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Effects of wood species on degradation rates and bacterial communities in a small-scale biodegradation system for garbage using wood matrices

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Abstract Simulated organic waste was biodegraded in a laboratory-scale machine using matrices prepared from four wood species to investigate the effects of wood species on the degradation rate and the bacterial community. The degradation rate, estimated by measuring weight loss and CO_2 evolution, was found to be equal among the four wood species. Changes in viable cell counts and microbial communities over time were examined. Viable cell counts were also similar among the wood species, but initial bacterial communities differed owing to differences in wood species, although these communities became similar with time. The sensitivity of isolates to wood extractives was examined using paper discs. The extractive-insensitive bacteria species were dominant at the initial stage of biodegradation. However, occupancy of sensitive bacteria increased with time. It was thought that antibacterial extractives were degraded or inactivated after some time.

Key words Wood species · Garbage · Biodegradation rate · Bacterial communities · Bacterial sensitivity

Introduction

The garbage automatic decomposer-extinguisher (GADE) machine, which quickly degrades organic waste aerobically by bacteria, is a waste-processing alternative to incineration, which generates chlorodibenzodioxins. In addition, it is a viable approach to developing a resource recirculation/recycling system.[1,2] A matrix of wood particles plays an important role in this machine as a structure for aeration and as a microbial carrier. Garbage is decomposed aerobically and rapidly.

Commercial wood species can be used as matrices in the GADE machine and are widely available in the form of lumber chips, offcuts, sawdust, and thinning cuts. Cypress, cedar, and Douglas fir are examples of widely used wood species that have antimicrobial activity. The antimicrobial activity of tropolones in cypress is well known, for example.[3,4] Microbial propagation may be considered to be affected by extractives in these wood species. When wood species that contain high quantities of antibacterial extractives are used as the matrices, it is feared that microbial growth is inhibited and that the microbial community is changed, resulting in slower degradation.

To clarify the effects of wood species on degradation rates and microbial communities, simulated waste was biodegraded in laboratory-scale equipment using wood matrices prepared from sapwood and heartwood of four species. Degradation rates and microorganism concentrations among the wood species were measured and compared. The relations between microbial communities and extractives were also examined.

Materials and methods

Preparation of wood matrix

Wood matrices were prepared from sapwood of *Cryptomeria japonica* D. Don (CJ), heartwood of CJ, *Thujopsis dolabrata* S. and Z. var *hondai* Mak (TD), *Chamaecyparis obtusa* (Sieb. et Zucc.) Endl (CO), and *Pseudotsuga menzeiesi* Franco (PM). These woods were cut using a cutting mill and sieved to grain diameters of 0.5–1.0 mm.

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Measurement of biodegradation rate

The biodegradation experiment was conducted according to the protocol described in a previous report.[5] Wood particles (60% moisture content on a wet-weight basis) were put separately in a 1000-ml Kjeldahl flask used as a degradation reactor. A formula rabbit feed (Hi-Pet, Osaka, Japan) composed of alfalfa, flour, delipidated soybean, and wheat germ was applied as the simulated waste. This feed contained crude protein, fat, fiber, and ash at ratios of 19%, 2%, 10%, and 9%, respectively. Every 24 h, 3.5 g of the simulated waste was added to each reactor. Each reactor was kept in a separate incubator at 30°C, rotated at 30 rpm for 2 min every 15 min, and aerated by 300 ml/min. The moisture content of each matrix was maintained at a constant initial level of 60%. The degradation experiment lasted for 30 days. There were triplicate flasks for each wood species. The rate of degradation was assessed by the rate of weight loss per gram of matrix per day (R_{-w}) and by the rate of CO_2 evolution (R_{-CO_2}), as described in a previous report.[5]

Viable cell count and isolation of bacteria

Every 5 days, 1 g of matrix was taken from its container and suspended in 100 ml of sterilized water. The suspension, diluted serially, was applied on a standard method agar (SMA) (Nissui, Tokyo, Japan) plate that contained yeast extract (0.25%), peptone (0.5%), glucose (0.1%), and agar (1.5%).[6] The viable cells were counted after several days of incubation at 30°C.

Each SMA plate on which 20–200 colonies appeared independently was chosen. The colonies were isolated, as they were considered to be the predominant species of the bacterial community in each matrix. Each isolate was confirmed to be pure culture and then subcultured. Isolates were identified by their cell morphology, gram staining, and utilization pattern of 95 carbon sources.[7,8]

Estimation of bacterial sensitivity to wood extractives

A 40-g aliquot of wood particles from each wood species was soaked and stirred in 1500 ml of 70% ethanol at room temperature for a week. After removal of wood solid, a portion of each solution was evaporated and freeze-dried for calculating the yield of extractives. The extractives from sapwood of CJ and from heartwood of CJ, TD, CO, and PM were 0.94%, 3.64%, 1.08%, 4.10%, and 1.91%, respectively. Depending on the yield, extractives were spotted on a paper disc (8 mm diameter, 30 mg weight) to obtain the extractive content equal to or five times that of the extractive content of the respective wood species. Isolates precultured on SMA plate medium were suspended in sterilized water. Cells in suspension were plated on top of the agar (0.8% agarose) in SMA agar plates to attain approximately 10^6 cells per plate. Paper discs spotted with the wood extractives were laid on top of the agar. The plates were incubated at 30°C for 2 days and examined for clear zones of growth inhibition.

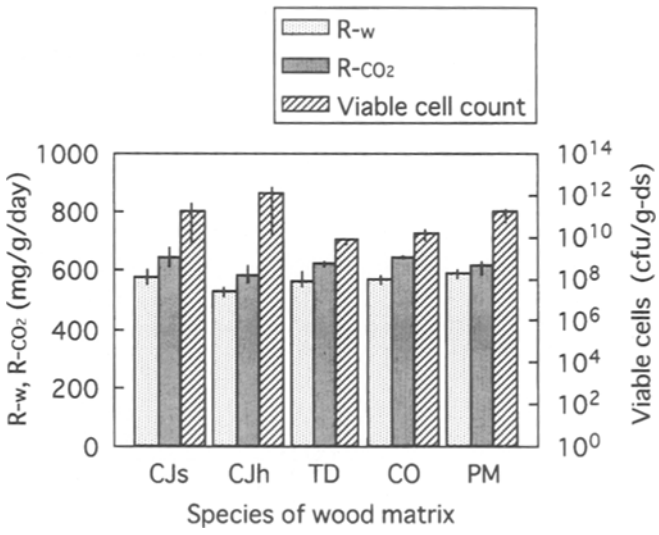

Fig. 1. Rates of weight loss (R_{-w}) and CO_2 evolution (R_{-CO_2}) and numbers of viable cells in a matrix of each wood species. *CJs*, sapwood of *Cryptomeria japonica*; *CJh*, heartwood of *Cryptomeria japonica*; *TD*, heartwood of *Thujopsis dolabrata*; *CO*, heartwood of *Chamaecyparis obtusa*; *PM*, heartwood of *Pseudotsuga menzeiesii*. *Bars* show maximum and minimum values of three plots

Results

Degradation rate, pH of matrix, and viable cell count

Weight loss (R_{-w}) and CO_2 evolution (R_{-CO_2}) increased gradually from the start of the experiment and reached a constant value after 5–7 days (data not shown). Analysis of variance ($P = 0.01$) on R_{-w} and R_{-CO_2} showed no significant differences among wood species (Fig. 1). The average values for R_{-w} and R_{-CO_2} were 566 mg/g/day and 622 mg/g/day, respectively. Except for PM, the viable cell count increased from the start of the experiment and reached a constant level after 5–10 days (Fig. 2). The viable cell count in the PM matrix after 3 days was extremely low relative to that of other wood species, but it increased and reached an equal value after 5 days. Average viable cell counts at a constant level (from the 10th to the 30th day) are shown in Fig. 1. There was no significant difference in viable cell count. Yeast and fungi colonies did not appear in significant levels.

The pH of matrices was 5.2–5.5 before the experiment began, except for PM, of which the pH was 3.3. The pH of matrices of all wood species increased steadily until reaching constant levels of around 8.5–9.0 (Fig. 3), a trend that was also observed in previous studies.[5,9]

Analysis of bacterial community

Results of identification and composition of isolates are shown in Table 1. In the matrices of CJ sapwood, regarded as a material low in antibacterial activity, *Bacillus subtilis* proliferated in the container at the initial stage of biodegradation and occupied almost 100% of the container. The bacterial community changed with time, and *Cellulomonas*

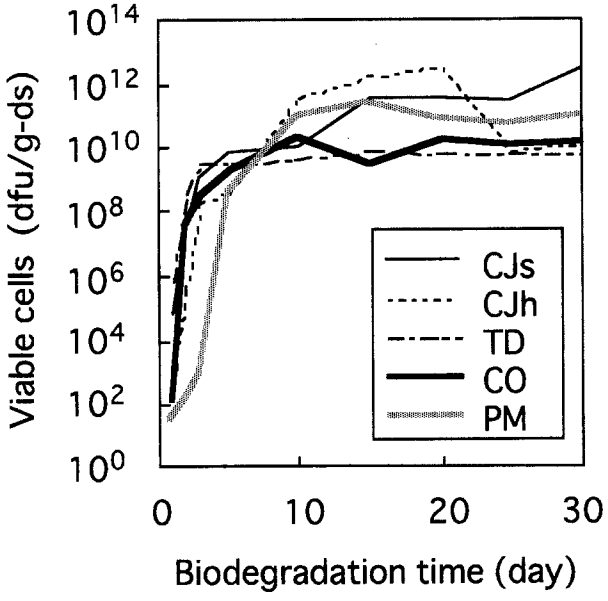

Fig. 2. Number of viable cells in wood matrix

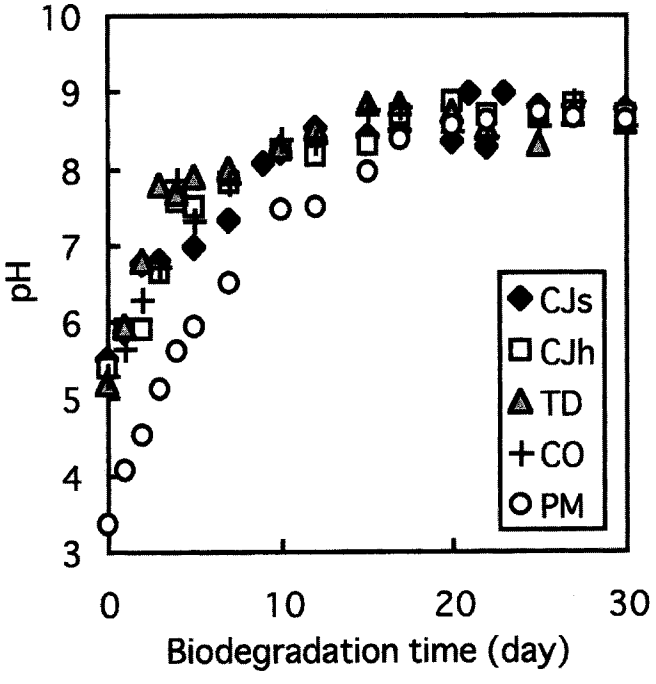

Fig. 3. Succession of pH of matrix

(formerly *Oerskovia*) *turbata* and *Xanthomonas campestris* became the dominant species during the later stage of biodegradation, when the conditions in the container stabilized. The triplicate determinations showed similar tendencies.

Pseudomonas spp. occupied almost 100% of the containers in heartwood of CJ, TD, and CO at the initial stage and *Burkholderia* (formerly *Pseudomonas*) *gladioli* in PM. However, *C. turbata* and *X. campestris* comprised major species at the later stages in the heartwood of those species and the sapwood of CJ.

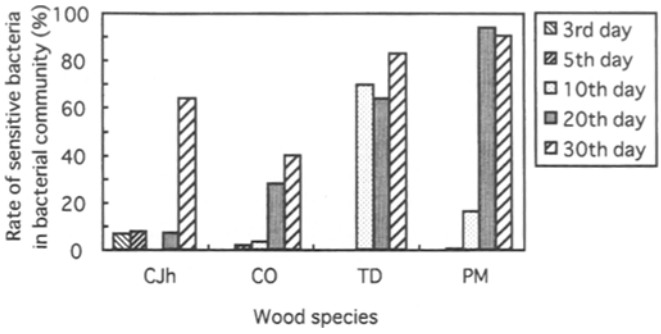

Fig. 4. Rate of sensitive bacteria in bacterial community from the 3rd, 5th, 10th, 20th, and 30th day matrices to wood extractives

Bacterial sensitivity to wood extractives

Isolates were assumed to be insensitive to wood extractives if they gave rise to an inhibition zone with a diameter of less than 9 mm in paper discs that spotted fivefold levels of extractives. A solution of 70% ethanol alone spotted on paper discs did not inhibit the growth of bacteria.

The extractives from the sapwood of CJ did not inhibit the growth of any isolates from any of the wood species. In the heartwood of each species, extractives did not inhibit the growth of isolates from each wood species at the initial stage, but isolates sensitive to extractives tended to increase with time (Fig. 4). *C. turbata* and *X. campestris*, which appeared regardless of wood species, were sensitive to extractives from the heartwood of each species.

Discussion

The results showed that the degradation rate did not vary among the matrices of wood species. This means that any of the wood species examined can be used as a matrix for the GADE machine. Bacterial communities propagated sufficiently in matrices from all the wood species and were initiated by the species of bacteria that were insensitive to wood extractives. These extractive-insensitive bacteria species were dominant during the initial stage. *B. subtilis* dominated at the initial stage in the matrix of CJ sapwood. However, its growth was inhibited in matrices of heartwood of CJ, TD, and CO; therefore, *Pseudomonas* sp., which was insensitive to heartwood extractives, became the pioneer bacterial species. For instance, β-thujaplicin, which is found in TD, has a broad antibacterial spectrum but has little influence on some gram-negative bacteria.[10] During an initial stage in the PM matrix, the count and community of bacteria were different from those of other wood species. The pH and extractives of the wood matrix were considered the reason. However, antibacterial extractives are thought to be degraded or inactivated over time because extractive-sensitive bacteria became dominant in the later stages. It is interesting that the bacterial communities became similar despite their initial differences, and that equal degradation

Table 1. Occupancy rate of isolated bacteria during biodegradation in each wood species

Isolate	Percent at 3–30 days of biodegradation				
	3	5	10	20	30
From CJs					
Bacillus subtilis	72	10	25	12	
Cellulomonas turbata	28	17	17	18	31
Enterobacter cloacae		52	29		5
Xanthomonas campestris		14	29	59	42
Xanthomonas maltophilia		3			
Sphingobacterium sp.		3			
Enterococcus sp.				6	14
Giraldi pink gram-negative rod				6	5
Comamonas sp.					3
From CJh					
Pseudomonas spp.	99	95			
B. subtilis	1	3			
Rhodospordium sp.		2			
Sphingomonas sp.			99	97	6
X. campestris			1	3	11
C. turbata					69
X. maltophilia					6
Alcaligenes xylosoxydans					6
Micrococcus sp.					3
From CO					
Pseudomonas spp.	100	99			
Enterobacter cloacae		1	5		
X. maltophia			95	47	17
X. campestris				28	25
C. turbata				10	42
Bacillus brevis				10	
Ochrobactrum anthropi				5	4
UI(CO1)[a]					13
From TD					
Pseudomonas spp.	100	12			
X. maltophilia		88	33	10	18
C. turbata			57	56	56
X. campestris			4	10	12
UI(TD1)[a]			4	7	
B. brevis			2		12
Micrococcus sp.				15	
Kingella kingae				2	
UI(TD2)[a]					3
From PM[b]					
Burkholderia gladioli		99	50		
B. brevis		1		7	
C. turbata			38	61	65
X. campestris			3	27	22
UI(PM1)[a]			3		
A. xylosoxydans			3		
Corynebacterium pseudodiphtheriticum			3		
Bacillus megaterium				2	
Acinetobacter radioresistens				2	
X. maltophilia					6
UI(PM2)[a]					4
Bacillus sphaericus					2

CJs, sapwood of *Cryptomeria japonica*, CJh, heartwood of *Cryptomeria japonica*; CO, *Chamaecyparis obtusa*; TD, heartwood of *Thujopsis dolabrata*; PM, heartwood of *Pseudotsuga menzeiesii*

[a] Unidentified isolates

[b] No isolates were obtained on day 3 for PM

rates resulted. It is speculated that the bacterial community composed mainly of *C. turbata* and *X. campestris* is stable. This stable community might be specific to rabbit feed (the simulated waste in this study). *C. turbata* is a gram-positive bacterium that is isolated from soil or decayed plants.[11] It was reported that *C. turbata* was dominant in aerobic waste treatment tanks and related to the solubilization, assimilation, and decomposition of starch, protein, and fats.[12–14] *X. campestris*, well known as a phytopathogen,[15] is detected during composting of organic agricultural substrates, such

as a mixtures of field-grown shredded maize plants (*Zea mays*), wood chips, and straw-bedded horse manure.[16] It is believed that these bacterial species were dominant because a vegetable-based rabbit feed was used as simulated waste. Improvement of the GADE machine's efficiency is therefore possible by identifying the most suitable environmental conditions for those bacterial communities.

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