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Effects of pH on Lignin and Cellulose Degradation by *Streptomyces viridosporus*†

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Lignocellulose degradation by *Streptomyces viridosporus* results in the oxidative depolymerization of lignin and the production of a water-soluble lignin polymer, acid-precipitable polymeric lignin (APPL). The effects of the culture pH on lignin and cellulose metabolism and APPL production by *S. viridosporus* are reported. Dry, ground, hot-water-extracted corn (*Zea mays*) lignocellulose was autoclaved in 1-liter reagent bottles (5 g per bottle) and inoculated with 50-ml volumes of *S. viridosporus* cells suspended in buffers of specific pH (pH 6.0 to 9.2 at 0.4 pH unit intervals). Four replicates of inoculated cultures and of uninoculated controls at each pH were incubated as solid-state fermentations at 37°C. After 6 weeks of incubation the percent loss of lignocellulose, lignin, and carbohydrate and the amount of APPL produced were determined for each replicate. Optimal lignocellulose degradation, as shown by substrate weight loss, was observed in the pH range of 8.4 to 8.8. Only minor differences were seen in the Klason lignin, carbohydrate, protein, and ash contents of the APPLS produced by cultures at each pH. The effects of pH on the degradation of a spruce (*Picea pungens*) [^{14}C-lignin]lignocellulose and a Douglas fir (*Pseudotsuga menziesii*) [^{14}C-glucan]-lignocellulose were also determined at pH values between 6.5 and 9.5 (0.5 pH unit intervals). The incubations were carried out for 3 weeks at 37°C with bubbler-tube cultures. The percentage of initial ^{14}C recovered as $^{14}CO_2$, ^{14}C-labeled water-soluble products, and [^{14}C]APPL was then determined. The mineralization of lignin and cellulose to CO_2 was optimal at pHs 6.5 and 7.0, respectively. However, the optimum for lignin and cellulose solubilization was pH 8.5, which correlated with the pH 8.5 optimum for APPL production. Overall, the data show that, whereas lignin mineralization is optimal at neutral to slightly acidic pHs, lignocellulose degradation with lignin solubilization and APPL production is promoted by alkaline pHs. These findings indicate that lignin-solubilizing actinomycetes may play an important role in the metabolism of lignin in neutral to alkaline soils in which ligninolytic fungi are not highly competitive.

In nature, the aromatic polymer lignin, which is covalently linked to cellulosic polysaccharides in lignocellulosic biomass, is resistant to degradation by most microorganisms. This recalcitrant phenylpropanoid polymer is degraded by a few fungi and filamentous bacteria (5, 7, 10–13, 15). *Streptomyces viridosporus*, a filamentous actinomycete, degrades lignin oxidatively, releasing a water-soluble lignin polymer, acid-precipitable polymeric lignin (APPL), as the initial intermediate of lignin catabolism (9). APPL is released as the major product of lignin degradation by *S. viridosporus* as it solubilizes lignin to gain access to plant polysaccharides (3). APPL is also a lignin degradation intermediate which was shown to be only slowly degraded by other known lignin degrading microorganisms (19).

In this paper we report the effects of initial culture pH on lignin and cellulose degradation by *S. viridosporus* and particularly its effect on the production of the APPL intermediate. The effect of pH on lignocellulose degradation was determined by using 6-week solid-state fermentations of corn lignocellulose and 3-week bubbler-tube cultures of [^{14}C-glucan]- and [^{14}C-lignin]lignocelluloses. The data show that while lignocellulose mineralization is optimal in an initially neutral to slightly acidic medium, lignin solubilization and APPL production are greatest when the initial pH is alkaline. These findings have implications regarding the niche that *S. viridosporus* occupies as a ligninolytic actinomycete in the soil.

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MATERIALS AND METHODS

Organism. Cultures for all experiments were inoculated with spores of *S. viridosporus* T7A (ATCC 39115) (21) from stock slants maintained on yeast extract-malt extract agar kept at 4°C for 2 to 12 weeks (20).

Solid-state fermentation of corn lignocellulose with *S. viridosporus*. Corn (*Zea mays*) stover was dried, ground in a Wiley mill to pass a 40-mesh screen, and then sequentially extracted with hot water, benzene-ethanol (1:1; vol/vol), ethanol, and hot water, after which it was air dried (9, 15). Dry 5-g samples of the lignocellulose were added to 1-liter reagent bottles, which were then autoclaved uncovered for 1 h and again for 15 min after being plugged with cotton. *S. viridosporus* T7A spores were inoculated into 2-liter flasks each containing 1 liter of pH 7.1 to 7.2 sterile 0.6% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) in mineral salts solution containing, per liter of deionized H_2O, 5.3 g of Na_2HPO_4, 1.98 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, 0.05 g of $CaCl_2 \cdot 2H_2O$, and 1 ml of trace elements [20]. After inoculation, cultures were incubated with shaking (100 rpm) at 37°C for 3 days. For harvest, cells were allowed to settle to the flask bottom, and 50-ml (0.1 ± 0.02 g [dry weight] of cells) aliquots of the gravity-concentrated cell suspension were added aseptically to sterile centrifuge bottles, which were then centrifuged at 16,000 × *g* for 30 min. The supernatant was then decanted, and 50 ml of sterile buffered 0.1% (wt/vol) yeast extract medium of specific pH was added. Two buffered media were used: 0.1 M phosphate buffer (13.61 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of

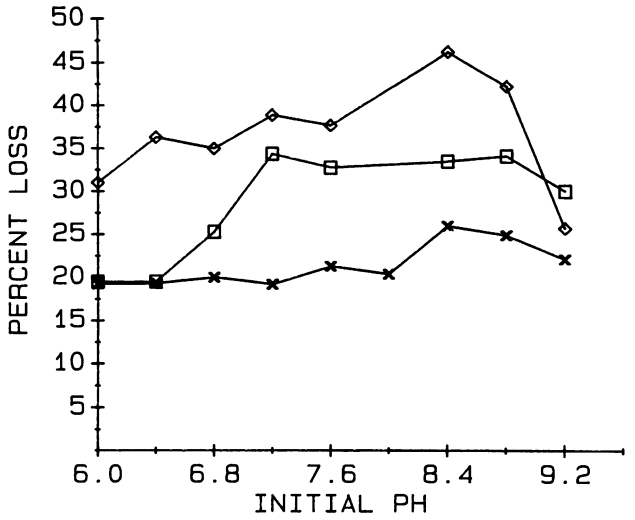

FIG. 1. Net changes for initial lignocellulose in percent weight loss (×), percent carbohydrate loss (◇), and percent lignin loss (□) as a result of corn lignocellulose degradation by *S. viridosporus* at different initial culture pHs after 6 weeks of incubation at 37°C as a solid-state fermentation. All values are the averages of four replicates minus the uninoculated control values, which had initial Klason lignin and carbohydrate contents of 27 and 45%, respectively.

NaCl, and 0.05 g of $CaCl_2 \cdot 2H_2O$ in 1 liter of deionized H_2O adjusted with NaOH to pH 6.0, 6.4, 6.8, 7.2, 7.6, or 8.0), and 0.1 M Tris buffer (12.11 g of Tris, 0.2 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, and 0.05 g of $CaCl_2 \cdot 2H_2O$ in 1 liter of deionized H_2O adjusted with HCl to pH 8.4, 8.8, or 9.2). Each 50-ml buffered cell suspension of specific pH was added to 5 g of sterile corn lignocellulose in a reagent bottle, and the inoculated lignocellulose was spread evenly over the inside wall of the bottle. The bottles were then incubated for 6 weeks at 37°C as solid-state fermentations. Four inoculated replicates and four uninoculated sterile controls were examined at each pH.

At harvest, 40 ml of distilled H_2O was added to each bottle and the final pH was determined. Then 50 ml of 0.2 M NaOH was added, and after being mixed the suspension was allowed to stand at room temperature for 1 to 3 h. The insoluble residues were then collected on preweighed filter paper disks (no. 54; Whatman, Inc., Clifton, N.J.), washed with distilled water, and air dried at 50 to 60°C for 48 h. After equilibration to room temperature, the residues were weighed so that total lignocellulose weight loss could be calculated. The filtrates were acidified to pH 1 to 2 with concentrated H_2SO_4. The APPLs which precipitated were collected in preweighed centrifuge bottles by centrifugation at 16,000 × *g* for 30 min, dried at 50 to 60°C for 48 h, and weighed (9). Lignocellulose weight loss and APPL production were determined in this manner for cultures grown at each pH. Lignin, carbohydrate, amino nitrogen, and ash contents of the residual lignocelluloses and APPLs were determined by the following methods: Klason lignin (16) and Smogyi carbohydrate determinations on Klason soluble fractions (2), organic nitrogen by the Kjeldahl procedure (14), and ash by combustion (9).

Degradation of [^{14}C-lignin]- and [^{14}C-glucan]lignocelluloses. A [^{14}C-lignin]lignocellulose was produced from spruce (*Picea pungens*) twigs by the L-[U-^{14}C]phenylalanine incorporation method, as previously described (6, 18). The [^{14}C-lignin]lignocellulose was also base extracted in 1 N NaOH at room temperature for 48 h before use to remove any ^{14}C-labeled esterified aromatic acids that might have interfered with the lignin biodegradation assay (5, 9). After extraction, the lignocellulose was collected by filtration, washed with water, and then air dried. The extraction was also important because it removed ^{14}C-labeled aromatic acids that would be solubilized at the higher pHs used, since this would have complicated the examination of pH effects on the degradation of the insoluble lignin polymer itself. The [^{14}C-lignin]lignocellulose had a specific activity of 990 dpm/mg, as determined by tube furnace combustion of 100- to 200-mg samples at 750°C (18, 19).

A [^{14}C-glucan]lignocellulose was produced by feeding D-[U-^{14}C]glucose to Douglas fir (*Pseudotsuga menziesii*) twigs by the glucose uptake method, as described previously (8). The [^{14}C-glucan]lignocellulose had a specific activity of 1,080 dpm/mg, determined by combustion as above.

To 30,000 dpm of base-extracted spruce [^{14}C-lignin]lignocellulose or fir [^{14}C-glucan]lignocellulose, enough unlabeled ground and extracted lignocellulose of the same type was added to bring the final weight to 50 mg. This mixture was transferred to a test tube, and the lignocellulose was autoclaved dry. Then 10 ml of sterile specifically buffered 0.2% (wt/vol) yeast extract medium also containing 0.1% (wt/vol) glucose was added. Phosphate buffer was used to establish cultures at pHs 6.5, 7.0, 7.5, and 8.0; Tris buffer was used for pH 8.5, and 0.025 M borate buffer (9.54 g of $Na_2B_4O_7 \cdot 10H_2O$, 0.2 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, and 0.05 of $CaCl_2 \cdot 2H_2O$ in 1 liter of deionized H_2O) was used for pHs 9.0 and 9.5. Each culture tube was inoculated with 0.1 ml of active-cell inoculum prepared from *S. viridosporus* cells which had been grown aerobically at 37°C in 0.6% yeast extract medium for 48 h. Sterile bubbler-tube assemblies were then fitted to each culture tube (18). For each pH, four replicate cultures and four sterile uninoculated controls were incubated at 37°C for 21 days

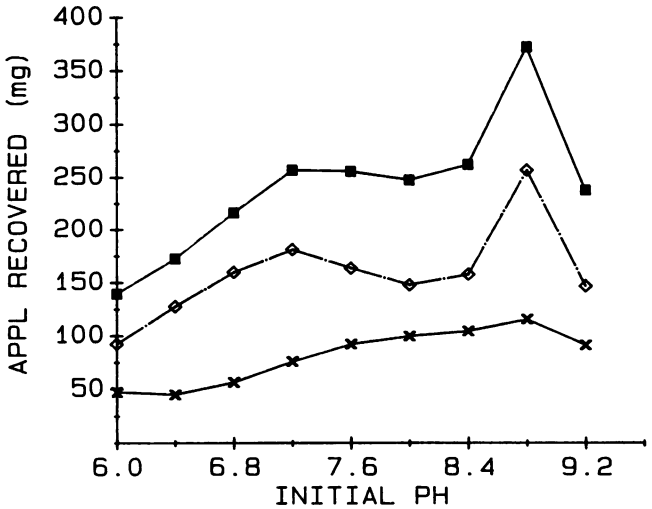

FIG. 2. Changes at different initial culture pHs in recovered APPL which was produced during corn lignocellulose degradation by *S. viridosporus* (■) compared with changes in APPL recovered from uninoculated sterile controls (×). The difference curve represents the inoculated culture values minus the uninoculated control values (◇). Values represent milligrams (dry weight) of APPL recovered per 5 g of initial lignocellulose after 6 weeks of incubation at 37°C as a solid-state fermentation.

with continuous bubbler-tube aeration. The percentage of initial ^{14}C evolved as $^{14}CO_2$ after 21 days was determined by liquid scintillation counting (18, 19). Also after 3 weeks, the final pH of each culture was determined and the residual lignocellulose was removed by filtration. Filtrates were acidified to pH 1 to 2, and precipitated APPLs were collected by centrifugation at 27,000 × *g* for 20 min. Supernatants were retained for separate analysis. The percentages of initial ^{14}C present in the APPL and water-soluble fractions from each culture were determined by liquid scintillation counting as previously described (19).

RESULTS

Effects of initial culture pH on the degradation of corn lignocellulose and on APPL production by *S. viridosporus*. The effects of pH on the percent loss of lignocellulose, carbohydrate, and lignin are presented in Fig. 1 (uninoculated sterile control values have been subtracted from the data). The greatest lignocellulose weight loss occurred at pH 8.4 (26%). There was very little difference in weight losses in cultures with initial pHs between 6.0 and 8.0 (averaging about 20%), and weight losses declined from the maximum at pHs above 8.5. Maximum carbohydrate degradation was also observed at pH 8.4 (46%), and there was a gradual increase in carbohydrate loss (totaling 15%) as the initial culture pH was increased from 6.0 to 8.4. At pHs above 8.4, there was a strong inhibition of carbohydrate degradation. Maximum lignin loss occurred over a wide range of initial pH values (7.2 to 8.8), with maximum losses averaging 32 to 34%. Between pHs 6.0 and 7.2, lignin loss rapidly increased from the 20% value observed at pH 6.0. Lignin loss declined slightly at pH 9.2 compared with the loss at pH 8.8. These data show that maximum lignocellulose weight loss and carbohydrate degradation occur at the same pH (8.4), whereas lignin degradation remains maximal over a wider range of initial pH values.

Maximum APPL production (averaging 372 mg; Fig. 2) was observed when the initial culture pH was 8.8. The unioculated controls accumulated small amounts of APPL, and the amount slowly increased with increasing pH (Fig. 2). The difference curve (inoculated minus control) shows that microbially mediated release of APPL increased linearly as the initial pH was raised from 6.0 to 7.2. Production appeared to reach a plateau or decline slightly between pHs 7.2 and 9.2, except for a marked and reproducible spike of APPL production when the initial pH was set at 8.8. The pH 8.8 maximum for APPL production was almost the same as that for maximum lignocellulose weight loss and carbohydrate loss (pH 8.4). However, lignin loss from the substrate as shown by the Klason assay (Fig. 1) was consistently about 33% between pHs 7.2 and 8.8, and no spike in Klason lignin loss was observed.

Final pH determinations on solid-state fermentation extracts showed that the buffers used did not maintain the initial pHs over the entire incubation period. A drop in pH occurred with each of the cultures except for the culture with an initial pH of 6.0 (Table 1). For cultures with initial pHs of 7.2 or higher, the final pH values ranged between 7.0 and 7.4. The pH drop was probably caused by an accumulation of organic acids and CO_2 released as a result of lignocellulose degradation by *S. viridosporus* (4).

The carbohydrate, lignin, nitrogen, and ash contents of each of the APPLs are shown in Table 1. Amino nitrogen and ash contents were not consistently affected by initial culture pH, with nitrogen content varying between 1.8 and 2.4% and ash content varying between 3.4 and 5.3%. The highest carbohydrate content for any APPL was for that recovered from the culture with initial pH 7.6 (3.5%). The rest of the

TABLE 1. Effects of the initial culture pH on the chemical composition of the APPL produced by *S. viridosporus* during degradation of corn lignocellulose after 6 weeks of incubation at 37°C as a solid-state fermentation

Initial pH	Final pH	Composition of APPL[a]			
		% Lignin	% Carbohydrate	% Nitrogen	% Ash
6.0	6.1	62.6	2.7	2.3	4.9
6.4	6.2	65.5	2.4	1.8	4.5
6.8	6.7	67.0	2.8	2.2	4.6
7.2	7.0	67.9	2.0	2.4	5.3
7.6	7.1	82.1	3.5	2.4	4.3
8.0	7.2	75.5	2.6	2.3	4.6
8.4[b]	7.2	65.5	2.2	1.9	3.4
8.8[b]	7.4	67.5	1.9	2.2	3.5
9.2[b]	7.4	68.5	1.7	2.6	3.8

[a] The mean standard deviations for each assay were ±5.11% for lignin, ±0.37% for carbohydrate, ±0.10% for amino nitrogen, and ±0.29% for ash.
[b] Change in buffer from phosphate to Tris.

TABLE 2. Effects of the initial culture pH on the degradation of spruce [^{14}C-lignin]lignocellulose by *S. viridosporus* T7A after 21 days of incubation at 37°C in bubbler tubes[a]

Initial pH	Final pH	% ^{14}C-labeled products recovered			Total ^{14}C recovered (%)
		CO_2	Water soluble	APPL	
6.5	6.9	16.5[b]	14.3	0.9	31.7[c]
7.0	7.4	12.5	12.7	1.4	26.6
7.5	7.8	9.9[b]	12.1	2.4[b]	24.4
8.0	8.2	7.0[b]	10.5	3.1	20.6
8.5	8.4	12.4	17.5[b]	1.1	31.0[c]
9.0	8.7	0.6	10.2	3.3	14.0[b]
9.5	9.1	1.1	11.5	5.8[b]	18.4

[a] Each value is the average of four replicates for each initial pH.
[b] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in these categories (columns), as determined by general linear model procedures (17).
[c] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in this category (column) but not from each other, as determined by general linear model procedures (17).

TABLE 3. Nonbiological degradation of spruce [^{14}C-lignin] lignocellulose in uninoculated sterile controls after 21 days of incubation at 37°C in bubbler tubes at different initial pHs[a]

Initial pH	Final pH	% ^{14}C-labeled product recovered			Total ^{14}C recovered (%)
		CO_2	Water soluble	APPL	
6.5	6.6	0.2	5.0	1.8	7.0[b]
7.0	7.1	0.4	5.6	2.6[b]	8.6
7.5	7.4	0.3	5.7	3.1	9.1
8.0	7.8	0.4	6.2	4.2[b]	10.8[c]
8.5	8.4	0.4	8.9[b]	1.9	11.2[c]
9.0	8.8	0.3	5.9	1.9	8.1
9.5	9.2	0.4	6.4	3.1	9.9

[a] Each value is the average of four replicates for each initial pH.
[b] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in these categories (columns), as determined by general linear model procedures (17).
[c] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in this category (column) but not from each other, as determined by general linear model procedures (17).

TABLE 4. Effects of initial culture pH on the degradation of Douglas fir [^{14}C-glucan]lignocellulose by *S. viridosporus* T7A after 21 days of incubation at 37°C in bubbler tubes[a]

Initial pH	Final pH	% ^{14}C-labeled produce recovered			Total ^{14}C recovered (%)
		CO_2	Water soluble	APPL	
6.5	6.7	16.9	13.0	0.8	30.7
7.0	7.2	20.6[b]	12.9	0.4	33.8
7.5	7.7	16.3	14.8	0.6	31.6
8.0	8.3	13.0[b]	16.6	0.5	30.1
8.5	8.4	16.3	20.2[c]	1.0	37.6[c]
9.0	9.0	15.2	21.6[c]	1.4	38.1[c]
9.5	9.2	2.4[b]	37.3[b]	5.7[b]	45.4[b]

[a] Each value is the average of four replicates for each initial pH.

[b] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in these categories (columns), as determined by general linear model procedures (17).

[c] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in this category (column) but not from each other, as determined by general linear model procedures (17).

APPLs had carbohydrate contents ranging between 1.7 and 2.8%. The highest Klason lignin contents were observed in the APPLs from the pH 7.6 and 8.0 cultures (82.1 and 75.5%, respectively), whereas the other APPLs contained 63 to 68% Klason lignin. This would suggest that *S. viridosporus* modified the basic structure of the lignin polymer less when the initial pH was 7.6 to 8.0 than when it was either higher or lower. The [^{14}C-lignin]lignocellulose degradation studies (see Tables 2 and 3 and discussion below) support this conclusion.

Effects of pH on spruce [^{14}C-lignin]lignocellulose degradation by *S. viridosporus*. The changes in pH from the initial values were negligible for the bubbler-tube cultures over the course of the incubation, and uninoculated controls evolved no appreciable $^{14}CO_2$ (Tables 2 and 3). The mineralization of the [^{14}C-lignin]lignocellulose to $^{14}CO_2$ by *S. viridosporus* was significantly influenced by initial culture pH. The greatest evolution of $^{14}CO_2$ was at pH 6.5 (16.5%), followed by pHs 7.0 and 8.5 (approximately 12%) (Table 2). The mineralization of the lignin virtually stopped at pH 9.0 or above and was statistically lower at pHs 7.5 and 8.0 (9.9 and 7.0%, respectively). A lower level of microbially mediated degradation at these pHs may in part explain the higher Klason lignin levels for the APPLs produced at pHs 7.5 and 8.0 (discussed above).

The accumulation of ^{14}C-labeled water-soluble products as a result of lignin metabolism by *S. viridosporus* is also shown in Table 2. The accumulation was significantly ($P < 0.05$) greater at pH 8.5 (17.5%) than at any other initial pH. The next highest value was at pH 6.5 (14.3%), and at all other pHs the values ranged between 10 and 12%. For the uninoculated controls, the greatest nonbiological release (through leaching) of ^{14}C-labeled water-soluble products was at pH 8.5 (8.9%), with lower values at the other pHs (5.0 to 6.4%). The greatest amount of [^{14}C-lignin]APPL recovered from inoculated cultures was at pH 9.5 (5.8%). Lignin mineralization by *S. viridosporus* stopped at pHs 9.0 and 9.5 (Table 2 and 3); therefore, this release must be considered nonbiological. The next highest values for inoculated cultures were at pHs 8.0 and 9.0, at which acid-precipitable ^{14}C in the filtrates averaged about 3.2% of the initial counts. The pH range of 8.0 to 9.0 corresponds to the value at which APPL recoveries were greatest in the solid-state fermentations (pH 8.8). The lowest amount of [^{14}C-lignin]APPL recovered was at pH 6.5 (0.9%), which corresponds to the pH at which the maximum $^{14}CO_2$ evolution occurred. The filtrates from the uninoculated controls also contained some acid-precipitable counts. The highest value was at pH 8.0 (4.2%), followed by pHs 7.5 and 9.5 (3.1%). The highest values for total percent ^{14}C recovered from the inoculated cultures were observed at pH 6.5 and 8.5 (31.7 and 31.0%, respectively). These recoveries were significantly ($P < 0.05$) higher than those at any other initial pH. At both pH 6.0 and 8.5, mineralization as well as solubilization values were high, and both contributed significantly to the high percentage of the total percent ^{14}C recovered.

Effects of pH on fir [^{14}C-glucan]-lignocellulose degradation by *S. viridosporus*. After 3 weeks of incubation, only minor changes in the final pHs of these bubbler-tube cultures were observed (Tables 4 and 5). Glucan mineralization to $^{14}CO_2$ by *S. viridosporus* was significantly ($P < 0.05$) higher at pH 7.0 (20.6%) than at any other initial pH. The next highest values were observed at pH 6.5, 7.5, and 8.5, at which recoveries of $^{14}CO_2$ averaged about 16% of the initial radioactivity. The lowest recovery of ^{14}C as $^{14}CO_2$ occurred at pH 8.0. This was also near the initial pH at which pH the APPLs produced had a higher carbohydrate content than the APPLs harvested from cultures of other initial pHs. Unlike lignin mineralization, which did not occur above pH 9.0, glucan mineralization was not dramatically inhibited until pH 9.5. $^{14}CO_2$ recovered from the uninoculated controls was negligible (0.2 to 1.6%). The greatest production of ^{14}C-labeled water-soluble products during [^{14}C-glucan]lignocellulose degradation by *S. viridosporus* was at pH 8.5 and 9.0 (20.2 and 21.6% of the initial ^{14}C, respectively). The lowest values were at pHs 6.5 and 7.0 (13.0 and 12.9%, respectively). The trend in recovery of ^{14}C-labeled water-soluble metabolites was the same for both the uninoculated controls and inoculated cultures. The lowest recoveries were at pH 7.0, and the highest were at pH 9.5. This indicates that as the culture pH becomes more alkaline, more of the glucan fraction is solubilized nonbiologically over the extended aerobic incubation period. The trend for recovery of [^{14}C-glucan]APPL (i.e., APPL containing labeled glucan contaminants) was also the same for the inoculated cultures and uninoculated controls, with the lowest recoveries at about pH 7.0 (0.4%) and the highest recoveries at pH 9.5 (5.7%). The dominant sugar in the carbohydrate fraction of these APPLs is xylose, but there is also some glucose (3). A low level of contamination of the APPLs with [^{14}C]glucose explains the values

TABLE 5. Nonbiological degradation of Douglas fir [^{14}C-glucan] lignocellulose in uninoculated sterile controls after 21 days of incubation at 37°C in bubbler tubes at different initial pHs[a]

Initial pH	Final pH	% ^{14}C-labeled product recovered			Total ^{14}C recovered (%)
		CO_2	Water soluble	APPL	
6.5	6.4	0.6	18.9	0.9	20.4
7.0	7.0	0.2	15.0	0.6	15.7
7.5	7.4	0.2	17.5	0.5	18.1
8.0	7.8	0.5	20.9	1.0	22.4
8.5	8.4	1.3	25.2	1.2	27.7[c]
9.0	8.8	1.3	26.0	1.3	28.6[c]
9.5	9.2	1.6	32.3[b]	4.9[b]	38.8[b]

[a] Each value is the average of four replicates for each initial pH.

[b] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in these categories (columns), as determined by general linear model procedures (17).

[c] Values were significantly different at the 0.05% level ($P < 0.05$) from all other values in this category (column) but not from each other, as determined by general linear model procedures (17).

observed for the total ^{14}C recovered as [^{14}C-glucan]APPL. The significantly ($P < 0.05$) highest values for total ^{14}C recovered as [^{14}C-glucan]APPL from the inoculated cultures and uninoculated controls were at pH 9.5 (5.7 and 4.9%, respectively). This was due to the alkaline pH and not to microbial activity, because cellulose mineralization stopped at pH 9.5 (Tables 4 and 5). The next highest values for [^{14}C-glucan]APPL were at pHs 8.5 and 9.0 and resulted from a high level of microbial solubilization as well as a high level of microbially mediated mineralization of the lignocellulose glucan component.

DISCUSSION

Previously, when we grew *S. viridosporus* on lignocelluloses in bubbler-tube cultures, we grew the cultures in a medium also containing 0.6% (wt/vol) yeast extract and 0.1% (wt/vol) glucose at an initial pH of 7.1 (9). During aerobic incubation, $^{14}CO_2$ evolution was very rapid at first and then slowly declined. The results reported here show that the initial pH in those cultures promoted lignin and cellulose mineralization. During those incubations, however, the medium became more alkaline, increasing to pH 7.8 during week 1 and stabilizing at pH 8.5 by the end of week 2. This increase in pH was probably caused by alkaline product accumulation resulting from the metabolism by the culture of a large amount of the yeast extract in the medium. The present results show that as the pevious cultures became more alkaline, conditions shifted from those optimal for mineralization to those which also optimized lignin solubilization and APPL accumulation.

An important finding of the present research is that *S. viridosporus* decomposed lignocelluloses actively at a wide range of pHs (between 6.5 and 9.0). Although the mineralization of lignocellulose was more dominant at the lower pHs, APPL production was more strongly promoted at alkaline pHs. In terms of the most optimal situation, total lignocellulose degradation and lignin solubilization were actually maximal when the initial pH was set in the 8.4 to 8.8 range. The high ligninolytic activity at high pHs provides this actinomycete with a potential degradative niche in the environment. The ability of this actinomycete to degrade lignin at pHs greater than 8 sharply contrasts with the patterns observed for ligninolytic white rot fungi, which, in lignin degradation studies (10), are typically incubated at pH 5.5 to 6.5. For example, *Phanerochaete chrysosporium* grows well only at low pHs and is optimally ligninolytic at pHs near 4.5 (13). Generally, filamentous fungi prefer a soil pH of 5.5 or less, and the filamentous bacteria (actinomycetes) prefer a more alkaline soil, of pH 8.0 (1). Therefore, in neutral and alkaline soil microenvironments, *S. viridosporus* is likely to be dominant over fungi as a decomposer of lignocellulose.

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