Fate of xenobiotics during composting

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ABSTRACT

A primary concern with composting is the lack of knowledge about the fate of widely used pesticides. Also, there is increasing interest in the use of composting as a bioremediation method for soils contaminated with xenobiotics such as TNT, PCBs, chlorophenols and PAHs. We used a laboratory scale composting system to determine the extent of mineralization, incorporation into humic matter, and volatilization, during the composting of yard trimmings amended with ^{14}C labeled pesticides such as 2,4-D, diazinon, and pendimethalin. Our results showed that the pesticides studied have very different fates during composting. For example, 2,4-D is rapidly mineralized and transformed into high molecular weight humic compounds. Diazinon, on the other hand, is not readily mineralized, but undergoes rapid transformation to water soluble metabolites, while pendimethalin appears to be converted primarily into unextractable residues. Atrazine mineralization during composting is limited and varies with different lignocellulosic substrates. Preliminary results show that limited degradation of PCBs occurs during the composting of contaminated soils. Our results also indicate that thermophilic microbial communities are involved in the mineralization and biotransformation of xenobiotics during composting.

Introduction

Composting is an aerobic thermophilic process widely used for the recycling of organic residues such as yard trimmings, agricultural wastes, food wastes, and biosolids. Gradients of oxygen, nutrients, and temperature that exist in compost support a diverse microbial population and rapid organic matter conversion (6). There is also a growing interest in using composting for the bioremediation of soils and sludges contaminated with xenobiotics (2,3,4,15,16). In this paper we present our work on the fate of xenobiotics such as pesticides and PCBs during composting, and describe efforts to identify thermophilic microorganisms involved in their degradation.

Pesticide Degradation during Composting

Pesticides are widely used in lawn care and agriculture in the US and around the world. In the U.S., over 30,000 tons of pesticides are used every year on lawns, turf farms and gardens. Although much is known about the fate and microbial degradation of pesticides in soils, little is known about their fate during composting (1,11). There is a general public concern about the possible persistence of pesticides in composts made from yard trimmings and agricultural residues. Our broad research goals are to study the fate of pesticides and other xenobiotics during composting and to advance our understanding of the microbial communities involved in the turn over of the organic matter and in the degradation of xenobiotics during composting.

We used a laboratory scale compost reactor system which simulates the temperature and aeration conditions typically found in windrows (7,8). Compost feed stock consisted of yard trimmings (2:1 leaves:grass, w/w) amended with ^{14}C ring-labeled pesticides at 10-20 ppm. The extent of pesticide conversion to CO_2, volatilization, humification, leaching, and biotransformation during composting were determined and these results are summarized here (Table 1).

Table 1. Fate of xenobiotics during composting.

		Concentration (ppm)		Fate of Xenobiotic (% conversion)			
Xenobiotic/ Pollutant[a]	Feed stock[b]	Initial	Final	CO_2	Humics[c]	Volatiles	*Ref.*
PESTICIDES							
2,4-D	YT	11	<0.1	48	49	<1	*7*
Atrazine	NP	500	<10	7	52	-[d]	*12*
Carbaryl	B, WC	2	< 0.1	4	95	-	*11*
Chlordane	M, SD	100	-	04	5	50	*13*
Diazinon	M, SD	100	-	02	39	22	*13*
Diazinon	YT	10	<0.3	11	52	<0.5	*8*
Diazinon	GC	9	<0.1	-	-	-	*5*
Pendimethalin	YT	10	<0.1	13	72	25	*9*
Pendimethalin	GC	7-40	<0.1	-	-	-	*5*
CHLORPHENOLS							
Chlorophenols	BK, S	210	15	-	-	-	*15*
Chlorophenols	BK, S	850	40	-	-	-	*4*
PCP	BK, S	-	-	63	-	-	*4*
PCP	BK, S	210	-	30-40		1-3	*15*
EXPLOSIVES							
TNT	M,SD,A, S	3135	<300	02	48	-	*10*
TNT	M,SD,A, S	11,700	3	-	-	-	*16*
POLYCHLORINATED BIPHENYLS (PCBs)							
Aroclor 1248	YT, S	12	9	-	-	-	[e]
Aroclor 1232	B	11	2	-	-	16	*2*
Aroclor 1221	NP,M,B,SD,S	500	175	-	-	-	*14*
POLYAROMATIC HYDROCARBONS							
Pyrene	B,WC, S	15	35	17-20	-	-	*3*
Phenanthrene	B,WC	15	13	1	16	-	*11*
Phenanthrene	B	1000	96	-	-	05	*2*

[a] TNT-trinitrotoluene, PCP-pentachlorophenol

[b] A-alfalfa, B- biosolids, BK-Bark, GC-grass clippings, M-manure, NP-newspaper, S-soil, SD-sawdust, WC- wood chips, YT- yard trimmings (leaves, grass and brush)

[c] Conversion to humic acids, fulvic acids and humin.

[d] - not determined

[e] Michel, FC, unpublished results

2,4-D, a post-emergence broad leaf herbicide, is the second most commonly used lawn care pesticide (approximately 3000 tons used per year) in the U. S. There have been varying reports on the levels of 2,4-D found in yard trimmings at composting facilities. One study reported that 2,4-D was detected in all samples at levels up to 5.5 ppm (9). Another study showed that after a standard application of 2,4-D to grass, 80 ppm was found on day 0, 45 ppm on day 14, and 6 ppm on day 56 (9). During composting with yard trimmings, 48% of the ^{14}C-2,4-D was mineralized to $^{14}CO_2$ after 50 days of composting (7). Most of the remaining 2,4-D was transformed into high molecular weight humic compounds or was unextractable.

Diazinon is a broad-spectrum insecticide which is the single most widely used lawn care pesticide. Approximately 5000 tons are used per year in the U. S. At yard trimmings composting sites, diazinon has been found in a majority of the incoming yard trimmings samples at concentrations up to 5 ppm. Typically, the amount of diazinon on grass cut within a week of a standard pesticide application is 5 to 9 ppm (5). When diazinon amended yard trimmings were composted, low amounts (11%) of diazinon were transformed to CO_2. The bulk of the diazinon carbon, however, was transformed into a potentially leachable but less toxic hydrolysis product (isopropyl methyl hydroxy pyrimidine), and into unextractable and/or high molecular weight residues which are presumed to have low bioavailability (8). After 10 days of composting, no diazinon was detectable in lab scale composts. Diazinon degradation was faster during composting than that reported in soils, apparently due to the higher temperatures and greater moisture content of compost as compared to soils. The extent of diazinon volatilization observed in composting studies varies greatly (Table 1), perhaps due to the level of diazinon added, the feedstock used, the amounts of compost in the bench scale reactors, and the moisture content of the compost (8,13).

Pendimethalin (trade name Prowl®) is a preemergence herbicide that is commonly used to control broadleaf and grassy weeds. Although 5 to 40 ppm pendimethalin are found on cut grass after a standard application (5), generally low levels (≤ 0.13 ppm) are found in yard trimmings at composting facilities (9). During composting of yard trimmings, little pendimethalin was mineralized (13%). However, much of the pendimethalin (73%) was transformed into humic and unextractable residues (9).

Atrazine is one of the most widely used agricultural pesticides in the U.S. accounting for 12 percent of all pesticides used. It has widespread use in the control of broad-leaf weeds in corn, sorghum and certain other crops, and for lawn care. Also, it is one of the most frequently detected pesticides in surface waters in the U.S. Pesticide laden rinse waters from agricultural sites can contain up to 2000 ppm atrazine and atrazine has been detected in well waters at levels as high as 25 ug/liter. Rao et al (12) employed ^{14}C-atrazine amended wood products to determine the extent of atrazine mineralization and biodegradation during composting. Their results showed that atrazine carbon was mostly chloroform-extractable before composting but after 40 days of composting, much of the atrazine carbon had been converted into methanol- and NaOH- extractable residues. TLC analysis showed no detectable atrazine in the methanol extracts. These findings indicate that atrazine is converted to polar metabolites and is largely incorporated into humic acids during composting. The amount of atrazine mineralization observed during composting was from 2 to 7%, depending on the feedstock used (12).

Thus varying degrees of mineralization and humification of different pesticides are found during composting. Humification appears to be largely mediated by compost microorganisms, because in sterilized control composters the extent of conversion of pesticide carbon to humates was negligible (7). It should be noted, however, that there is little definitive information on the nature of pesticide-humate complexes, the amounts of pesticide carbon released over time from such complexes, and the relative toxicity of the pesticide carbon released. It is generally believed that the release of pesticide carbon from pesticide-humate complexes is a relatively slow process.

Bioremediation of Xenobiotics in Soils via Composting

Composts and composting have recently been used for the bioremediation of soils contaminated with explosives. In a field scale demonstration, Williams et al., (16) showed that explosives in contaminated soils such as TNT, RDX, nitrocellulose and HMX were reduced from greater than 10,000 ppm to less than 50 ppm during composting. In a succeeding study, Pennington (10) studied the fate of from ^{14}C TNT during composting and showed that almost no $^{14}CO_2$ was produced. Much of the ^{14}C-TNT was converted to amino derivatives of TNT and to conjugation products with cellulose and humin.

Chlorophenols were widely used as wood preservatives. These are important pollutants which persist in the environment, especially in soils near wood-processing facilities. Laine et al (4) showed that ^{14}C chlorophenols are converted primarily (60%) to $^{14}CO_2$ during bench scale composting of contaminated soils with softwood bark. During field scale composting, chlorophenol concentrations decreased from 850 ppm to less than 50 ppm with the accumulation of various chlorophenol metabolites. Inoculation of some soils with a chlorophenol degrading enrichment culture improved the initial rate of chlorophenol degradation. Collectively, these studies showed that in general composting is less costly than incineration or other remediation alternatives. Another advantage of composting is that it generates topsoil for site restoration.

In recent work, we have conducted field and laboratory scale tests on the bioremediation of PCB contaminated soils via composting. The microbial degradation of PCBs occurs primarily via cometabolism. In this process, PCBs are transformed, yet the organisms responsible are unable to grow on the PCBs and do not derive energy or carbon from their transformation. Therefore, a co-substrate is needed for microbial growth and activity. Composting can potentially provide both a highly diverse microbial environment, and the needed cosubstrates for PCB metabolism.

Few investigations on the effectiveness of composting in degrading recalcitrant pollutants such as polychlorinated biphenyls (PCBs) have been reported. Snell (14) reported that as much as 75% of a lower chlorinated PCB mixture (Aroclor 1221) added at a concentration of 500 mg/kg disappeared after 30 days of composting. In another study, Hogan et al. (2) reported 83% disappearance of Aroclor 1232 (11 mg/kg) and 1.6% volatilization after 35 days of composting at 50°C. These early studies suggested that PCB degradation occurs at a faster rate during composting than in soils alone; however, congener specific analysis and ^{14}C fate studies on PCB degradation were not done.

We recently initiated a study to determine the effect of yard trimmings amendment on PCB degradation during composting of a contaminated soil. The contaminated soil was located at a former paper mill and had a PCB concentration of 51± 21 ppm (identified as

Aroclor 1242), a moisture content of 65%, bulk density of 800 kg/m3, carbon content of 26%, C:N ratio of 50, and pH of 6.7. The contaminated soil was mixed with various levels of yard trimmings (1:1:1 leaves:grass:brush) and a shredder was used to mix the composts. The effect of amendment level on composting temperature, oxygen content, organic matter loss, and other environmental parameters which influence microbial activity were determined. These experiments showed that as little as 10% amendment (by weight) led to the development of active composting with temperatures greater than 50°C whereas little temperature change or oxygen uptake was observed in control piles containing soil alone. Degradation of PCB congeners in the composted soil was monitored by direct organic extraction and congener specific analysis. These results indicated a significant correlation between the level of amendment added to the contaminated soil and the overall PCB loss during composting. Less-chlorinated PCB congeners (1-3 chlorines) were degraded more than highly chlorinated congeners. Overall, however, the rate and extent of PCB degradation during composting was much less than that observed for pesticides and other organic contaminants.

Microbiology of Thermophilic Xenobiotic Degradation

An interesting question regarding xenobiotic degradation during composting is how thermophilic microorganisms and their enzymes responsible for this process differ from those active in mesophilic environments. We isolated thermophiles from lab scale composts that were actively mineralizing 2,4-D [Michel, Jr., FC, Topp E, Forney LJ, Reddy CA (1997) Abstr Ann Mtg Amer Soc Microbiol]. The isolates grew on 2,4-D as the sole carbon and energy source at 60° C, but not at 37° or 25°, and were Gram positive, spore-forming, motile rods. Sequencing of 16S rDNAs amplified from these isolates indicated that they were closely related to group 5 thermophilic Bacilli. The total DNA from these isolates was analyzed by Southern hybridization using 2,4-D degradation pathway gene probes *tfdA, tfdB, tfdC, tfdD* and *tfdE* cloned from plasmid *pJP4* of the mesophile *Alcaligenes eutrophus* JMP134. No hybridization was observed at high stringency (>90% homology) with any of the strains. However, all of the probes hybridized at medium stringency (70-90% homology) with total DNA from one isolate (X2), and hybridized at low stringency (>60% homology) with total DNA from another (X3). These results suggest that genes from thermophilic 2,4-D degraders are somewhat similar to 2,4-D degradation genes widely found in gram negative mesophiles from soils. Future studies will aim to sequence these thermophilic 2,4-D degradation genes for comparison with mesophilic genes.

Aerobic PCB degradation is believed to occur by cometabolism of biphenyl and similar compounds such as terpenes. A biphenyl dioxygenase (*bphA*) is known to be conserved in many PCB degrading mesophilic bacterial strains. However, the biphenyl degradation genes used by thermophilic PCB degraders are not known. By using primers corresponding to *bphA* genes from mesophiles, we recently PCR-amplified *bphA* genes using total DNA isolated from thermophilic composts made from PCB contaminated soils amended with yard trimmings [LaMontagne, MG, Quensen III JF, Tiedje JM, Reddy CA (1998) Abstr Ann Mtg Amer Soc Microbiol]. The *bphA* gene sequence was also amplifiable from thermophilic yard trimmings compost made without PCB contaminated soil, indicating the ubiquitous nature of *bphA* like genes. The sequences of the amplified *bphA* gene fragments found in composts were closely related to those from mesophilic PCB degrading strains.

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