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**EXPERIMENTAL ARTICLES**

# Microbiological and Biochemical Investigation of Succession in Lignin-Containing Compost Piles

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**Abstract**—The investigation of microbiological succession and changes in the enzymatic activity, temperature, pH, and phytotoxicity of lignin during its composting showed that the addition of a starter culture (a specially developed association of microorganisms) affects degradational succession in the compost pile. The process of composting can be monitored either microbiologically or biochemically, by measuring the activity of some enzymes. The compost is ready for use when the activity of oxidoreductases (particularly polyphenol oxidases) falls and the activity of invertase stabilizes at a certain level.

*Key words*: kraft lignin, composting, enzymatic indication, enzymatic activity, degradational succession.

Lignocellulosic waste materials are large-capacity byproducts of the wood-chemical industry. One such byproduct is lignin, which is not recycled and is deposited in huge amounts in dumps. The lignin dumps are ecologically hazardous because of their inflammability and high content of acids (the pH of lignin is about 2.5), which are washed out by the rain runoff and carried to soil and then to groundwater. The atmosphere is also polluted by the small-grain fractions of lignin, which are lifted into the air with winds. The lignin dumps of the Irkutsk region alone contain more than 2 million tons of lignin [1]. Great efforts are now being directed toward decreasing the impact of lignin dumps on the environment and developing economically efficient methods of lignin salvage. However, almost all of the methods proposed cannot be employed on a large scale and are energy- and labor-consuming [2, 3]. One of the most feasible methods of lignin reprocessing is composting [4–6].

The natural composting of the lignocellulosic refuse is a very slow process, which can, however, be accelerated by the addition of a starter culture, i.e., a specially developed association of microorganisms isolated from the lignin dumps [7].

The aim of the present work was to study microbial succession in compost and to develop reliable methods for monitoring its maturity.

## MATERIALS AND METHODS

The experimental compost pile was made late in May using fresh lignin from a wood-chemical plant [7]. The compost was mixed 3, 7, and 10 weeks after the onset of the experiment, when the temperature measured inside the compost pile decreased. The control pile contained the same components as the experimental compost pile except for the starter culture. The process of composting was monitored by measuring the compost temperature, the pH of the salt liquor and the aqueous solution of compost, its microbiological composition, enzymatic activity, and phytotoxicity.

The temperature was measured two times a week at depths of 0–10, 30–40, 60–70, 90–100, and 120–130 cm from the surface on the southern and northern sides of the piles. Compost samples were taken at 1- to 2-week intervals from depths of 0–10, 20–30, and 50–60 cm at 15 sites throughout the pile and were combined for analysis.

The pH of the salt liquor and aqueous solutions was measured using an EB-74 universal ion-meter [8]. Microorganisms were enumerated by the standard procedures using the following agar media [9]: nutrient agar for the total bacteria, Sabouraud's medium for fungi, Czapek medium for actinomycetes, Winogradsky medium for nitrifying bacteria, nutrient broth for ammonifying bacteria, Giltey medium for denitrifying bacteria, Hutchinson medium for aerobic cellulolytics, and Kosmacheva medium for thermophiles. All measurements were carried out in 3–4 replicates.

Catalase was assayed with permanganate. Polyphenol oxidase and peroxidase were assayed with iodine [10]. The activity of invertase, cellulase, and urease was measured as described by Khaziev [11]. Phytotoxicity was estimated by the Ten Khak Mun method [12].

## RESULTS AND DISCUSSION

The fermentation of plant debris in a compost pile is considered to be terminated when the compost temperature is equal to the surrounding temperature even after

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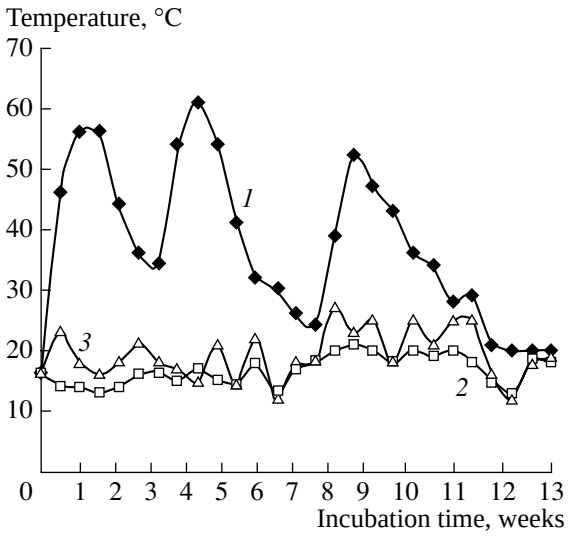


**Fig. 1.** Temperature dynamics during the composting of lignin: (*1*) compost; (*2*) lignin; and (*3*) air.

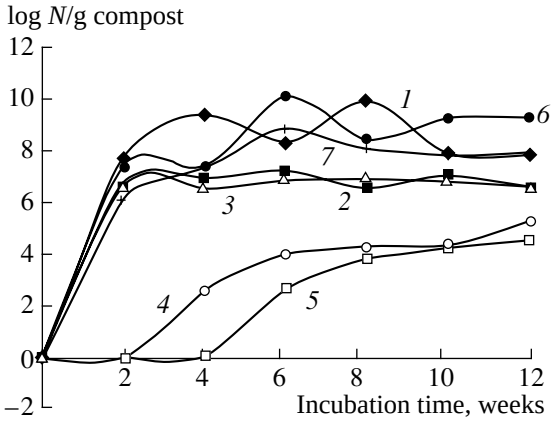


**Fig. 2.** Dynamics of the abundance (*N*) of different groups of microorganisms during the composting of lignin: (*1*) total bacteria; (*2*) fungi; (*3*) actinomycetes; (*4*) $NO_2$-nitrifying bacteria; (*5*) $NO_3$-nitrifying bacteria; (*6*) ammonifying bacteria; and (*7*) denitrifying bacteria. Microorganism were enumerated before the addition of the starter.

mixing the compost [12]. In our experiments, the compost temperature began increasing 1 day after introducing a starter culture and elevated temperature persisted for about 10 days (Fig. 1). Then the temperature tended to decrease, indicating a decline in the intensity of microbiological processes and a deficiency of oxygen. After mixing the compost, its temperature again increased. The maximum temperature (more than 60°C) was inside the compost pile, at a depth of 60–70 cm from the top. In the surface compost layer, the temperature was lower (no more than 42°C on the southern side) and less variable due to heat dissipation into the surroundings. In the bottom layer of the compost pile, the temperature did not exceed 31°C. This was likely due to airless conditions, which are unfavorable for microbial fermentation. In the control pile, to which the starter was not added, the temperature virtually did not differ from the surrounding temperature.

After introducing lime, the pH of the compost increased to 8.9, but as soon as after 1 week, it decreased to 6.0–6.5 (due to the lime neutralization by the acids present in the lignin) and persisted at this level throughout the rest of the experiment. The pH of the salt liquor and the aqueous solution of the compost differed insignificantly. The pH of the control pile varied negligibly, which can be accounted for either by the absence of the effect of the introduced microflora or by the establishment of the pH value that was optimal for the fermentation of the lignocellulosic material.

The total number of fungi, bacteria, and actinomycetes in the experimental compost drastically increased two weeks after the introduction of the starter (Fig. 2). The number of nitrifiers, particularly ammonia-oxidizing bacteria, increased at a lower rate. Nitrite-oxidizing bacteria appeared only by the 6th week. Peaks in the number of aerobic cellulolytics and thermophiles coincided with the peaks of the compost temperature (Fig. 3). Such a coincidence was not observed for the other groups of the microorganisms present in the compost (Fig. 2). The coincidence of the dynamics of the total number of bacteria with the dynamics of cellulolytics and thermophiles between the 6th and 12th weeks of incubation suggests that the cellulolytics and thermophiles are primarily represented by bacteria. The total number of microorganisms in the compost stabilized at a considerable level by the 3rd month of incubation, indicating the development of a stable microbial community and, hence, compost maturity.

Microbial succession in the control pile was entirely different. After 2 weeks of incubation, this compost was dominated by actinomycetes, whereas the number of bacteria and fungi had remained insignificant for a long time. The number of all microbial groups tended to slowly increase in the course of time.

The microbiological analysis of composts is very informative but labor- and time-consuming. For this reason, we attempted to develop an enzymatic approach to the analysis of the compost maturity. Tested were urease (an enzyme of nitrogen metabolism), invertase (an enzyme of carbon metabolism), cellulase (the enzyme catalyzing the degradation of the cellulosic moiety of lignocellulosic materials), as well as catalase, peroxidase, and polyphenol oxidase (enzymes involved in oxidative metabolism).

The high activity of oxidoreductases was observed as soon as after 2 weeks of incubation (Figs. 4a and 4b). Oxidase activity correlated with the temperature dynamics of composting. By the end of composting, the

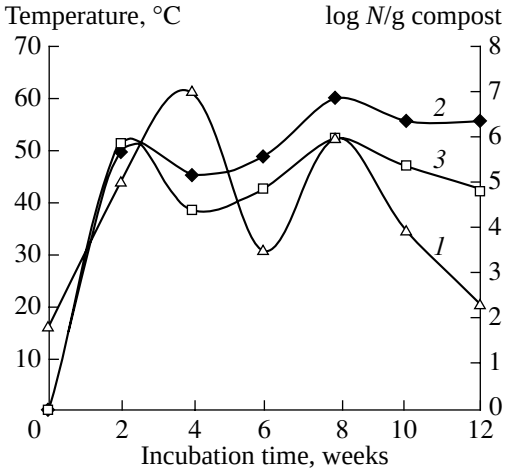


**Fig. 3.** Dynamics of the abundance (*N*) of thermophiles and aerobic cellulolytics during the composting of lignin: (*1*) temperature; (*2*) aerobic cellulolytics; and (*3*) thermophiles.

activity of the oxidoreductases decreased to nearly zero.

In the control pile, the activity of polyphenol oxidase reached a detectable level only by the 6th week of incubation and then followed the dynamics observed in the experimental compost, albeit at a lower level. The activities peroxidase and catalase were low throughout the composting period. The activity of peroxidase peaked in the 11th week of incubation, when the peroxidase activity of the experimental compost was already close to zero.

The dynamics of cellulase activity coincided with the dynamics of temperature and the number of aerobic cellulolytic bacteria (Fig. 5). The activity of invertase showed a small peak in the 4th week of incubation and then tended to increase. By the 13th week, it stabilized at a high level. It should be noted that high invertase activity is typical of fertile soils [13]. Urease was at a maximum in the 8th week of incubation, but then tended to decrease to approximately zero by the end of the composting period.

Cellulase and invertase activities in the control pile were lower than in the experimental pile but had similar dynamics, except for the absence of the invertase activity peak in the 4th week of incubation. Urease activity showed two peaks (in the 2nd and 11th weeks of incubation) and did not correlate either with the compost temperature or with the abundance of any microbial group.

The phytotoxicity of the experimental compost correlated with the activity of the enzymes tested. This observation is in agreement with the results of the laboratory experiment [7]. Initially, lignin was not phytotoxic but showed a high phytotoxicity as soon as after 2 weeks of composting (Fig. 6). By the end of the

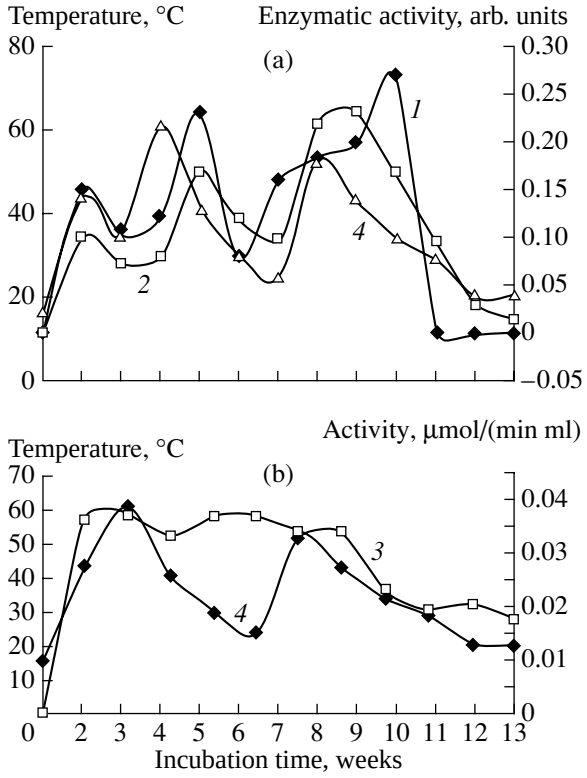


**Fig. 4.** Dynamics of (*1*) peroxidase, (*2*) polyphenol oxidase, (*3*) catalase, and (*4*) temperature during the composting of lignin.

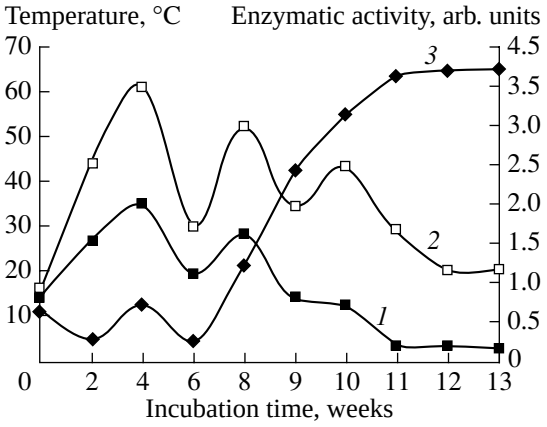


**Fig. 5.** Dynamics of (*1*) invertase, (*2*) cellulase, and (*3*) temperature during the composting of lignin.

experiment, the phytotoxicity of the compost decreased to a level typical of soil.

Thus, the addition of a starter culture affects degradational succession in the compost pile. The process of composting can be monitored either microbiologically or by measuring the activity of some enzymes. The compost is ready for use when the activity of oxidoreductases (the most appropriate indicator enzymes

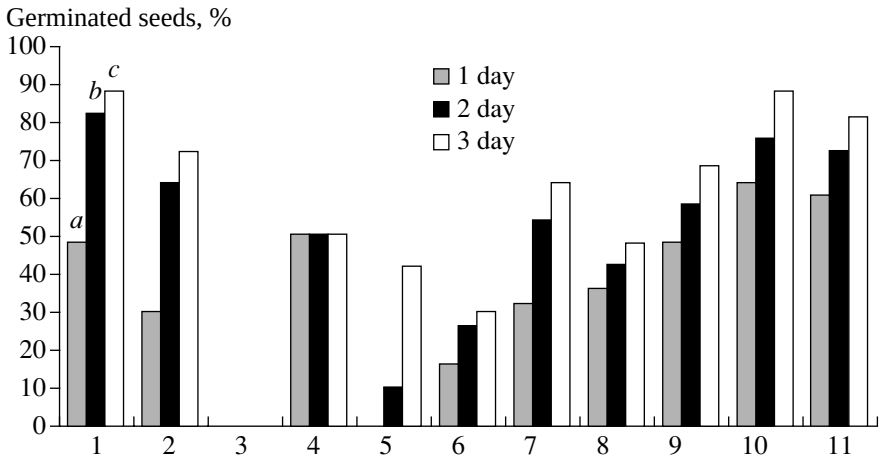


**Fig. 6.** The phytotoxicity of (1) lignin in the control pile, (2) untreated lignin, (3) untreated lignin with mineral supplements, (4) soil, and lignin subjected to composting for (5) 2, (6) 4, (7) 6, (8) 8, (9) 10, (10) 12, and (11) 13 weeks. The phytotoxicity was expressed as a percentage of seeds germinated after (a) 1, (b) 2, and (c) 3 days of incubation.

are polyphenol oxidases) falls and the activity of invertase stabilizes at a certain level. Some other enzymes and the phytotoxicity of compost can be used as the auxiliary indicators of compost maturity. With this approach, there is no need to perform microbiological and other time- and labor-consuming analyses.

## REFERENCES

1. *O sostoyanii okruzhayushchei prirodnoi sredy Irkutskoi oblasti v 1996 godu* (On the State of the Irkutsk Region Environment in 1996), Irkutsk, 1997.
2. Epshtein, Ya.V., Akhmina, E.I., and Raskin, M.N., Efficient Utilization of Kraft Lignin, *Khim. Drev.*, 1977, no. 5, pp. 24–44.
3. Sergeeva, V.N., Efficient Utilization of Kraft Lignin and Lignosulfonates, in *Perspektivy ispol'zovaniya drevesiny v kachestve organicheskogo syr'ya* (Prospects in Wood Utilization as an Organic Raw Material), Riga: Zinatne, 1982, pp. 105–125.
4. Strakhov, V.L., Lignin and Crop, *Gidrol. Lesokhim. Prom.*, 1986, no. 4, pp. 2–3.
5. Korol', G.S., Shakun, M.L., Rak, M.V., Gornostai, O.M., Yushkevich, I.A., and Mozheiko, F.F., Method for Manufacture of an Organic Fertilizer, USSR Inventor's Certificate no. 1638139, *Byull. Izobret.*, 1991, no. 12.
6. Imranova, E.L., Microbial Succession during the Degradation of Plant Residues, *Cand. Sc. (Biol.) Dissertation*, Irkutsk, 1998.
7. Volchatova, I.V., Medvedeva, S.A., Korzhova, L.F., and Rudykh, N.V., Changes in the Composition of Hydrolyzed Lignin during Composting, *Prikl. Biokhim. Mikrobiol.*, vol. 36, no. 3, pp. 293–298.
8. Arinushkina, E.V., *Rukovodstvo po khimicheskomu analizu pochv* (Handbook on the Chemical Analysis of Soils), Moscow: Mosk. Gos. Univ., 1970.
9. *Rukovodstvo k prakticheskim zanyatiyam po mikrobiologii* (Manual for a Practical Course in Microbiology), Egorov, N.S., Ed., Moscow: Mosk. Gos. Univ., 1995.
10. Khaziev, F.Kh., *Metody pochvennoi enzimologii* (The Methods of Soil Enzymology), Moscow: Nauka, 1990.
11. *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Zvyagintsev, D.G., Ed., Moscow: Mosk. Gos. Univ., 1991.
12. Ten Khak Mun, Fermentation of Organic Residues into Fertilizers, *Metodicheskie rekomendatsii* (Methodological Recommendations), Khabarovsk: Inst. Vodn. Ekol. Probl., 1988.