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Decomposition of sawdust and bark treated with cellulose-decomposing microorganisms

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Summary. The decomposition of coniferous sawdust and bark with added N and P was studied in relation to its capacity to serve as a substrate for plant growth. With sawdust as a substrate, there was more microbial biomass, greater CO_2 evolution, more ammonification and more actinomycetes but less nitrification and less fungi compared with bark. All groups and activities were greater in sawdust and bark compared with soil used as the substrate. Inoculation with cellulolytic strains of *Bacillus* sp. *Cephalosporium* sp. and *Streptomyces* sp. sometimes increased these activities but only marginally. The derived sawdust and bark composts increased the yields of tomato compared with soil to which the same nutrients had been added.

Key words: Compost – Sawdust – Bark – Cellulolysis – Ammonification – Nitrification

In the last few years composts from different kinds of waste have been widely used in agriculture in many countries (Greenland 1980; Parr and Wilson 1980; Simidchieve et al. 1981; Subba Rao 1984; Kardinalovskaya 1986; Lynch 1987; Steiner et al. 1987). They are used as manures and as substrates in the greenhouse production of vegetables and flowers (Hoitink and Poole 1980; Romano 1984; Verdonck et al. 1985). In some areas there is now a shortage of materials, particularly manure, resulting from new technologies in stock-breeding and the use of straw in the cellulose industry and for a fodder. This has required a search for new sources of organic substances. One possibility is waste products from the timber industry (Klett et al. 1972; Cappaert et al. 1975; Schulser et al. 1977; Solbraa 1979a, b). The microflora and microbiological processes connected with wood waste mineralization as well as the possibilities of their acceleration by the application of inoculants have received little attention.

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In the present study we investigated microbial populations and processes during the mineralization of composted spruce sawdust and bark, and examined the opportunities for acceleration of these processes using an association of active cellulose-decomposing microorganisms isolated from roots and soil. We also investigated the suitability of the derived composts for use as substrates in the container production of tomatoes.

Materials and methods

The wood products were derived from spruce (*Picea excelsa* Link). The bark fragments were 2–15 mm in size and the sawdust chips 1–5 mm. The chemical composition of the wood wastes is shown in Table 1. The analysis comprised the determination of total C and N, by the method of Ponomarova and Plotnicova (1975); P, by the molybdic-vanadate method (Mincheva and Brashnarova 1975); K, by flame photometry; and the percentage of water-soluble substances, cellulose and lignin (Christov et al. 1954). The soil used was a controlled leached meadow Eutric Cambisol soil with the following composition (mg $100\,g^{-1}$): NH_4^+-N, 0.48; NO_3^--N, 0.25; $P(P_2O_5)$, 15; $K(K_2O)$, 4.05; pH (in KCl), 5.8; total C, 0.83 g $100\,g^{-1}$.

A laboratory experiment was carried out with the following treatments: (1) soil; (2) sawdust; (3) sawdust + an association of active cellulose-decomposing microorganisms as inoculants; (4) bark; (5) bark + inoculants. The wood wastes had been composted in pots with 7 g N (as H_2NCONH_2) and 7 g P (as double superphosphate, 35–40% P_2O_5 with $Ca(H_2PO_4)_2 \cdot H_2O$ as the major component) per 1 kg substrate. The association of active cellulose-decomposing isolates used as inoculants comprised a *Streptomyces* sp., a *Bacillus* sp., and a *Cephalosporium* sp. Some degradative activities of the isolates used are given in Table 2. Cellulolytic ability was determined by measuring CO_2 production from cellulose by gas chromatography; the determination was carried out in brown glass bottles with a volume of 125 ml and 30 ml Hutchinson's media of composition (g dm^{-3}): filter paper (12×1.4 cm) cellulose, 0.1; K_2HPO_4, 1; $CaCl_2$, 0.1; $MgSO_4$, 0.3; NaCl, 0.1; $FeSO_4$, 0.01; $NaNO_3$, 2.5. The ligninolytic ability of the isolates was determined by the decomposition of tannic acid as shown by the zone of clearing on Petri dishes (Subba Rao 1984). The phytine decomposable ability of the isolates was determined by the zone of clearing on Petri dishes containing glucose-aspartate media with 0.05% phytine (Subba Rao 1984).

The mixed microbial inoculants were used at 10^4 to 10^5 cells for each isolate g^{-1} substrate. However, in a second experiment to study the effect on plants only, inoculants were added individually at 10^9

Table 1. Chemical analyses of the initial substrates

Substrate	Chemical elements				Chemical compounds			
	C	N	P	K	Cellulose	Lignin	Substances soluble in cold water	Substances soluble in hot water
	%							
Sawdust	42.6	0.09	0.008	0.07	48.7	26.1	1.62	2.82
Bark	42.7	0.17	0.021	0.15	26.7	51.3	0.91	4.89

Table 2. Cellulose, lignin, and phytine decomposable abilities of the isolates used as inoculants

Place of isolation	Genera	Cellulolytic ability (ml CO_2 14 days)	Ligninolytic ability (zone of clearing)	Phytine decomposable (zone of clearing)
Roots of maize	*Cephalosporium* sp.	13.30 ± 1.4	+	+
Roots of grass	*Streptomyces griseus*	10.94 ± 1.9	–	–
Smolnitza soil	*Bacillus* sp.	9.43 ± 1.7	–	–

cells g^{-1} substrate. The composts (2 kg) were incubated in plastic vessels for 3 and 6 months at 16–20 °C and 60–70% w:w moisture, and the soil was incubated similarly in 12-kg amounts. To improve aeration the sawdust and bark composts were turned over every 2 weeks. Every 2 months $CaCO_3$ was added to the composts to maintain pH between 5.5 and 7.0. CO_2 production was determined by gas chromatography, initially every 72 h, and then weekly after the 2nd month. The values for CO_2 evolved were averaged for each month. On days 30, 60, 120, and 180 the following were determined: (1) Microbial biomass (Anderson and Domsch 1978); (2) NH_4^+-N and NO_3^--N (Bremner and Keeney 1965); (3) ammonifying microorganisms, fungi, actinomycetes, and cellulose-decomposing microorganisms by serial dilution (Pochon 1954). After finishing the composting (180 days) the N_2-fixing activity of the composts was determined by the acetylene reduction method (Turner and Gibson 1980). The N_2-fixing activity of the composts was determined in brown glass bottles (capacity 125 ml). Each sample (5 g) was placed in a bottle with a rubber seal and acetylene was introduced (10% v:v). After 3 days of incubation at room temperature (20–22 °C) the ethylene generated was determined by gas chromatography.

The composts derived were used as substrates for container growing of tomatoes (*Lycopersicon esculentum* L., variety "Triumph") in glasshouse conditions with daily watering. Before the seedlings were planted, the substrates, including soil, were analyzed for N, P, and K. The mineral status of the substrates was optimized for plant productivity (Rankov et al. 1983) with ammonium nitrate, double superphosphate, and potassium sulphate, on the basis of necessary nutritional elements, to yield 1 kg fruit (3.3 g N, 0.5 g P, and 2.7 g K). The necessary quantity of N, P, and K was determined according to the coefficient of assimilation of these elements from the fertilizers by the plants, allowing for the availability of nutritional elements in the composts.

Results and discussion

The dynamics of CO_2 evolution from the composts are shown in Fig. 1a. In the soil (treatment 1) CO_2 production was low, varying from 0.49 to 0.75 mg CO_2 for 24 h per 100 g dry matter. In the wood wastes, CO_2 evolution was higher in the beginning but decreased after the 2nd month in the sawdust and after the 1st month in the bark. This prolonged support of maximum respiration activity by the sawdust and the higher values of CO_2 production throughout the period of composting are probably due to the presence of more accessible energy sources in the sawdust compared with the bark (Baumann 1977). Inoculation with active cellulose-decomposing microorganisms further increased respiration in the sawdust throughout the 1st and 2nd months of composting. A similar but lesser effect was obtained with the bark in the 1st month, probably due to the greater lignin content of the substrate. The bark did not respond well to the inoculants, and may have contained more inhibitors of decomposition, such as tannins and phenols (Gartner et al. 1974; Still et al. 1976). The effect of inoculation with cellulose-decomposing microorganisms may have been governed in part by the differences inoculum potential, the bark possibly being richer in natural cellulose-decomposing microorganisms than the sawdust. Our results with the bark are in accord with the results of Solbraa (1984), who studied the influence of different kinds of compost starters on the mineralization process in composted spruce bark. This author found no substantial positive effect and suggested that bark itself normally contains sufficient microbes for composting, including strongly competitive groups such as *Aspergillus*, *Penicillium*, and *Streptomyces* spp. The results shown in Table 3 support this conclusion.

The C:N ratio of the bark decreased from 251 at the beginning of the composting to 62 after the 6-month period of decomposition, indicating substantial microbial activity. In the sawdust this ratio decreased from 473 to 41. The microbial biomass present after the 1st month of composting was probably related to the presence of more easily assimilated substances (Fig. 1b). The subsequent biomass decline probably reflects the exhaustion of this substrate.

The dynamics of NH_4^+-N and NO_3^--N in the decomposition of wood wastes are shown in Fig. 1c and d. In the sawdust, NH_4^+-N reached maximal values during the 4th month of the composting and then decreased. In the bark, the maximum NH_4^+-N value occurred during the 1st month. On the day 120 NH_4^+-N was very low, at 2–5 mg 100 g^{-1} dry weight. The NO_3^--N in the bark reached a maximum during the 2nd month (Fig. 1d).

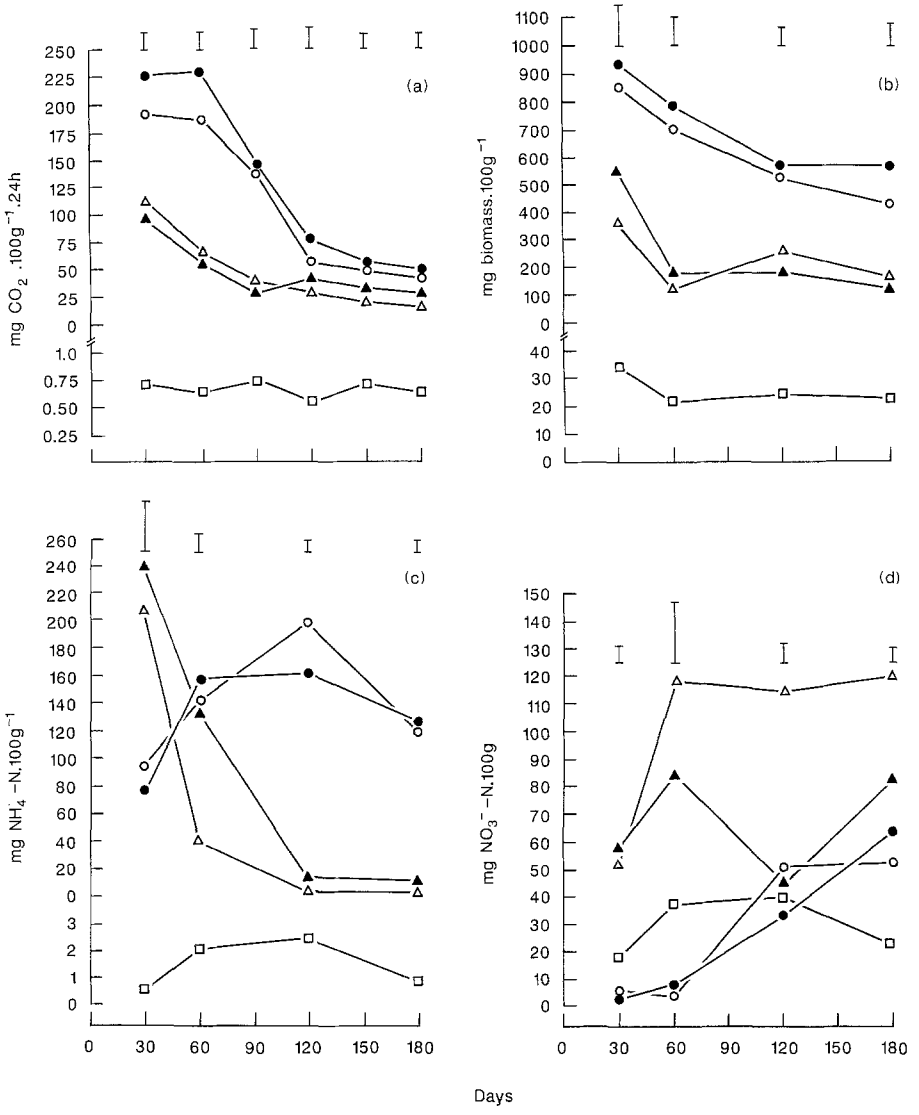

Fig. 1a–d. Microbial biomass and respiration: **(a)** CO_2 evolution; **(b)** microbial biomass; **(c)** NH_4^+-N; **(d)** NO_3^--N; □–□, soil; ○–○, sawdust; ●–●, sawdust+mixed inoculants; △–△ bark; ▲–▲, bark+mixed inoculants; *error bars* represent least significant difference (P = 0.05)

Probably because of greater immobilization, these values were lower in the inoculated treatments. Better aeration gave greater nitrification in the bark compared with the sawdust. Further, the reduced intensity of decomposition probably gave a lower concentration of organic substances in the compost solution. Consequently, a smaller quantitity of these substances may have suppressed the nitrification processes. Differences in the degree of N transformation in the two materials were related to differences in chemical structure, and in the level of microbiological activity and organic matter derivation that inhibited nitrification. The presence of more accessible substances in the sawdust increased the N demand of the microorganisms, so that part of the NH_4^+-N released was immobilized by the microorganisms and was then released gradually after decomposition of the accessible energetic sources. At the end of the composting, the concentration of available N (formed from mineral and biomass N) per 100 g dry matter was 243 mg for the non-inoculated sawdust, 279 mg for the inoculated sawdust, 159 mg for the non-inoculated bark and 113 for the inoculated bark. The greater N loss from the bark in comparison with the sawdust has not been satisfactorily explained.

Table 3 and Fig. 2 show the dynamics of the ammonifying microorganisms, fungi, cellulose-decomposing microorganisms, and actinomycetes. The fact that bark has more fungal propagules could account for some of the increased activity. In both types of wood wastes, as mineralization proceeded, the number of ammonifying microorganisms, fungi and cellulose-decomposing microorganisms declined after reaching a peak, probably due to de-

Table 3. Number of microorganisms in substrates before composting

Substrate	Ammonifying (10^6 g^{-1})	Actinomycetes (10^6 g^{-1})	Fungi (10^6 g^{-1})	Cellulose-decomposing microorganisms (10^3 g^{-1})
Sawdust	0.38	0.02	0.11	20
Bark	0.37	0.38	0.33	38
LSD (0.05)	0.24	0.28	0.08	19

LSD, least significant difference

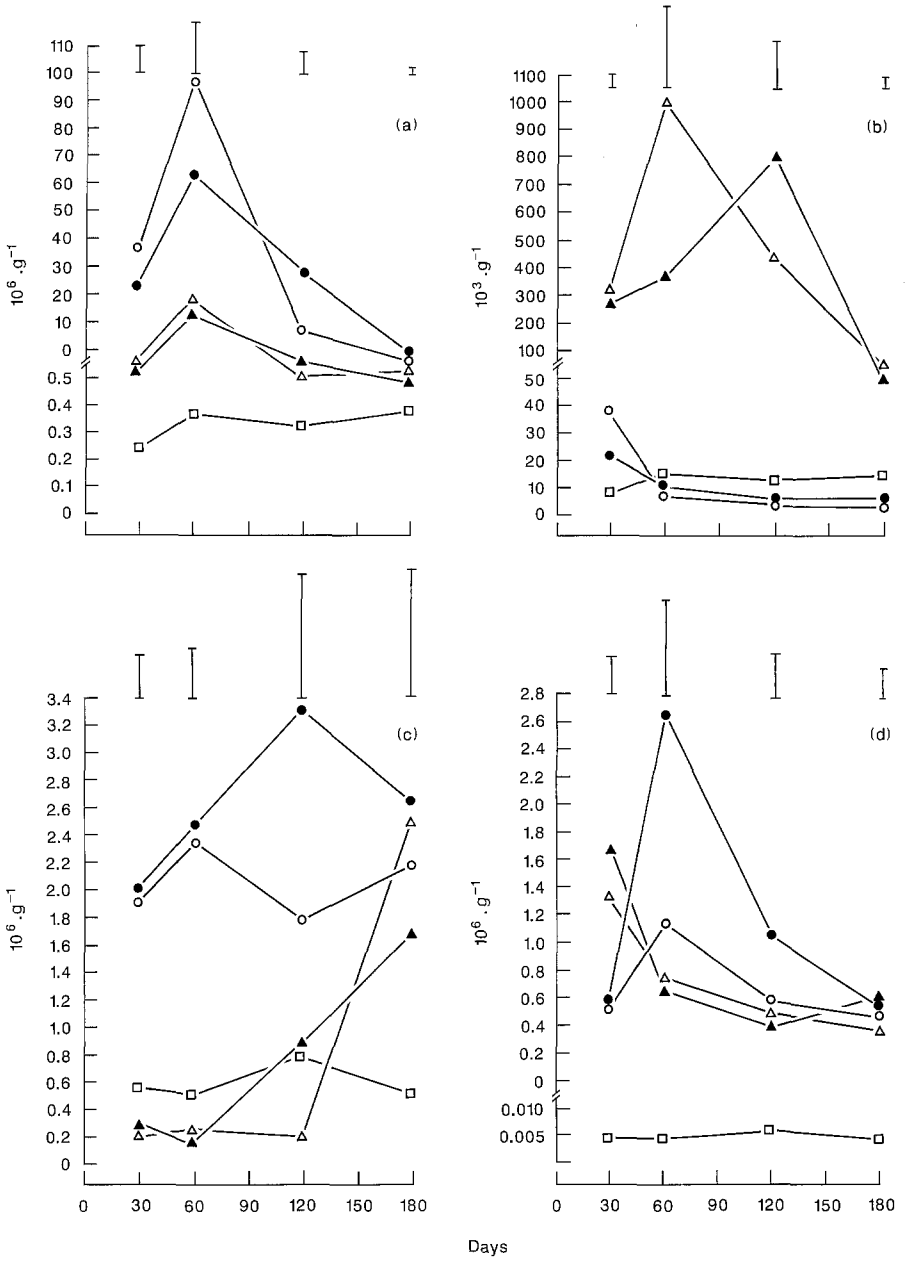

Fig. 2a–d. Dynamics of the microbial populations: **(a)** Ammonifying bacteria; **(b)** fungi; **(c)** actinomycetes; **(d)** cellulose-decomposing microorganisms; for explanation of symbols, see Fig. 1

pletion of the accessible energy sources. In the bark the number of actinomycetes increased with the longer period of composting. These results are consistent with those of Ten Khak Mun et al. (1988) and Grishkova and Tranina (1971) in the composting of coniferous bark. In the present study there were more ammonifying microorganisms and actinomycetes in the sawdust than the bark but the bark had several times the number of fungi in the sawdust. The maximum number of cellulose-decomposing microorganisms occurred in the 1st month in the bark, but in the 2nd month in the sawdust. The difference in the dynamics of the microorganisms in each case can be explained by the higher quantity of decomposable cellulose in the sawdust, which increased the length of the mineralization period.

The N_2-fixing activity of the composts is shown in Table 4. The activity was not determined until 180 days after the composting had started because of the influence of mineral N and the presence of inhibiting substances. The inoculated wood wastes showed higher N_2-fixing activity, probably because the experimental conditions fa-

Table 4. N_2-fixing activity of composts after 180 days

	Ethylene (n*M* 100 g dry matter^{-1} 24 h^{-1})
Soil	0
Sawdust	199
Sawdust + mixed inoculant	574
Bark	306
Bark + mixed inoculant	732
LSD (0.05)	173

LSD, least significant difference

voured the development of free-living N_2-fixing microorganisms. Decomposition has been promoted in another lignocellulosic substrate (wheat straw) by inoculation with cellulolytic fungi with the anaerobic N_2-fixing bacterium *Clostridium butyricum* (Lynch and Harper 1985), and the cellulolytic bacterium *Cellulomonas* spp. with the microaerophilic *Azospirillum brasilense* (Halsall and Gibson 1986). With the bark in the present study, it was possible to derive the measured N_2-fixation using either of these types of process.

The derived composts were used as substrates for the container cultivation of tomatoes. Fruit yields are shown in Table 5. The yields from the plants cultivated on the composts from wood bark and sawdust were higher than those from the control soil. The soil was incubated for the same period and lost some nutrient elements, but nutrients were added to both soil and composts to optimize production. The highest production without inoculation was obtained from the plants cultivated on wood bark. Cotter (1974) also obtained higher yields from tomatoes from plants that were cultivated on bark compared with sawdust. The author suggested that the bark possessed better base-exchange capacity. The sawdust composted for 3 months without inoculation gave a reduced yield of tomatoes (−28% compared to the control), probably because of the low level of mineralization and the accumulation in the compost of toxic substances like tannic acid and phenols (Solbraa et al. 1983). The yield increase obtained with inoculation of the sawdust may be attributed to the capacity of *Cephalosporium* sp. to decompose phenolic compounds to such a low level that plant growth is stimulated (Solbraa 1979a, b; Solbraa et al. 1983). It was interesting, therefore, that in a second experiment to determine the effect of individual components of the inoculant mixture on yield, *Cephalosporium* appeared to be most active (Table 6). At the end of the composting, the pH of the bark compost was highest, that of the sawdust a little less and the soil pH was rather low (Table 7). This could contribute to the effect of the substrates on plant growth. However, the overall mechanism of the beneficial effect of compost on plant growth warrants further investigation.

Table 5. Yield of tomatoes grown in different substrates

	Yield	
	g pot^{-1}	Change (%)
Three months of composting		
Soil	1135.5	0.00
Sawdust	813.5	−28.4
Sawdust + mixed inoculant	1657.5	45.9
Bark	1495.2	31.7
Bark + mixed inoculant	1662.5	46.4
Six months of composting		
Soil	821.3	0.00
Sawdust	1079.8	31.46
Sawdust + mixed inoculant	1616.0	96.8
Bark	1692.0	106.0
Bark + mixed inoculant	1432.5	74.4
LSD (0.05)	561.42	

LSD, least significant difference

Table 6. Yields of tomatoes grown on sawdust

	Yield	
	g pot^{-1}	Increase (%)
Sawdust	1017.3	0.00
Sawdust + *Bacillus* sp.	1165.8	14.60
Sawdust + *Streptomyces griseus*	1134.5	11.52
Sawdust + *Cephalosporium* sp.	1364.3	34.11
LSD (0.05)	289.19	

LSD, least significant difference

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Table 7. Dynamics of pH values measured in water or potassium chloride during composting for 15–180 days

	15 days		30 days		60 days		120 days		180 days	
	H_2O	KCl	H_2O	KCl	H_2O	KCl	H_2O	KCl	H_2O	KCl
Soil	5.4	4.8	5.8	4.9	5.4	4.8	5.4	4.8	5.5	4.9
Sawdust	8.3	7.6	6.4	5.6	7.1	6.5	6.1	5.4	6.0	5.8
Sawdust + mixed inoculant	7.7	7.1	6.8	6.0	7.0	6.4	6.3	5.5	6.2	5.8
Bark	7.5	6.5	5.4	4.9	4.2	4.0	5.9	5.2	6.5	6.4
Bark + mixed inoculant	7.6	6.7	5.9	5.4	4.3	4.0	5.5	5.3	6.7	6.5

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