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Changes in the Composition of Hydrolyzed Lignin during Composting

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Abstract—Microbial assemblages were composed for composting hydrolyzed lignin. Data on bioconversion of aromatic compounds with various types of substitution in the ring were used for this purpose. Composting of hydrolyzed lignin reduced the contents of lignin, low-hydrolyzable polysaccharides, resins, and low-molecular-weight phenols and resulted in accumulation of humic acids. The resulting compost showed no phytotoxicity.

The problem of environmental pollution with wastes of the paper-pulp and wood-chemical industries is urgent in the Irkutsk Region, where paper-pulp enterprises produce about 400 000 tons of hydrolyzed lignin (HL) waste [1]. This waste occupies vast expanses and pollutes areas adjacent to the enterprises.

Economic methods for HL utilization have been sought virtually since the origin of the paper-pulp industry, but many projects have not been implemented because of the complexity and variability of lignin composition, insufficient technological development and instrumentation of some stages, etc. For example, HL is proposed to be used for production of phenol-formaldehyde resins, sorbents for purification of waste water, and a drug (Polyfelan). However, only 2.7% of HL are utilized. Use of HL as a fuel may be promising only for its removal because of its low calorific value, but its potential as a natural organic material remains untapped. Microbial composting can restore HL to the natural carbon circulation.

Hydrolyzed lignin doped with alkali admixtures, such as dung, was proposed to be used as a fertilizer [2]. Nevertheless, the cementing property of HL is known to impair soil texture [3]. Therefore, it is insufficient to mix HL with organic additives to obtain a good fertilizer.

One of the approaches to conversion of industrial lignin to organomineral fertilizers involves the use of microorganisms and their enzymes as delignifying agents, because lignin degradation is one of the main stages of composting. The microbiological method for utilization of lignin waste is ecologically sound but very slow under natural conditions.

The goal of the study is to develop a method for rapid composting of HL yielding an organomineral fertilizer and to investigate lignin conversion in this process.

MATERIALS AND METHODS

Hydrolyzed lignin from the Zima pulp works was used in the study. The following microbial species were used for lignin fermentation: *Phanerochaete chrysosporium* Burds. 1 MR-1, *Penicillium citreo-viride* Biourge, *Penicillium* sp., *Aspergillus niger* van Tieghem, *Cephalosporium* sp. (kindly supplied by Dr. Ten, Institute of Water and Ecological Problems, Far East Division of RAS, Khabarovsk), *Phanerochaete chrysosporium* Burds. ATCC 24725 (All-Russian VNPO Gidrolizprom, St. Petersburg), *Trichosporon cutaneum* (DeBeurm. *et al.*) Ota D-46, *Trichosporon cutaneum* (DeBeurm. *et al.*) Ota 5, and *Streptomyces asterosporus* Krassilnikov (kindly supplied by E.I. Kolomiets, Institute of Microbiology, Belarussian Academy of Sciences, Minsk, Belarus). Mutual antagonistic effects of microorganisms were tested as in [4].

High-performance liquid chromatography of phenolic substrates was carried out on a Milichrom-1 chromatograph (Russia) equipped with a column filled with Silasorb C-18. Elution was performed with 30 and 70% solutions of methanol in 0.01 M phosphate buffer pH 4. The components were detected at 280 nm.

For composting under laboratory conditions, 1 kg of HL was supplemented with 1.3 g of urea, 1 g of K_2HPO_4, and 0.7 g of KCl. After sterilization, HL samples were inoculated with equal amounts of 4-day cultures of microorganisms: two strains of *T. cutaneum* (cultivated on a medium for yeastlike fungi [5]), *S. asterosporus* (medium for lignin-degrading actinomycetes [6]), *P. chrysosporium* 1 MR-1, *P. citreo-viride*, *Cephalosporium* sp., and *A. niger* (on the Kirk medium for fungi [7]). The mixture was stored at 30°C for two months.

A large-scale experiment was carried out in an outdoor area in June 1996. Three tons of HL were supplemented with 37 kg of lime, 21 kg of KCl, 75 kg of $(NH_4)_2SO_4$, and 27 kg of ammophos. The composting mixture was placed in a 1.5-m-high pile. Temperature

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was measured weekly. The pile was mixed when the temperature began to fall (twice in the summer). Samples were taken after 3 weeks, 3 months, and 15 months.

The contents of lignin, resins, and easily (EHP) and low-hydrolyzable polysaccharides (HHP) in HL and compost samples were assayed as in [9]. Humic acids were assayed according to [10], and protein according to [11]. The components of the alkali-soluble fraction of HL were analyzed according to [12]. The enzymatic composition of the composts was determined as in [13], and phytotoxicity as in [8]. Samples for UV spectroscopy were prepared as follows: a 2-g HL sample was extracted three times with diethyl ether, the extract was filtered, and ether was evaporated. The residue was dissolved in 3 ml of methanol and analyzed on an SF-20 spectrophotometer (Russia).

RESULTS AND DISCUSSION

The efficiency of microbial transformation of lignin-pulp substrates, including HL, depends on many factors. The main factors are the enzymatic activity of microflora and the rate of its propagation in the substrate. Both problems can be solved by choosing an active association of cultures and inoculating the substrate with a sufficient amount of it. This is the foundation of composting technology.

For our study, we chose microorganisms isolated mainly from hydrolyzed lignin stored for decades in disposal areas and capable of growth on various lignin-containing substrates [5, 14–16]. Of particular importance is the yeastlike fungus *T. cutaneum* possessing growth-stimulating activity [17].

To evaluate the activity and contribution of each culture during lignin degradation, we simulated its bioconversion on aromatic compounds with various types of ring substitutions: 3,4-dihydroxy-(pyrocatechol), 3-methoxy-4-hydroxy-(vanillin, guaiacol, and guaiacylpropanol), and 3,4-dimethoxy-(veratrole).

Most cultures intensely metabolized aromatic compounds. For example, *P. citreo-viride*, *S. asterosporus*, *T. cutaneum* D-46, and *T. cutaneum* 5 completed the conversion of phenolic compounds after 7–8 days of incubation. A slower conversion was demonstrated by *A. niger* and *P. chrysosporium* 1 MR-1: nearly all substrates were present on day 7 in culture filtrates in amounts of 10 to 40% (Fig. 1; exemplified by two cultures). In the context of microbial lignin degradation, conversion of guaiacylpropanol (simulating a typical phenylpropane unit of lignin) and veratrole (which can be considered an approximation of a phenolic hydroxyl, either substituted or protected with an ether bond) is the most illustrative.

Most cultures metabolized guaiacylpropanol more slowly than simple phenols. For example, culture filtrates of *T. cutaneum* 5 contained 82% of unprocessed guaiacylpropanol on day 8, and *A. niger* did not utilize it at all. In the contrast, *S. asterosporus* fully utilized guaiacylpropanol on day 8, and *Cephalosporium* sp. completed this task as early as day 4. It is known that compounds bearing no free phenolic hydroxyl are most resistant to microbial degradation. Our cultures—*T. cutaneum* D-46, *A. niger*, *S. asterosporus*, and *P. citreo-viride*—metabolized veratrole, although slowly. *Cephalosporium* sp. and *T. cutaneum* 5 were more active. The former converted 65% of veratrole by day 5–7, and the latter utilized it completely by day 8. *Penicillium* sp. was inactive in our tests and was excluded from further investigation. Thus, the combined action of *A. niger* and *S. asterosporus* ensured transformation of simple phenols, whereas *P. citreo-viride*, *T. cutaneum* D-46, *T. cutaneum* 5, *P. chrysosporium* 1 MR-1, and *Cephalosporium* sp. are capable of converting guaiacylpropanic compounds and compounds bearing substituted phenolic hydroxyls.

Table 1. Variation in the chemical composition of hydrolyzed lignin during composting

Time of composting	Element, %					
	C	H	N	S	Ash	C/N
HL	52.05	5.09	3.03	0.66	12.57	17.18
HL + mineral dopes	48.91	5.10	6.40	3.18	18.12	7.64
3 weeks	48.32	5.10	6.46	3.25	18.27	7.48
3 months	46.01	5.00	6.65	2.93	21.13	6.92
15 months	48.40	5.58	6.72	2.22	17.48	7.20

To compose a microbial starter, we determined the antagonistic relationships between the cultures. We found that virtually all tested microorganisms were compatible with each other. An insignificant retardation of *S. asterosporus* in the presence of *Cephalosporium* sp. was observed. The fungus *P. chrysosporium* ATCC 24725 completely inhibited *S. asterosporus* and, in turn, was inhibited by another strain of the latter. Hence, all strains except for *P. chrysosporium* ATCC 24725, can be used for inoculating compost.

To sum up, our chemical and microbiological studies demonstrated that the tested cultures, with the exception of *Penicillium* sp. and *P. chrysosporium* ATCC 24725, can be used for composing an artificial microbial association.

The variation in the chemical, group, and fractional composition of compost obtained under laboratory conditions was investigated to trace HL conversion during composting. The chemical analysis demonstrated that microbial treatment resulted in an increase in ash content due to consumption of carbon sources. Ash content in HL with mineral additives was 5.46% and in the compost, 7.24%. Carbon contents were 59.91 and 55.70%, respectively. From the chemical viewpoint, biological treatment of HL resulted in a decrease in the contents of lignin and resins and in accumulation of humic acids.

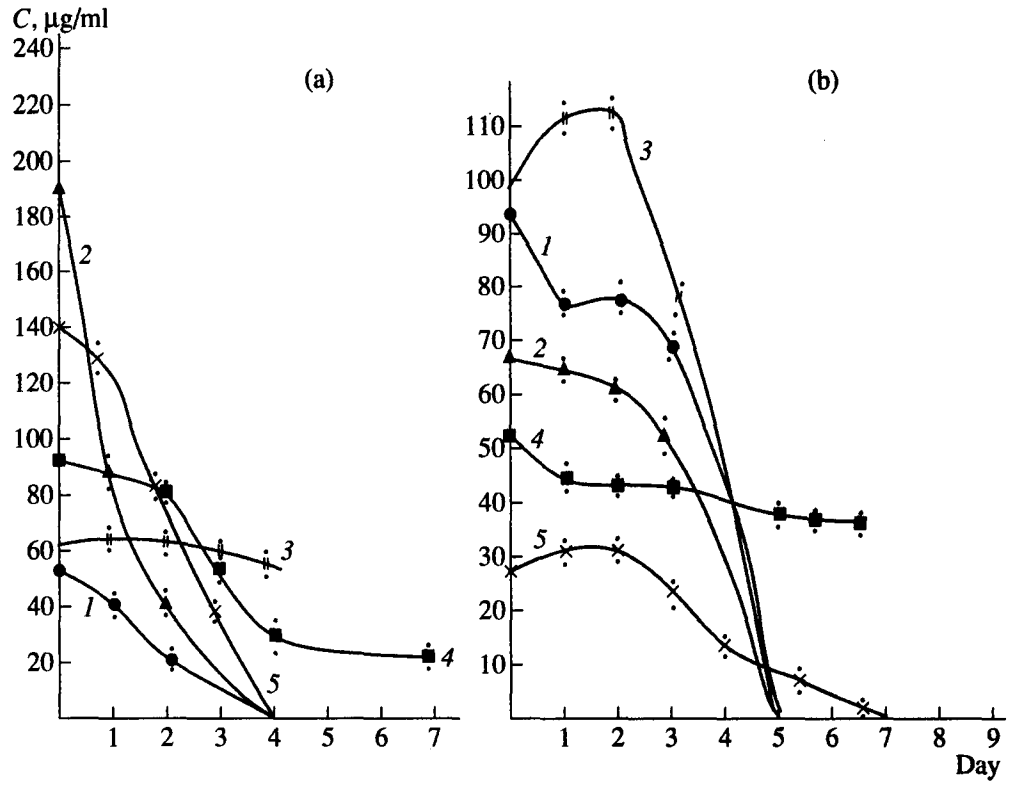

Fig. 1. Degradation of phenolic substrates by (a) *Cephalosporium* sp. and (b) *Penicillium citreo-viride*: (*1*) pyrocatechol, (*2*) vanillin, (*3*) guaiacol, (*4*) veratrole, (*5*) guaiacylpropanol-1.

Separation of the alkali-soluble low-molecular-weight components of HL demonstrated that microbial treatment considerably reduced the contents of phenols (by an order of magnitude). This is worth noting, because they are undesirable in compost. After application to soil, phenol-oxidizing microorganisms compete with plants for nitrogen and phosphorus and suppress plant growth.

The change in the content of low-molecular-weight phenolic components was confirmed spectrophotometrically. As is seen from UV spectra of ether extracts from HL samples obtained by composting (Fig. 2), an increase in absorption at 270–280 nm took place for the first seven weeks. This was related to low-molecular-weight phenols accumulating as a result of HL degradation. After nine weeks, only a slight inflection was recorded in this UV region, because the low-molecular-weight phenols began to be metabolized by microorganisms.

We traced the change in the activity of extracellular enzymes produced by the microorganisms for ten weeks of laboratory composting. The data shown in Fig. 3 demonstrate different time patterns of formation of redox enzymes. The maximum peroxidase activity was observed after two weeks of composting, and that of polyphenol oxidase activity after four weeks. Catalase activity fell to a minimum after six weeks of microbial treatment and then rose again. Taking into account the fact that lignin-degrading enzymes, including polyphenol oxidase and peroxidase, are inducible, the dramatic decrease in their activity after 8–10 weeks may be related to the decrease in the content of low-molecular-weight phenols and degradable polymer fragments in the substrate.

Hence, our artificial microbial association produces oxidase enzymes, is capable of HL bioconversion, and thus can be used for lignin composting.

The pilot experiment was conducted under conditions simulating industrial ones. Under these conditions, the microorganisms reproduced intensely. Protein content was 24, 72, and 182 ng/g of the compost after 3 weeks, 3 months, and 15 months, respectively. The data of chemical analysis of HL and compost samples shown in Table 1 demonstrate that carbon content decreased and ash content increased after 3 months of composting (a dramatic change in the 3-week sample is related to addition of mineral components). As this took place, the C/N ratio required for composting (which should be no more than 40) was maintained [8]. Because the outdoor pile was exposed to precipitation, the mineral components were eluted, the content of ash in the compost decreased, and the content of carbon correspondingly increased. As a result of microbial treatment (already after 3 months), the compost blackened and the specific HL odor vanished. This indicates that HL components passed to the compost. Another way to evaluate the maturity of compost is to test its toxicity by crop germination. Using peas as test seeds,

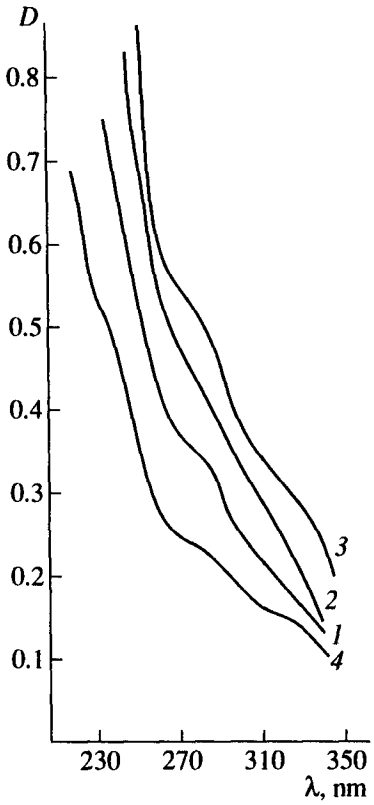

Fig. 2. Variation in UV spectra of the ether-soluble fraction of hydrolyzed lignin during composting: (*1*) starting HL, (*2*) 4 weeks, (*3*) 7 weeks, (*4*) 9 weeks.

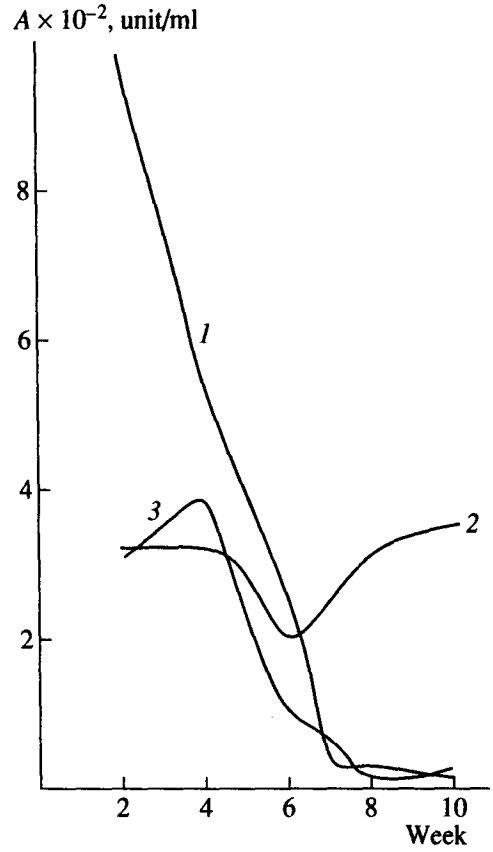

Fig. 3. Variation in enzymatic activity during composting of hydrolyzed lignin: (*1*) peroxidase, (*2*) catalase, (*3*) polyphenol oxidase.

we found that their germination on the 3-month and 15-month compost samples was no less than in the control, whereas no more than 13% of seeds germinated on the starting HL and the 3-week sample.

As for variation in the fractional composition of HL, one should note an increase in the content of humic acids in compost samples (Table 2) and a decrease in lignin content. Lignin degradation is accompanied by degradation of low-hydrolyzable polysaccharides. Taken together, these facts are indicative of humification, along with biodegradation of macrocomponents. This is of particular importance, because the effect of organic fertilizers is attributed, first of all, to the presence of humic acids.

The group composition of the low-molecular-weight fraction of HL shows that phenols accumulate at early stages of composting, as in the laboratory experiment. This is an additional piece of evidence that the 3-week sample cannot be regarded as compost or used for fertilization. Further composting resulted in a decrease in phenol content, although lower than under laboratory conditions. Utilization of low-molecular-weight phenols can be achieved by their degradation to CO_2 and H_2O and condensation yielding lignin-like or humic compounds, which is related to the presence of

Table 2. Variation in the fractional composition of hydrolyzed lignin during composting

Fraction	HL, %		
	starting	3 weeks	3 months
Lignin	62.10	61.70	56.40
EHP*	0.04	0.04	0.05
HHP*	10.08	7.74	7.20
Resins	3.00	0.83	0.97
Humic acids	6.20	6.30	8.17

* Easily- and hardly hydrolyzable polysaccharides.

Table 3. Variation in the group composition of the alkali-soluble fraction of hydrolyzed lignin during composting

Group of compounds	HL, %			
	starting	3 weeks	3 months	15 months
Phenols	0.08	0.21	0.07	0.02
Acids	0.11	0.16	0.25	0.40
Neutral compounds	0.03	0.02	0.03	0.03
Lignin-like substances	0.38	0.55	0.65	1.04

phenol oxidase, which is capable of both degradation and oxidative polymerization of phenolic compounds [18].

Thus, we demonstrated that bioconversion of HL can be accomplished by using an artificial microbial association. This association degrades its low-molecular-weight components, including phenolic compounds toxic to plants, and macrocomponents (polysaccharides and lignin itself). Secondary processes yield humic compounds, whose content increases with time. The resulting product has altered chemical and morphological properties, ceases to have phytotoxicity, and can be regarded as compost. According to physicochemical properties and phytotoxicity, the optimum term of composting is three months.

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