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Microbial Decomposition of Synthetic ^{14}C-Labeled Lignins in Nature: Lignin Biodegradation in a Variety of Natural Materials

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Lignin biodegradation in a variety of natural materials was examined using specifically labeled synthetic ^{14}C-lignins. Natural materials included soils, sediments, silage, steer bedding, and rumen contents. Both aerobic and anaerobic incubations were used. No ^{14}C-labeled lignin biodegradation to labeled gaseous products under anaerobic conditions was observed. Aerobic ^{14}C-labeled lignin mineralization varied with respect to type of natural material used, site, soil type and horizon, and temperature. The greatest observed degradation occurred in a soil from Yellowstone National Park and amounted to over 42% conversion of total radioactivity to $^{14}CO_2$ during 78 days of incubation. Amounts of ^{14}C-labeled lignin mineralization in Wisconsin soils and sediments were significantly correlated with organic carbon, organic nitrogen, nitrate nitrogen, exchangeable calcium, and exchangeable potassium.

Lignin is a major structural polymer of vacuolar plants and functions to impart rigidity to cell walls, decrease water permeation across cell walls of xylem tissue, and impede microbiological invasion of plant tissues (21). Lignin, the second most abundant naturally occurring organic polymer (11), represents a significant component of the carbon cycle. Lignin also serves to regulate the cycling of other carbonaceous compounds, such as plant proteins and polysaccharides, by limiting the digestibility of these materials (12).

The structure and biosynthesis of lignins have been elucidated (8, 21), but the microbiological and biochemical aspects of lignin biodegradation have yet to be well defined (16). Efforts to clarify the biodegradation process have been hindered by the lack of a representative substrate and a specific and sensitive assay for lignin biodegradation (17). Three major methods for monitoring lignin biodegradation have been described. One approach involves the analysis of physicochemical changes that occur during the microbial degradation of lignins extracted from unlabeled plant materials (28). Another approach utilizes lignocelluloses extracted from radiolabeled plant materials (6, 10). A third approach utilizes synthetic lignins ("dehydrogenative polymerizates") specifically labeled with ^{14}C in aromatic nuclei, propyl side chains, or methoxyl groups of phenylpropane units of the polymer (10, 17). These synthetic lignins are dehydrogenative polymers of specifically labeled coniferyl alcohol and are considered to be useful model polymers for lignin biodegradation studies. We describe here the biodegradation of these chemically characterized (17) synthetic lignins during incubation with a variety of natural materials.

MATERIALS AND METHODS

Description of sampling sites. Samples of natural materials were taken from sites in the University of Wisconsin Arboretum, Lake Mendota sediment, Yellowstone National Park, Mexico, and Central America. Rumen contents, silage, and stacked steer bedding were also collected.

(i) University of Wisconsin Arboretum sites. Five sites, which included three forest sites, a fen site, and a prairie site, were chosen for ^{14}C-labeled lignin biodegradation studies. The soil profiles of these sites were previously characterized by soil scientists (25) with respect to texture, color, soil type, and parent material. One of the three forests, Noe Woods, is an oak-hickory forest with a well-drained Dodge silt loam soil. The Black Cherry and Aspen Woods covers a well-drained Juneau silt loam, which overlies a poorly drained Kibbie silt loam. Wingra Woods is an oak-hickory forest with a well-drained Dodge loam soil. Wingra Fen has a lake plain physiography and a poorly drained Adrian muck soil. Curtis Prairie has a native prairie vegetation and a well-drained Dodge silt loam soil type. Other Arboretum sites included a site near a rotting log in Wingra Woods, a site in a conifer stand, and a site in Teal Pond, a shallow aquatic environment.

(ii) **Other sites.** Lake Mendota sediment was collected off Picnic Point under 18 m of water. This portion of Lake Mendota has been studied by several investigators (5, 29). Silage samples were obtained from the University of Wisconsin Electric Farm. Two-year-old stacked steer bedding was obtained from a barn in Sun Prairie, Wis. Samples of rumen contents were taken from fistulated cattle of the University of Wisconsin herd. Soil samples from a 25°C site and a 35°C site in the lower geyser basin of Yellowstone National Park were collected. Grab samples of soils from Mexico, Costa Rica, and Guatemala were also utilized in this study.

Sampling procedures. Soils and sediment collected in the University of Wisconsin Arboretum were sampled with a manual soil corer capable of taking 50- by 2.5-cm cores. Cores from sites with known soil profiles were divided according to horizon; soil from each horizon was separately homogenized manually. All other natural materials were collected as surface samples. Samples remained moist throughout handling. Lake Mendota sediment was collected by dredge sampling as described by Zeikus and Winfrey (29). All samples assayed for anaerobic ^{14}C-labeled lignin biodegradation were collected and manipulated anaerobically by using the technique described by Hungate (13), modified for collection and manipulation procedures. Samples of natural materials from Wisconsin, Mexico, and Central America were collected in mid-June. Yellowstone soils were collected in mid-July.

Analysis of soils. Determinations of soil pH were made by a glass-electrode method (23). The following standard analytical procedures were used for the chemical analyses of Wisconsin soils and sediments: organic N (4), nitrate N (15), total organic carbon (26), and exchangeable Na, Mg, Ca, and K (23).

^{14}C-labeled lignin biodegradation assay. Synthetic ^{14}C-labeled lignins, specifically labeled in alkyl (β and γ carbons of the propyl side chain), aryl (aromatic nuclei), or methoxyl positions, were prepared and characterized as described previously (17). Specific activities of ^{14}C-labeled lignins were determined by scintillation counting in 10 ml of a solution containing 10.0 g of PPO (2,5-diphenyloxazole), 250 mg of dimethyl-POPOP {1,4-bis [2-(4-methyl-5-phenyloxazoly)]benzene} and 100.0 g of naphthalene per liter of 1,4-dioxane. Scintillation counting was performed using Packard Tri-Carb 3003 and 3375 scintillation spectrophotometers. Counting efficiencies were determined by automatic external standardization, internal standardization with [^{14}C]toluene, and channels ratio procedures. All data were corrected for background and efficiency. Specific activities for ^{14}C-labeled lignins used in this study were as follows: alkyl-^{14}C-labeled lignin, 216,000 dpm/mg; aryl-^{14}C-labeled lignin, 93,300 dpm/mg; and methoxyl-^{14}C-labeled lignin, 100,400 dpm/mg.

Synthetic ^{14}C-labeled lignins were incubated with natural materials in glass vessels under aerobic or anaerobic conditions. Natural materials, except for Central American, Mexican, and Yellowstone samples, were collected and utilized the same day. All natural materials except rumen contents were incubated with 1.60 mg of aryl-^{14}C-labeled lignin (149,300 dpm) under aerobic conditions. With the exceptions of the two Yellowstone soils and rumen contents, natural materials were incubated at 30°C. Yellowstone soils were incubated at their in situ temperatures of 25 or 35°C. Rumen contents were incubated with aryl-^{14}C-labeled lignin (1.60 mg; 149,300 dpm), alkyl-^{14}C-labeled lignin (0.78 mg; 168,500 dpm), and methoxyl-^{14}C-labeled lignin (1.07 mg; 107,400 dpm) anaerobically, without added nitrate, at 37°C. Silage, steer bedding, and sediments were also incubated anaerobically, with and without added nitrate, at 30°C. Lake Mendota sediment was incubated aerobically and anaerobically at both 30 and 20°C (in situ temperature). Wisconsin samples were incubated for 41 days aerobically or for 60 days when incubated anaerobically. Mexican and Central American samples were incubated for 30 days, and Yellowstone samples were incubated for 78 days.

Results of ^{14}C-labeled lignin biodegradation experiments described here are reproducible with natural materials obtained from the same site at the same time of year. Percent standard deviations of the mean for replicate studies were usually less than 10% and always less than 20%. Results presented for natural materials from Wisconsin are representative of results obtained over a 2-year period.

Aerobic incubation procedure. One gram (wet weight) of natural material was incubated with 0.5 ml of sterile glass-distilled water and ^{14}C-labeled lignin in 20-ml serum-stoppered vials. Vials were incubated without shaking, in the dark. At approximately 3-day intervals, vials were flushed for 5 min with sterile humidified CO_2-free air (10 ml/min). $^{14}CO_2$ flushed from vials was trapped directly in 10 ml of a scintillation solution that contained 5 ml of an ethanolamine-anhydrous methanol solution (1:4, vol/vol) and 5 ml of a toluene cocktail containing 4.0 g of PPO and 0.1 g of POPOP {*p*-bis-[2-(5-phenyloxazolyl)]-benzene} per liter of toluene. At the end of incubation, samples were acidified with 2 ml of 4 N H_2SO_4 and then flushed to release $^{14}CO_2$ dissolved as bicarbonate. Controls were prepared by autoclaving natural material at 15 lb/in^2 for 1 h and adding 1 ml of formalin (37% formaldehyde) to the natural material prior to the addition of ^{14}C-labeled lignins.

The aerobic procedure was shown to be quantitative for atmospheric $^{14}CO_2$ by the use of an acidified $NaH^{14}CO_3$ standard. Double-trapping experiments showed that all $^{14}CO_2$ was contained in the first trap. In all cases of aerobic incubation, $^{14}CO_2$ was contained in the first trap. In all cases of aerobic incubation, $^{14}CO_2$ was identified by gas chromatography and gas proportional counting (20) as the only detected radioactive product in the gas phase. The continued presence of oxygen in incubation vials between flushings was demonstrated by gas chromatography. Extraction studies of soils incubated with ^{14}C-labeled lignins revealed that loss of $^{14}CO_2$ during incubation was negligible; most residual radioactivity was recoverable by extraction. All data from aerobic incubations were corrected for background and efficiency (determined by automatic external standardization). When plots of total $^{14}CO_2$ trapped (percentage of total radioactivity) as a func-

tion of incubation time were nonlinear, data were presented graphically. When $^{14}CO_2$ evolution was a linear function of incubation time, linear regression by the method of least squares was used to fit experimental data. The slopes of the lines thus obtained were the rates of mineralization of total radioactivity, in units of percent per day.

Anaerobic incubation procedure. The anaerobic procedure involved a serum bottle modification of the Hungate technique for the cultivation of obligate anaerobes (19). One gram (wet weight) of natural material was incubated with ^{14}C-labeled lignin in each 5-ml serum-stoppered vial. One milliliter of sterile glass-distilled water or 1.0% $NaNO_3$ solution was added to each vial to allow a 4-ml gas space. Vials were gassed with N_2 and incubated without shaking, in the dark. At weekly intervals, 0.4 cm^3 of atmosphere was withdrawn from each vial and analyzed for the presence of $^{14}CO_2$ and/or $^{14}CH_4$ by gas chromatography and gas proportional counting, as described by Nelson and Zeikus (20). The conversion of 0.1% of the total radioactivity of ^{14}C-labeled lignins to labeled gases was readily detectable with this procedure. Controls that contained natural material, ^{14}C-labeled lignin, 10% formalin (vol/vol, final concentration), and 1% chloroform (vol/vol, final concentration) were used.

Radioactive chemicals. The synthesis and chemical characterization of specifically labeled ^{14}C-lignins used in this study has been described (17). Other radioactive compounds used were a $NaH^{14}CO_3$ standard (specific activity, 687 μCi/mg; Amersham/Searle) and a [^{14}C]toluene standard (specific activity, 4.24×10^5 dpm/ml; New England Nuclear Corp).

RESULTS

Chemical characterization of sampling sites. Wisconsin soils and sediments examined for lignin biodegradation varied with respect to pH, organic carbon, organic nitrogen, nitrate, and soil cations present (Table 1). Numerical values were determined on a wet-weight basis. Organic C/N ratios from 4.93 to 36.88. Yellowstone soil from the 25°C site had a pH of 7.2 and contained 8.10% organic carbon on a dry-weight basis. Yellowstone soil from the 35°C site had a ph of 8.3 and contained 31.94% organic carbon on a dry-weight basis. Other natural materials used in this study were not chemically characterized.

Biodegradation of ^{14}C-labeled lignins in selected natural materials. Results of ^{14}C-la-

TABLE 1. *Chemical analyses of Wisconsin soils and sediments*

Depth (cm)	Horizon	pH	% Solids	% Organic N	μg of NO_3-N/g	% Total organic C	Organic C/organic N	kg/hectare			
								Ca	Mg	Na	K
Noe Woods											
0-7	A1	6.3	54.5	0.177	16.13	2.79	15.75	31,258	5,708	299	1,875
7-15	A21	5.1	80.5	0.109	9.20	1.66	15.15	6,523	2,174	217	897
15-30	A22	4.9	82.5	0.050	3.22	0.59	12.00	6,523	2,174	217	1,033
Wingra Woods											
0-14	A1	5.1	74.0	0.132	9.03	2.79	8.27	7,339	1,631	163	1,441
14-33	A2	4.4	82.5	0.045	0.42	0.76	11.30	2,174	1,740	174	1,169
Soil near rotting log											
0-20		5.1	51.0	0.175	1.79	6.45	36.88	19,027	1,903	190	2,582
Teal Pond											
0-10 (under 10 cm of water)		6.4	63.9	0.211	3.20	1.32	13.06	36,694	10,872	1087	1,903
Conifer Stand											
0-15		5.5	76.9	0.117	8.84	1.58	13.55	10,872	2,174	217	1,033
Lake Mendota sediment											
0-8 (under 18 m of water)		7.2	20.0	0.109	3.40	1.36	12.39	57,080	5,164	516	1,196
Black Cherry and Aspen Woods											
0-5	A11+	6.1	82.5	0.165	8.42	2.10	12.70	13,591	2,174	217	1,359
5-20	A12+	5.6	88.1	0.079	2.03	0.93	11.67	9,513	1,740	174	897
20-45	A13 & IIA1b	5.8	90.8	0.062	3.27	0.65	10.59	9,242	1,740	174	652
Wingra Fen											
0-20	Oa1	6.5	66.9	0.867	43.28	12.07	13.92	64,147	3,262	326	2,718
20-25	Oa2	6.5	58.3	0.792	23.26	11.23	14.19	86,979	3,534	353	1,631
25-50	Oa3	6.8	80.3	0.177	−0.01	0.88	4.93	29,899	3,262	326	2,365
West Curtis Prairie											
0-15	Ap	5.7	75.9	0.108	1.60	1.12	10.42	10,601	1,903	190	1,142
15-28	A2	5.5	81.6	0.047	−0.01	0.50	10.52	16,309	2,718	272	1,631
28-36	B1	5.4	81.6	0.033	1.63	0.56	17.25	21,201	3,262	326	2,446

beled lignin biodegradation studies for several natural materials are displayed in Table 2. No mineralization of ^{14}C-labeled lignin under anaerobic conditions was observed, although unlabeled gaseous products of anaerobic metabolism were produced. The production of CO_2 and/or CH_4 indicated that an active microflora was present. Sediments and steer bedding evolved methane when incubated in the absence, but not in the presence, of nitrate. Rumen contents evolved unlabeled CO_2 and CH_4, but neither $^{14}CO_2$ nor $^{14}CH_4$ was produced during incubation with alkyl-, aryl-, or methoxyl-^{14}C-labeled lignin. Biodegradation of ^{14}C-labeled lignins to labeled gaseous products did not occur in silage incubated anaerobically or aerobically, although unlabeled CO_2 was evolved from silage under both aerobic and anaerobic conditions. Controls showed no ^{14}C-labeled lignin degradation to gaseous products.

Total $^{14}CO_2$ evolved from ^{14}C-labeled lignin plotted as a function of incubation time was linear for all aerobic incubations shown in Table 2, except for steer bedding. $^{14}CO_2$ evolution from aerobically incubated steer bedding during the first 12 days amounted to 0.7% of the total radioactivity present. After 12 days, ^{14}C-labeled lignin mineralization occurred at a reduced rate, and an additional 0.3% of the total radioactivity was converted to $^{14}CO_2$. The mineralization of ^{14}C-labeled lignin to $^{14}CO_2$ occurred in all aerobically incubated natural materials with the exception of silage, but rates of mineralization were slow. Controls incubated aerobically exhibited no $^{14}CO_2$ evolution.

The effect of aerobicity upon ^{14}C-labeled lignin mineralization was most evident during aerobic incubation of sediments. Although the two sediments studied exhibited no observed ^{14}C-labeled lignin mineralization under anaerobic conditions, both exhibited conversion of ^{14}C-labeled lignin to $^{14}CO_2$ under aerobic conditions. Lake Mendota sediment incubated aerobically showed a temperature-dependent conversion of ^{14}C-labeled lignin to $^{14}CO_2$ (Table 2). Greater mineralization occurred during incubation at 30°C than at 20°C, the in situ temperature.

Effect of soil site and horizon on ^{14}C-labeled lignin mineralization. The biodegradation of aryl-^{14}C-labeled lignin to $^{14}CO_2$ in soils from five University of Wisconsin Arboretum sites varied with soil site and horizon. Total $^{14}CO_2$ evolution as a function of incubation time was linear for horizons of four sites, but rates of ^{14}C-labeled lignin mineralization varied considerably (Table 3). Although ^{14}C-labeled lignin

TABLE 3. *Effect of soil site and horizon on aryl-^{14}C-labeled lignin biodegradation*

Site	Horizon	% of total radioactivity converted to $^{14}CO_2$ (41 days)	Rate of mineralization (%/day)
Black Cherry and Aspen Woods	A11+	5.1	0.114
	A12+	3.6	0.080
	A13 & IIA1b	4.0	0.094
West Curtis Prairie	Ap	5.3	0.125
	A2	3.1	0.079
	B1	9.3	0.208
Noe Woods	A1	7.3	0.167
	A21	6.0	0.139
	A22	3.4	0.083
Wingra Woods	A1	3.3	0.079
	A2	4.2	0.099

TABLE 2. *^{14}C-labeled lignin biodegradation in selected natural materials*

Sample	Label position[a]	Incubation temp (°C)	Aerobic/anaerobic	% of total radioactivity converted to $^{14}CO_2$ and/or $^{14}CH_4$ (41 days)	Rate of mineralization (%/day)
Soil near a rotting log (0 to 20 cm)	r	30	Aerobic	3.7	0.077
Teal Pond sediment (0 to 10 cm under 10 cm of water)	r	30	Anaerobic ± nitrate	None observed	
	r	30	aerobic	9.2	0.204
Lake Mendota sediment (0 to 8 cm under 18 m of water)	r	20, 30	Anaerobic ± nitrate	None observed	
	r	20, 30	aerobic	3.3, 5.6	0.075, 0.125
Stacked steer bedding	r	30	Anaerobic ± nitrate	None observed	
	r	30	aerobic	1.0	
Rumen contents	r, s, m	37	Anaerobic − nitrate	None observed	
Soil from conifer stand (0 to 15 cm)	r	30	Aerobic	3.7	0.084

[a] r, Aryl; s, alkyl; m, methoxyl.

mineralization varied with site and horizon, mineralization did not always decrease with increased soil depth.

^{14}C-labeled lignin mineralization was not linear with time in incubations of soil from Wingra Fen horizons (Fig. 1). The Oa1 horizon soil showed the greatest total conversion of aryl-^{14}C-labeled lignin to $^{14}CO_2$ (22.2%) of any Wisconsin soil examined. The Oa2 and Oa3 horizons of this site exhibited 7.9 and 7.5% mineralization, respectively.

Chemical parameters correlated with ^{14}C-labeled lignin mineralization in Wisconsin soils and sediments. In soils from Noe Woods and Black Cherry and Aspen Woods, ^{14}C-labeled lignin mineralization was significantly linearly correlated with chemical parameters of soil horizons within the individual sites themselves. Linear correlation coefficients (r) were determined after 15, 30, and 41 days of incubation using soil chemical parameters determined for horizons of these individual sites (Table 1) as independent variables and percentage of conversion of total radioactivity to trapped $^{14}CO_2$ as the dependent variable. The number of values (n) for each independent variable is equal to the number of horizons in each site, since one numerical value for each chemical parameter (e.g., nitrate N, organic C, etc.) was obtained for each horizon. Several chemical parameters were significantly correlated at the 99% ($n = 3$, $r \geq 0.990$) or 95% ($n = 3$, $r \geq 0.950$) confidence levels with ^{14}C-labeled lignin mineralization in the horizons of the Noe Woods and Black Cherry and Aspen Woods sites. In the Noe Woods site, these parameters included organic N ($r = 0.993$ at 15 days, $r = 0.983$ at 30 days, and $r = 0.971$ at 41 days), nitrate N ($r = 0.993$, 0.983, and 0.971, respectively), organic C ($r = 0.996$, 0.987, and 0.977), and organic C/N ($r = 0.956$, 0.973, and 0.985). In the Black Cherry and Aspen Woods site, chemical parameters significantly correlated with ^{14}C-labeled lignin mineralization included nitrate N ($r = 0.983$, 0.991, and 0.998), exchangeable magnesium ($r = 1.000$, 0.951, and 0.971), and exchangeable sodium ($r = 1.000$, 0.951, and 0.971).

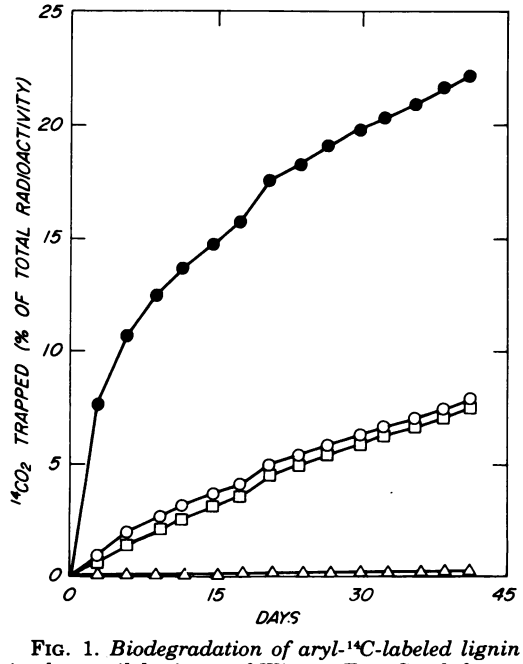

FIG. 1. *Biodegradation of aryl-^{14}C-labeled lignin in three soil horizons of Wingra Fen. Symbols: ●, Oa1 horizon; ○, Oa2 horizon; □, Oa3 horizon; △, controls.*

Linear correlation coefficients were also determined after 15, 30, and 41 days using chemical parameters for all 18 horizons and sites ($n = 18$) shown in Table 1 as independent variables and percentage of conversion of total radioactivity to $^{14}CO_2$ as the dependent variable. Five chemical parameters were significantly correlated at the 99% ($n = 18$, $r \geq 0.575$) or 95% ($n = 18$, $r \geq 0.456$) confidence levels with ^{14}C-labeled lignin mineralization in all aerobic 30°C incubations of Wisconsin soils and sediments considered together. These five parameters presented with linear correlation coefficients for 15, 30, and 41 days were: organic N ($r = 0.775$, 0.764, and 0.756), nitrate N ($r = 0.844$, 0.817, and 0.801), organic C ($r = 0.693$, 0.663, and 0.648), exchangeable potassium ($r = 0.558$, 0.604, and 0.618), and exchangeable calcium ($r = 0.574$, 0.576, and 0.611). No significant linear correlations of soil pH (or H^+ concentration) or organic C/N with ^{14}C-labeled lignin mineralization were found when all 18 Wisconsin soils and sediments were considered together.

^{14}C-labeled lignin biodegradation in other soils. The mineralization of synthetic aryl-^{14}C-labeled lignin in two soils from the lower geyser basin of Yellowstone National Park is shown in Fig. 2. The 25°C, pH 7.2 soil exhibited 8.8% conversion of total radioactivity to $^{14}CO_2$ after 78 days of incubation. The 35°C, pH 8.3 soil exhibited 42.4% conversion, the greatest percentage observed in this study. Mineralization of ^{14}C-labeled lignin in 10 Mexican and Central American soils is shown in Fig. 3. Neither the rates nor the extents of ^{14}C-labeled lignin mineralization in these soils were consistently greater than those of temperate soils.

DISCUSSION

The absence of observed ^{14}C-labeled lignin biodegradation to labeled gaseous decomposi-

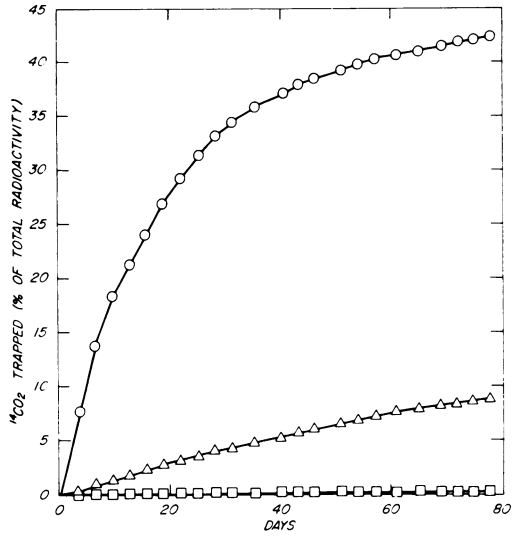

FIG. 2. *Biodegradation of aryl-^{14}C-labeled lignin in two Yellowstone National Park soils. Symbols: ○, 35°C Yellowstone soil (pH 8.3); △, 25°C Yellowstone soil (pH 7.2); □, controls.*

tion products during anaerobic incubation may have profound environmental implications. The gradual accumulation in anaerobic environments of lignin and lignin-derived materials over extended periods of time might form the basis for peat and coal deposits (24). The observed degradation of ^{14}C-labeled lignin to $^{14}CO_2$ in aerobically incubated Lake Mendota sediments implies that lignin degradation in lake sediments could occur when molecular oxygen is present, as occurs during and after lake turnovers. Mineralization of ^{14}C-labeled lignin in aerobically but not anaerobically incubated Teal Pond sediment suggests that lignin degradation to CO_2 in organic-rich, transient, shallow aquatic environments might occur on a seasonal basis, when such environments dry and sediments are exposed to oxygen. Anaerobic incubations of rumen contents with alkyl-, aryl-, and methoxyl-^{14}C-labeled lignins indicate that conversion of various portions of the lignin molecule to CO_2 or CH_4 does not occur anaerobically in this natural material. These observations are in agreement with reports that increased lignin content lowers the nutritional value of forage (12).

Aerobic ^{14}C-labeled lignin mineralization in soil horizons of five University of Wisconsin Arboretum sites varied with site, soil type, and soil horizon. Mineralization did not always decrease with increased soil depth. In Noe Woods and the Black Cherry and Aspen Woods, the extent of ^{14}C-labeled lignin mineralization cor-

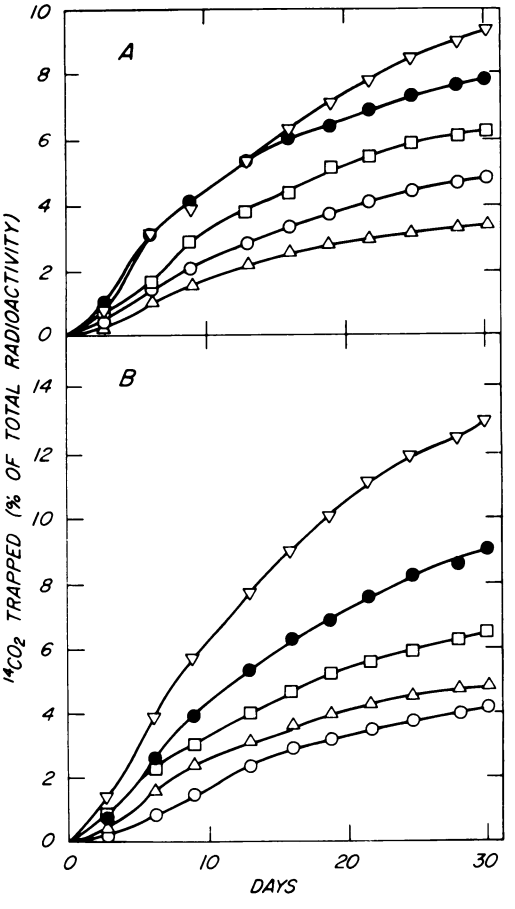

FIG. 3. *Biodegradation of aryl-^{14}C-labeled lignin in 10 Mexican and Central American soils. Symbols (A): ▽, edge of tropical forest near ruins of Palenque (Chiapas, Mexico); ●, pine forest near Lake Atitlan (Patrizia, Guatemala); □, forest meadow (Turrialba, Costa Rica); ○, tropical forest riverbank (Ritu, Guatemala); △, coffee plantation (Turrialba, Costa Rica). Symbols (B): ▽, beneath leaf litter near Hacienda Uxmal (Yucatan, Mexico); ●, tropical forest hillside near ruins of Palenque (Chiapas, Mexico); □, beneath bombax tree (Turrialba, Costa Rica); △, beneath cedar tree (Turrialba, Costa Rica); ○, unburned pine forest west of Rio Frio (Mexico, Mexico).*

related significantly with chemical parameters of soil horizons in the individual sites themselves, but this was not the case with other sites. Organic C/N, which correlated with ^{14}C-labeled lignin mineralization at the 95% confidence level in horizons of Noe Woods, was not significantly correlated with ^{14}C-labeled mineralization when 18 different Wisconsin soils and sediments were considered. These observations suggest that lignin decomposition in a given

site may be related to a variety of chemical, physical, and biological parameters interacting in a site-specific manner not readily applicable to other individual sites.

When all Wisconsin soils and sediments incubated aerobically at 30°C were considered together, the extent of ^{14}C-labeled lignin mineralization correlated highly with the organic N, nitrate N, organic C, exchangeable Ca^{2+}, and exchangeable K^+ contents of these natural materials. Correlations were significant (99% or 95% confidence level) at 15, 30, and 41 days of incubation. Levels of organic C and organic N may reflect the presence of soil biota and plant-derived materials. Higher levels of ^{14}C-labeled lignin biodegradation in soils and sediments that contain greater amounts of living biomass and decomposing organic materials might be expected. Organic N and particularly nitrate N were highly correlated with ^{14}C-labeled lignin mineralization throughout the course of incubation, but correlation coefficients decreased with time. This finding is in accord with observations that high nitrogen content in litter material promotes litter decomposition, particularly in the early stages (27). Nitrogen supplementation has been shown to cause increased decomposition of hardwoods and softwoods incubated in soil (1, 2). The high positive correlations of ^{14}C-labeled lignin mineralization with exchangeable calcium and potassium ions may reflect the complexing of these ions with soil organic matter. The greater amounts of exchangeable Ca^{2+} than Mg^{2+} and K^+ than Na^+ in soils and sediments chemically analyzed for these ions suggests that Ca^{2+} may predominate over Mg^{2+} in the occupation of divalent cation sites on soil organic matter or clay minerals. Similarly, K^+ may predominate over Na^+ in the occupation of monovalent sites. A predominance of exchangeable Ca^{2+} over Mg^{2+} and of K^+ over Na^+ is common in clay minerals (3) and the binding of clays with soil humic substances is well known (9, 22). Thus, the high positive correlations of ^{14}C-labeled lignin mineralization with exchangeable calcium and potassium might reflect the binding of these cations to organic materials already highly correlated with ^{14}C-labeled lignin biodegradation.

^{14}C-labeled lignin mineralization in non-Wisconsin soils varied widely with respect to extent of mineralization. The extensive biodegradation of ^{14}C-labeled lignin in the 35°C Yellowstone soil may reflect the high in situ temperature and organic content. Central American and Mexican soil incubations did not exhibit consistently greater ^{14}C-labeled lignin mineralization than incubations of temperate soils despite reports (27) of more rapid mineralization of organic matter in tropical soils. These results could reflect the site-specific nature of lignin decomposition, since some Central American and Mexican soils were more active in ^{14}C-labeled lignin mineralization than some temperate soils.

Percentage of mineralization data obtained for synthetic ^{14}C-labeled lignins incubated with natural materials represent minimum values for lignin biodegradation because lignin degradation by the process of humification (7), adsorption of lignin-derived material onto soil colloids (14), and the assimilation of lignin decomposition products by microorganisms were not measured. The results presented here demonstrate that the biodegradation of lignin in nature is a slow process. Amounts of ^{14}C-labeled lignin mineralization reported in this study are in general accord with values obtained by Wojtas-Wasilewska et al. (28) for the model humification of Bjorkman lignin from rye straw. These authors reported approximately 7% weight loss of their lignin preparation after 40 days of incubation and 40% weight loss after 180 days of incubation in soil. Little loss was observed between 180 and 300 days of incubation. Our data are also in agreement with biodegradation data for a variety of ^{14}C-labeled plant materials incubated in soils (14) with respect to amounts of decomposition reported.

^{14}C-labeled lignin mineralization described here is slower that that reported for the lignin component of ^{14}C-labeled lignocelluloses in a recent study by Crawford and Crawford (6). These authors described rather rapid decomposition of ^{14}C-labeled lignocelluloses in shake-flask incubations inoculated with soil. Flasks were incubated with shaking, at 35°C. More efficient aeration due to shaking, generally higher incubation temperatures, and the binding of lignin with carbohydrates in ^{14}C-labeled lignocelluloses might explain the higher rates observed by these authors. Carbohydrates bound to lignin could serve as growth substrates. The apparent requirement for a growth substrate by certain lignin-degrading fungi has been reported (18). Although both extracted ^{14}C-labeled lignocelluloses and synthetic ^{14}C-labeled lignins can serve as useful polymeric models for lignin biodegradation studies, synthetic ^{14}C-labeled lignins are chemically characterized (17) and can be specifically labeled with ^{14}C in known positions in the polymer. Additional characterization of extracted ^{14}C-labeled lignocelluloses is necessary before assuming that no plant-mediated molecular rearrangements of labeled precursors have occurred during attempts to produce specifically labeled lignocelluloses biosynthetically (6).

We (17) have described the desirability of establishing minimum values of lignin mineralization in order to ascribe lignin-degrading activity to a given organism or mixed culture. In the present study, aerobic incubation of ^{14}C-labeled lignin with steer bedding (Table 2) resulted in the mineralization of 0.7% of the total radioactivity present during the first 12 days of incubation, followed by much decreased mineralization throughout the rest of incubation. Such results could indicate the presence of an easily degraded ^{14}C-labeled lignin component. These observations might suggest a minimum value of approximately 0.7% conversion of total radioactivity to $^{14}CO_2$ as an acceptable criterion for lignin biodegradation in this study. This suggestion is in accord with observations (17) that less than 1% of synthetic ^{14}C-labeled lignins consists of molecules smaller than tetramers. The limited degradation of ^{14}C-labeled lignin incubated with steer bedding could conceivably indicate the depletion of a nutrient required by lignin-degrading microorganisms.

In summation, the results presented here indicate the usefulness of synthetic ^{14}C-labeled lignins in studies of lignin biodegradation in natural materials. The extent of ^{14}C-labeled lignin mineralization varied with the nature of the material studied, site, soil type, and horizon. Aerobicity was a critical factor in observed ^{14}C-labeled lignin mineralization. Significant linear correlations of certain chemical parameters with ^{14}C-labeled lignin mineralization suggest that certain chemical factors are related to lignin mineralization generally, but that lignin decomposition in a particular natural environment may be related to a variety of parameters interacting in a manner not readily applicable to other individual environments. Thus, lignin biodegradation should be studied on a site-by-site basis to determine which factors influence lignin decomposition in each site chosen.

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