Samples were analyzed in quintuplicate, and changes in specific radioactivity of the residue were determined by sample oxidation before and after protease incubation.

Study site and sample collection. Wood samples used as inocula for [^{14}C]lignocellulose degradation experiments were obtained from Mack Creek, a third-order stream in an old-growth section of the H. J. Andrews Experimental Ecological Reserve, located at an elevation of 830 m in the Cascade Mountain Range, Oreg. Surface scrapings were obtained with a carpentry plane, and cores were taken with a 12-mm-diameter increment borer (Forestry Suppliers, Inc., Jackson, Miss.) from bark-free, stream-wetted portions of an old-growth Douglas fir log that fell into the stream in 1977. Samples were placed in sterile Whirlpak bags, stored on ice, transported to the laboratory, and processed within 48 h. Wood cores were split in the laboratory with a sterile knife and subsampled to avoid contamination that may have been carried down from the surface by use of the increment borer.

Sample preparation. Wood samples were homogenized in sterile distilled water for 8 min at a setting of 30 on a VirTis model 45 homogenizer (VirTis Co., Inc., Gardiner, N.Y.). This duration of homogenization had been previously determined to yield the highest numbers of microorganisms from wood samples by use of standard microbiological plate counts on diluted tryptic soy agar (data not shown). Experimental treatments were prepared by placing 1-ml portions of homogenate into 60-ml glass serum bottles containing 20 ml of an incubation medium and fitted with glass capillary (2-mm-diameter) bubbler tubes and sleeve-type rubber stoppers. The treatments were bubbled either continuously or once per day for 15 min with filtered, humidified, CO_2-free air. Incubations were carried out at room temperature (21 ± 2°C). Outflow gas from the incubation bottles was passed through 8% (wt/vol) NaOH to absorb $^{14}CO_2$, radioactivity was determined by acidification of the alkali with concentrated H_2SO_4, and the $^{14}CO_2$ was trapped on filter paper soaked with β-phenylethylamine (free base, Sigma). Filter papers were then placed in 15 ml of liquid scintillation cocktail and their radioactivity was counted on a Beckman model LS8000 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Counting efficiency was determined by using a quench series and the external standard technique, with data corrected for efficiency and background. The liquid scintillation cocktail consisted of Spectrafluor (Amersham), methanol, and toluene (16:100:125, vol/vol). The removal of $^{14}CO_2$ from the alkali traps

was shown to be complete by using known quantities of $Na_2{}^{14}CO_3$.

Spatial distribution and [^{14}C]lignocellulose decomposition experiments. The spatial distribution of the microbial community in the study log was determined by obtaining surface scrapings and core samples from a depth of 25 cm in July and September 1981. Plate counts were performed on samples of homogenate by spreading 0.1 ml of serial dilutions (3×10^{-2} to 3×10^{-4} in distilled water) of wood homogenate on the surface of one-tenth-strength tryptic soy agar plates. Incubation was carried out at 30°C for 5 to 7 days, under both aerobic and anaerobic conditions. Anaerobic incubation conditions were achieved by incubating plates inside GasPak anaerobic jars (BBL Microbiology Systems, Cockeysville, Md.) placed in a 30°C incubator. Preliminary studies had revealed that diluted tryptic soy agar yielded a higher recovery of microorganisms from the homogenate than did full-strength media.

Wood homogenates from the surface scrapings and core samples were also incubated in the presence of 10 kdpm of either [^{14}C-lignin]- or [^{14}C-cellulose]lignocellulose for 27 days in distilled water, with the evolved $^{14}CO_2$ trapped and quantified at ca. 7-day intervals. Portions of the sample obtained before incubation were prepared for scanning electron microscopy (SEM) by fixation with 0.33% (vol/vol) glutaraldehyde solution, dehydrated in a graded ethanol series, and dried in a model DCP-1 critical-point dryer (Denton Vacuum, Inc., Cherry Hill, N.J.). Samples were then mounted on stubs, coated with 10 nm of gold-palladium, and observed on an ETEC Autoscan scanning electron microscope.

The effects of various amounts of added [^{14}C-lignin]-lignocellulose on $^{14}CO_2$ evolution were determined by incubating 0.026-g (dry weight) portions of wood inoculum (collected in October 1982) with 10, 20, 30, and 40 kdpm of labeled substrate in distilled water.

The effects of various medium supplements on [^{14}C]lignocellulose mineralization were examined by incubating 0.013 and 0.025 g (dry weight) of wood inoculum (collected in February and April 1983, respectively) in media supplemented with organic and inorganic components. All incubation treatments contained either 20 kdpm of [^{14}C-lignin]lignocellulose or 10 kdpm of [^{14}C-cellulose]lignocellulose as the labeled substrate. The various incubation media (20 ml) used in the experiments were distilled water alone and mineral salts solution alone and in combination with one of the following amendments (final concentrations in grams per liter): $(NH_4)_2SO_4$, 3.0; yeast extract, 9.0; glucose, 1.8; KNO_3, 2.3; or NH_4NO_3, 0.9. The mineral salts solution was composed of the following components (grams per liter of distilled water): $CaCl_2$, 0.6; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; ferric citrate, 0.1; $K_2HPO_4 \cdot 3H_2O$, 0.5; KH_2PO_4, 0.188; and 10 ml of a trace elements solution containing (milligrams per liter) H_3BO_3, 143; $MnSO_4 \cdot 4H_2O$, 102; $ZnSO_4 \cdot 7H_2O$, 22; $CuSO_4 \cdot 5H_2O$, 8; $CoCl_2 \cdot 4H_2O$, 10; and $Na_2MoO_4 \cdot 2H_2O$, 5. Controls for all experiments consisted of distilled water and the appropriate labeled lignocellulose. Incubations were conducted in quintuplicate for 3 to 4 weeks at room temperature, and the $^{14}CO_2$ evolved was determined at weekly intervals. Results were plotted as accumulated kilodisintegrations per minute per gram (dry weight) of wood inoculum, and the standard error of the mean was determined for values obtained at each sampling time.

RESULTS

[^{14}C]lignocellulose characterization. The specific activities and distribution of ^{14}C within the labeled lignocellulose are summarized in Table 1 and correspond favorably to values reported in the literature for Douglas fir [^{14}C]lignocelluloses, as do the results of the Klason analysis (11). Chromatographic analysis of the Klason filtrate of the [^{14}C-cellulose]lignocellulose demonstrated that all of the radioactivity in the hydrolysate was found in the three sugars analyzed (glucose, mannose, and xylose), with 82% residing in the glucose fraction. In contrast, only 42% of the total radioactivity of the [^{14}C]lignin Klason filtrate could be accounted for in the three sugars, with 22% found in the glucose fraction (presumably from the acid hydrolysis of contaminating labeled cellulose). Similar patterns of label distribution within the Klason filtrate are reported by Maccubbin and Hodson for pine lignocelluloses (25) and by Crawford and Crawford (10) for lignocelluloses from several different sources. The 58% of the radioactivity in the [^{14}C]lignin Klason filtrate that is unaccounted for by the carbohydrate analysis is presumably in the form of either acid-soluble lignin or amino acids liberated by acid hydrolysis. In fact, it has been noted that a serious drawback of the Klason analysis is that considerable 72% H_2SO_4-soluble lignin is present in many lignocelluloses (9, 11). Protease digestion of the labeled lignocelluloses used in this study resulted in a 5% loss of radioactivity when compared with undigested substrate. A value for total protein content of 3.5%, assuming that protein = N × 6.25, was obtained from micro-Kjeldahl analysis.

TABLE 1. Distribution of ^{14}C within [^{14}C]lignocellulose

Substrate	Sp act (dpm/mg)	% Original radioactivity			
		In Klason residue	In Klason filtrate	Recovered	After protease digestion
[^{14}C-lignin]lignocellulose	1,211	76.5	27.6	104.1	94.5
[^{14}C-cellulose]lignocellulose	560	28.8	72.1	100.9	95.0

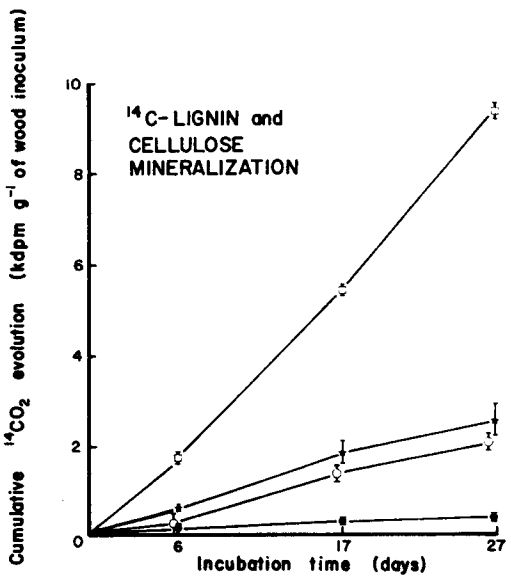

FIG. 1. [^{14}C]lignocellulose degradation in surface scrapings and core samples (25-cm depth) from a wetted log. Symbols: □, surface [^{14}C]cellulose; ○, core [^{14}C]cellulose; ★, surface [^{14}C]lignin; ●, core [^{14}C]lignin. Each point is the mean of two replicates. Bars, Standard errors of the means.

Spatial distribution and [^{14}C]lignocellulose decomposition experiments. A series of samples taken to investigate the spatial distribution of the microbial community on decaying wood showed that most of the colonization and activity occurred on the outer surface of the log. Plate counts revealed 1.44×10^7 CFU/g of wood from the aerobically incubated surface samples and 2.22×10^6 CFU/g of wood from the anaerobically incubated surface samples. Wood samples obtained from a depth of 25 cm in the log yielded no CFU under either aerobic or anaerobic conditions.

Evolution of $^{14}CO_2$ from the ^{14}C incubation experiments demonstrated that the greatest lignocellulose mineralization activity was in the surface sample incubated with [^{14}C]cellulose (Fig. 1). Decomposition of the [^{14}C]cellulose in the surface samples was more than four times that of the interior sample, with a similar relationship observed for the [^{14}C]lignin treatments. The SEM study also revealed a colonization pattern consistent with the results reported above (Fig. 2). One can see evidence of microbial colonization on the exposed side of the surface scraping, including individual bacterial cells, actinomycete-like filaments, and possibly fungal hyphae (Fig. 2A). There is very little microbial colonization apparent on the underside of surface scrapings or on the sample obtained from a depth of 25 cm (Fig. 2B and C).

Preliminary experimentation indicated that in the case of the [^{14}C-lignin]lignocellulose, caution must be exercised in the selection of the appro-

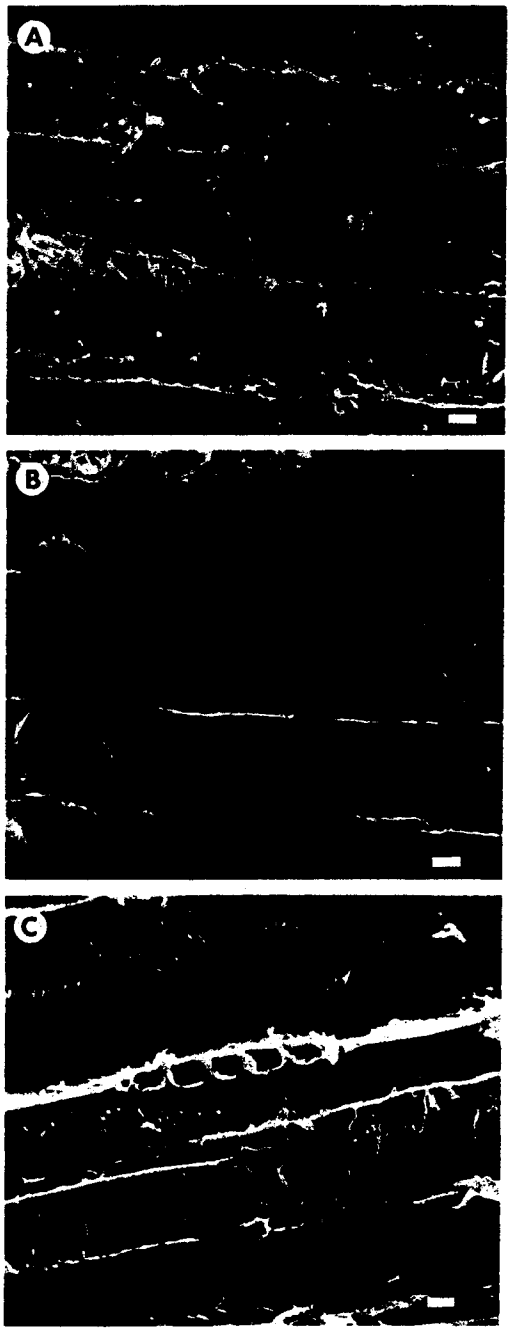

FIG. 2. SEM of wood samples from the study log. (A) Exposed side of a surface scraping, (B) underside of a surface scraping, and (C) core sample from a 25-cm depth. Bars, 10 μm.

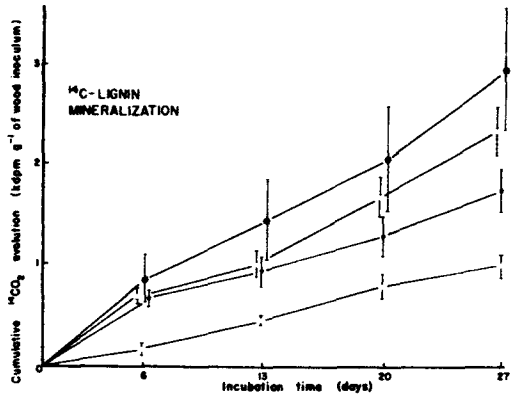

FIG. 3. Effects of various amounts of [^{14}C-lignin]-lignocellulose on mineralization rates with a fixed quantity of wood inoculum. Symbols: ○, 10-kdpm addition; ★, 20-kdpm addition; □, 30-kdpm addition; *, 40-kdpm addition. Each point is the mean of five replicates. Bars, Standard errors of the means.

priate amounts of radiolabel added to incubation treatments. Generally accepted amounts (10 to 30 kdpm per treatment) may result in suboptimal mineralization rates, depending on the type and amount of inoculum added. Results of an experiment illustrating the effect of various amounts of labeled substrate with a fixed quantity of inoculum are presented in Fig. 3. Increasing rates of [^{14}C-lignin]lignocellulose mineralization were evident with increasing ^{14}C substrate additions. Significant differences were observed between the 10-kdpm addition and the other three treatments.

Supplementation of the incubation treatments with either inorganic or organic nitrogen sources resulted in [^{14}C]lignocelluloses labeled in the cellulose fraction being mineralized to $^{14}CO_2$ at rates three- to ninefold faster than those of the [^{14}C]lignin fraction (Fig. 4). Organic and inorganic nitrogen supplements affected the rates of mineralization of the [^{14}C]lignin more than [^{14}C]cellulose mineralization rates when compared with samples incubated in either distilled water or nitrogenless mineral salts solution. Glucose had a substantial inhibitory effect on [^{14}C]cellulose mineralization and less of an effect on [^{14}C]lignin mineralization. Incubation with the nitrogenless mineral salts solution was only slightly more favorable than with distilled water for [^{14}C]lignin mineralization and resulted in no difference for the [^{14}C]cellulose treatment. The greatest stimulation of degradation was due to the addition of $(NH_4)_2SO_4$, which increased the [^{14}C]lignin and [^{14}C]cellulose mineralization rates by factors of 12 and 5, respectively, above that of the distilled water control.

Incubation of a different set of wood samples with [^{14}C]lignocelluloses in the presence of three different forms of inorganic nitrogen, $(NH_4)_2SO_4$, KNO_3, and NH_4NO_3, again demonstrated substantial increases in the rates of mineralization (Fig. 5). [^{14}C]cellulose was mineralized to $^{14}CO_2$ at rates four to six times those of [^{14}C]lignin mineralization. Here, in contrast to the previous experiment, rates of [^{14}C]cellulose mineralization were enhanced more than the lignin rates by medium supplements when compared with incubation in mineral salts solution alone. Of particular interest is the observation that the KNO_3 addition was the most favorable for [^{14}C]lignin decomposition, whereas all three inorganic nitrogen supplements were equally favorable to [^{14}C]cellulose decomposition. Addition of NH_4NO_3 resulted in [^{14}C]lignin mineralization rates similar to those of the $(NH_4)_2SO_4$ treatment.

DISCUSSION

Degradation of [^{14}C]lignocelluloses has been shown by this study to be a sensitive and useful

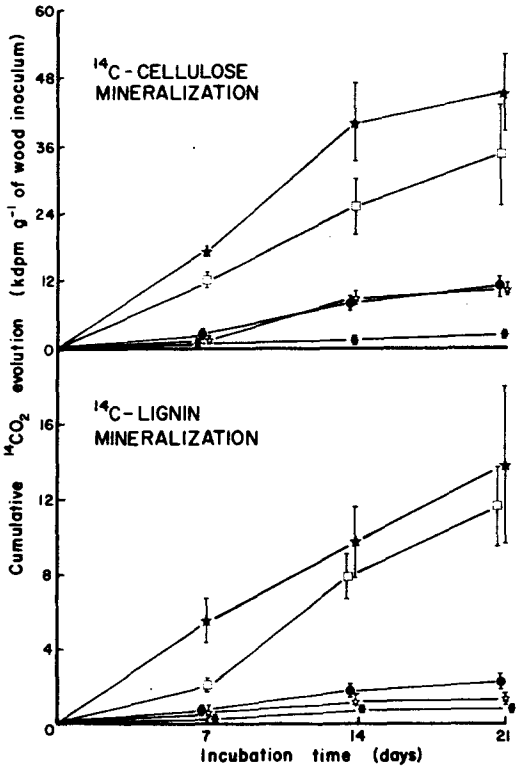

FIG. 4. Effects of medium supplements on [^{14}C]lignocellulose mineralization. Symbols: ☆, distilled water alone; ●, mineral salts solution; *, salts plus glucose; □, salts plus yeast extract; ★, salts plus $(NH_4)_2SO_4$. Each point is the mean of five replicates. Bars, Standard errors of the means.

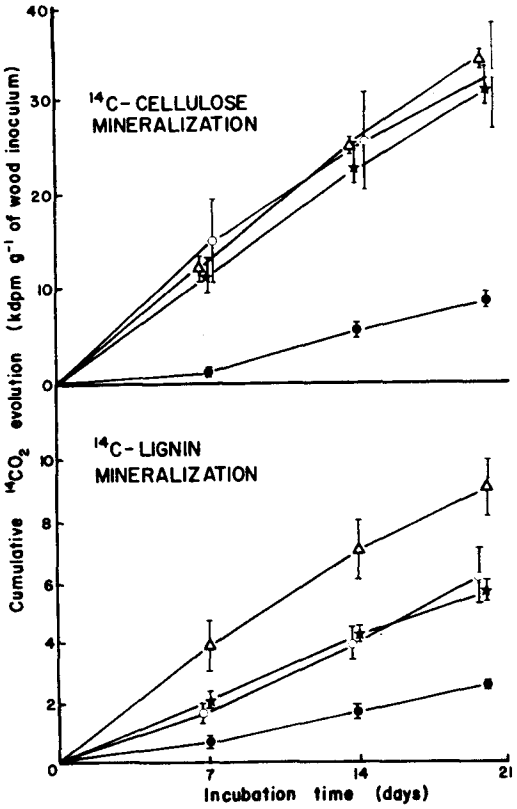

FIG. 5. Effects of various inorganic nitrogen species on [^{14}C]lignocellulose mineralization. Symbols: ●, mineral salts solution; ★, salts plus $(NH_4)_2SO_4$; ○, salts plus NH_4NO_3; △, salts plus KNO_3. Each point is the mean of five replicates. Bars, Standard errors of the means.

tool for assessing relative patterns of lignocellulose decay and microbial activity in wood. Application of this technique should prove particularly attractive to those interested in studying wood decay when it is compared with conventional weight loss studies. Measurements of wood decomposition by substrate weight loss may require many months of substrate incubation and provide no information on decay rates of specific chemical components.

Before the radiotracer technique is adopted for use in a particular experimental system, however, it is essential that a thorough chemical characterization of the [^{14}C]lignocellulose be obtained. For example, degradation of small amounts of contaminating ^{14}C-labeled protein residing in the lignin label could result in nonlignin-derived ^{14}C being evolved, leading to an overestimate of lignin-degrading capability. The quantities of ^{14}C-labeled protein contained in the lignin label used here were low (≤5%) and were well within ranges reported by other investigators using Douglas fir [^{14}C]lignocelluloses (5, 11). The results of Klason and carbohydrate analyses of the labeled material and the ratios between lignin and cellulose mineralization rates were also similar to other values reported in the literature and indicate that evolution of $^{14}CO_2$ from the treatments represents lignocellulose mineralization (5, 7, 10, 11, 25).

Rates of in vivo [^{14}C]lignin decomposition were maximized so that the effects of physical and chemical manipulations could be more readily measured. This was first accomplished by varying the amount of ^{14}C label with a fixed inoculum size (Fig. 3). Even though the 30- and 40-kdpm additions were slightly more favorable than the 20-kdpm addition by week 4 of incubation, the latter amount was selected to conserve labeled substrate and to restrict incubations to a maximum of 3 weeks. The appropriate level of ^{14}C substrate addition should be determined for all applications of the described technique and, surprisingly, has only recently been considered in the literature (3).

Degradation of [^{14}C]lignocelluloses, plate counts, and SEM all demonstrated that microbial colonization on the log was mainly a surface phenomenon. This supports suggestions in the literature that microbial activity is generally restricted to the surface of decomposing wood in aquatic environments (2, 12, 32, 36). The observed pattern of surface-related microbial activity probably reflects the lack of gallery-forming insect activity in aquatic wood, a factor considered a potentially important microbial distribution mechanism in logs decomposing in terrestrial environments (2, 12). Waterlogging and the absence of tunneling insects may limit the access of oxygen into the log, which is a requirement for significant microbial breakdown of natural lignin (9, 38). Oxygen limitation would also restrict the growth of the hyphae of aerobic lignin-degrading fungi into the inner parts of the wood (24). Restriction of microbial activity to the surface of aquatic wood results in extremely slow rates of decomposition given the small surface area-to-volume ratio of large logs and the recalcitrant nature of lignocellulose.

Though significant [^{14}C]lignocellulose decay occurred during incubation in distilled water, rates of breakdown were greatly enhanced by supplementation of media with inorganic nitrogen (Fig. 4). The observation that NO_3^- N enrichment enhanced [^{14}C]lignin degradation more so than did $(NH_4)_2SO_4$ or NH_4NO_3 is particularly worthy of note. Preliminary experimental evidence obtained by us suggests that nitrate ammonification may be occurring in the KNO_3-enriched incubation treatment (data not published). This observation warrants further study to determine whether it is related to the

favorable effect of NO_3^- N addition on lignin degradation. When NO_3^- N is supplied in combination with NH_4^+ N, however, [^{14}C]lignin mineralization rates are similar to those of the $(NH_4)_2SO_4$ treatment alone, suggesting that a classical repression of NO_3^- metabolism by NH_4^+ may be in effect (35).

In contrast to the stimulatory effects of nitrogen supplementation, the repression resulting from glucose addition suggests that the microbial community present in decaying wood will utilize more favorable carbon and energy sources when they are available. To know whether or not this repressive effect is directly on the ligninolytic community per se or indirectly on other members of the community requires further study. Repression of lignocellulose mineralization by simple sugars has been noted before, in both environmental samples and pure culture work (1, 34).

There are few published results of studies concerning the effect of nutrient amendments on [^{14}C]lignocellulose decomposition in natural samples, and none exist for wood substrates to the best of our knowledge. Positive correlations have been observed between synthetic [^{14}C]lignin degradation rates and NO_3^- N concentrations in sediments, and additions of nitrogen to arctic lake sediments enhanced [^{14}C]cellulose mineralization and had no effect on [^{14}C]lignin decay (14, 18). Pure culture work on the physiology of white-rot fungi and lignin-degrading actinomycetes also show contrasting effects of nitrogen amendments (4, 22, 24, 30). In nonradioactively labeled decomposition studies, however, several investigators report that added N can stimulate wood decomposition by fungi (15, 20, 33).

The enhancement of lignocellulose degradation by mineral nitrogen supplementation reported here suggests that nitrogen limitation may exist in decomposing wood in Pacific Northwest streams. Nitrogen concentrations in wood are usually low, with faster decay rates correlating with higher nitrogen content of the woody tissue (28). Even in the later stages of decomposition when nitrogen content has been shown to increase, it may not be in a form available for use by microorganisms (29, 31). These observations indicate that the microbiota on decaying wood should respond favorably to an external source of N. Streamwater sources of nitrogen are low in lotic ecosystems of the Pacific Northwest where nitrogen limitation has been observed (37). This could be responsible for the extremely slow biological processing rates of wood in these natural environments. Obviously, further studies in situ will need to be carried out.

Other nutritionally important mineral elements do not appear to be limiting to the degradation process, as evidenced by the lack of substantial increase in ^{14}C mineralization rates in the presence of a nitrogenless complete mineral salts and trace elements source (Fig. 4). Correlative studies in other stream ecosystems, however, have suggested that decay rates of wood may respond to increased phosphorus concentrations in streamwater (27) and to the initial quality of the substrate (16, 26).

Results from this study have demonstrated the applicability of the [^{14}C]lignocellulose technique in assessing the activity of lignocellulolytic microbial communities in decaying wood. Now that optimal conditions have been established for laboratory mineralization studies, this technique can be used in conjuction with other methodologies to further characterize the microbial communities involved in wood decay in the stream environment and the physicochemical and nutritional factors that affect these rates of activity.

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