Microorganisms Aerobically Degrading Skatole or Indole in Composting Processes

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Abstract The concentrations of skatole (3-methylindole) and indole were determined in the manure in chicken or pig waste composting processes, and microorganisms that aerobically degrade these indolic compounds were isolated. The concentration of skatole in the manure decreased as the treatment progressed in both composting processes. The concentration of indole decreased largely at the fermentation stage in the pig waste composting system ; however, that was almost stable in the chicken waste composting system. The numbers of total aerobes and skatole- or indole-degrading microbes decreased at the fermentation stage and increased again at the finishing stage in both composting systems. In the chicken waste system, the largest number of skatole- and indole-degrading microbes was occupied by facultatively anaerobic gram-positive cocci at the pretreatment and fermentation stages, and by aerobic gram-positive cocci at the finishing stage. In the pig waste system, that was occupied by facultatively anaerobic gram-positive cocci at any stage. Enzyme preparations which were extracted from cultures of skatole-degrading isolates or indole-degrading isolates showed degradation activities of skatole or indole, respectively.

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Composting has been widely used as an animal manure treatment system, and is effective methods for manure recycling¹⁵⁾. Recently, unpleasant smell from composting facilities is an important issue of a nuisance. Indole and its derivatives such as skatole (3-methylindole) are major malodorous compounds in the feces and sewage, and resistant to degradation^{3,4)}. Although some microorganisms have been known to degrade these indolic compounds^{1,2,6,7,11,13)}, a few studies were performed for microbes in composting processes¹⁵⁾. We had isolated anaerobic microbes from composting processes, and made clear their ability to degrade indolic compounds anaerobically^{9,10)}. Although the composting is widely believed to be an aerobic system of waste treatment, the aerobic degradation of indolic compounds in composting processes has not been clarified yet.

In the present study, we observed the change of

skatole and indole in the manure in composting processes. We also investigated microbes which aerobically degraded these indolic compounds in composting processes.

Materials and Methods

Sample

Samples were obtained from a chicken waste composting system and a pig waste composting system as mentioned previously⁹⁾. The water content of manure (%) at the pretreatment, fermentation, and finishing stage was 51.6, 61.3 and 20.1 in the chicken waste composting system, and 59.6, 63.0 and 24.9 in the pig waste composting system, respectively. Temperature of the manure (°C) at each stage was 50, 60 and 20 in the chicken waste composting system, and 40, 75 and 20 in the pig waste composting system, respectively.

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Measurement of skatole or indole

Concentrations of skatole and indole were measured by gas chromatography as mentioned previously¹⁰⁾.

Bacterial count

Aerobic microbes were counted by 10-fold dilution methods using a sterilized saline and a cultivation on trypticase soy agar plates added with 10% compost extracted solution. The compost extracted solution was prepared as described by Gordon and Rynearson⁵⁾. The solution extracted from the chicken or pig waste compost was used for cultivation of microbes from the chicken or pig waste composting system, respectively. The number of skatole or indole-degrading aerobic microbes was determined by colony counts on media which contained skatole or indole as a sole source of carbon, respectively. The media contained the following nutrients; skatole or indole 0.3 g, NaCl 0.1 g, KH₂PO₄ 0.5 g, KHPO₄ 1.5 g, MgSO₄ 0.35 g, agar 15 g, (pH 7.0) in 1 l of water. Skatole and indole were dissolved in a minimum amount of methanol and added to the autoclaved medium as previously mentioned⁹⁾. Typing of microbes were performed by the methods of Mitsuoka *et al.*¹²⁾. These cultures were incubated 7 days at room temperature (25–30°C) or 37°C.

Enzyme activity

Among microbes aerobically degrading skatole, three isolates were chosen; *i.e.* SC-54 (facultatively anaerobic gram-positive coccus), SP-06 (facultatively anaerobic gram-positive endospore-forming rod), and SC-53 (facultatively anaerobic gram-positive endospore-forming rod). Among microbes aerobically degrading indole, three isolates were chosen; *i.e.* IC-55 (facultatively anaerobic gram-positive coccus), IP-03 (aerobic gram-positive coccus), and IC-53 (aerobic gram-positive endospore-forming rod). SC-54, SC-53, IC-55 and IC-53 were isolated from the chicken waste composting process, and SP-06 and IP-03 were isolated from the pig waste composting process. Isolates were incubated in trypticase soy broth for 42 h at 37°C. The culture media and microbes were French-pressed together, and centrifuged at 10,000×g and the supernatant was filtrated through 0.45 µm millipore filter. Skatole and indole was added to the filtrate at

the final concentration of 30 mg/l. Concentrations of skatole and indole were determined after incubation at 37°C for 42 h.

Results

Decomposition of skatole and indole in the waste composting processes

Figure 1 showed the concentrations of skatole and indole in the manure in the composting processes. In the chicken waste composting system, concentration of skatole decreased as the treatment progressed, and that of indole was almost stable in the process. In the pig waste composting system, concentration of skatole decreased as the treatment progressed; however, that of indole decreased largely at the fermentation stage and was stable after that stage. Although the concentration of these indolic compounds was higher in the pig waste than that in chicken one at the pretreatment stage, the concentration of these compounds was similar level at the finishing stage.

Microbes aerobically degrading skatole or indole in the composting systems

Figure 2 showed the numbers of aerobic microbes in the standard culture plate which did not contain any indolic compounds. The numbers decreased at the fermentation stage and increased again at the finishing stage in both composting systems. We tried to detect microbes at two cultivation temperature, *i.e.* room temperature as the environmental condition and 37°C

Fig. 1. Concentrations of skatole (■) and indole (○) in the composting processes of chicken waste (A) and pig waste (B). Samples were collected from the pretreatment stage (Pre), the fermentation stage (Fer) and the finishing stage (Fin). The concentrations of indolic compounds were measured as described in the text.

Fig. 2. Numbers of aerobes in the composting processes of chicken waste (A) and pig waste (B). Samples were collected from the pretreatment stage (Pre), the fermentation stage (Fer) and the finishing stage (Fin), and cultivated on the standard culture plates without any indolic compounds at room temperature (25–30°C : ●) or at 37°C (○).

as the excreted fecal condition. There was little difference between the number in the culture at room temperature and the number at 37°C at each stage.

Microbes which aerobically degrade skatole or indole were detected from all stages of composting (Fig. 3). The numbers of skatole- or indole-degrading microbes decreased at the fermentation stage and increased again at the finishing stage in both composting systems. The number of skatole-degrading microbes and that of indole-degrading microbes were similar levels at each stage. There was little difference between the number in the culture at room temperature and the number at 37°C at each stage in the chicken waste composting; however, in the pig waste composting relatively larger numbers were observed in the culture at 37°C than at room temperature at the fermentation stage and the finishing stage. The numbers of skatole- or indole-degrading microbes were in the range of 1/1,000 to 1/100,000 of the number of aerobes in the standard culture at each stage.

Types of microbes aerobically degrading skatole or indole

Types of microbes degrading these indolic compounds were investigated among colonies. In the chicken waste composting (Table 1), skatole degrading microbes were classified into 3 types, *i.e.* facultatively anaerobic gram-positive cocci, aerobic gram-positive cocci, and facultatively anaerobic gram-

Fig. 3. Numbers of microbes aerobically degrading skatole (■, □) or indole (●, ○) in the composting processes of chicken waste (A, B) and pig waste (C, D). Samples were collected from the pretreatment stage (Pre), the fermentation stage (Fer) and the finishing stage (Fin), and were cultivated on the plates containing skatole or indole at room temperature (25–30°C : ■, ●) or at 37°C (□, ○).

positive endospore-forming rods. Indole-degrading microbes were classified into 2 types, *i.e.* facultatively anaerobic gram-positive cocci and aerobic gram-positive endospore-forming rods. At the pretreatment and fermentation stages, facultatively anaerobic gram-positive cocci were the largest in number among both skatole- and indole-degrading microbes. At the finishing stage, aerobic gram-positive cocci or aerobic gram-positive endospore-forming rods were the largest in number among skatole- or indole-degrading microbes, respectively.

In the pig waste composting (Table 2), 3 types of skatole degrading microbes were detected, *i.e.* facultatively anaerobic gram-positive cocci, aerobic gram-positive cocci, and facultatively anaerobic gram-positive endospore-forming rods. Indole-degrading microbes were classified into 3 types, *i.e.* facultatively anaerobic gram-positive cocci, aerobic gram-positive cocci and aerobic gram-positive endospore-forming

rods. Facultatively anaerobic gram-positive cocci were the largest in number at any stage among both skatole- and indole-degrading microbes.

Neither of gram-negative bacteria, actinomycetes or fungi were detected at any stage in the chicken and pig composting systems among microbes degrading

Table 1. Types of microbes aerobically degrading skatole or indole in the chicken waste composting process

Types of microbes ¹⁾	Pretreatment stage		Fermentation stage		Finishing stage	
	Number ²⁾	% ³⁾	Number	%	Number	%
Skatole-degrading microbes						
FPC	5.90	48.3	2.10	62.5	4.05	32.8
APC	5.50	19.0	1.40	12.5	4.35	67.2
FPER	5.74	32.8	1.70	25.0	3.23	5.0
Indole-degrading microbes						
FPC	6.25	74.1	3.56	95.0	4.25	25.0
APER	5.86	28.6	2.28	5.0	4.73	75.0

¹⁾ FPC, facultatively anaerobic gram-positive cocci ; APC, aerobic gram-positive cocci ; FPER, facultatively anaerobic gram-positive endospore-forming rods ; APER, aerobic gram-positive endospore-forming rods.

²⁾ Mean of log CFU/g of the manure wet weight.

³⁾ % of the total number of skatole- or indole-degrading microbes.

Table 2. Types of microbes aerobically degrading skatole or indole in the pig waste composting process

Types of microbes ¹⁾	Pretreatment stage		Fermentation stage		Finishing stage	
	Number ²⁾	% ³⁾	Number	%	Number	%
Skatole-degrading microbes						
FPC	4.78	42.4	4.34	88.1	5.34	88.0
APC	4.49	21.8	2.94	3.5	4.40	10.0
FPER	4.70	35.4	3.32	8.4	3.70	2.0
Indole-degrading microbes						
FPC	5.30	46.3	3.01	84.7	5.31	54.5
APC	4.81	14.9	0	0	0	0
APER	5.22	38.8	2.26	15.3	5.23	45.5

¹⁾ FPC, facultatively anaerobic gram-positive cocci ; APC, aerobic gram-positive cocci ; FPER, facultatively anaerobic gram-positive endospore-forming rods ; APER, aerobic gram-positive endospore-forming rods.

²⁾ Mean of log CFU/g of the manure wet weight.

³⁾ % of the total number of skatole- or indole-degrading microbes.

skatole or indole.

Enzyme activity

The enzyme activity of crude extract of cultures of isolates was measured (Table 3). As the concentration of the substrates, 30 mg/l of skatole or indole which was as high as their concentration at the pretreatment stage of the pig waste composting system was used. Extracts of cultures of microbes which were isolated from the skatole medium degraded 9.0–20.4% of skatole as the substrate, and that from the indole medium degraded 19.6–34.1% of indole. The results directly indicated that these isolates had the ability to degrade these indolic compounds.

Discussion

The concentrations of skatole or indole in the manure especially in the pig waste composting process decreased as the treatment progressed (Fig. 1). It suggests that these indolic compounds were degraded through the composting process. Higher numbers of microbes degrading skatole or indole were observed at the pretreatment stages (Fig. 3). They may degrade indolic compounds during the stage from pretreatment to fermentation. Their numbers decreased in the fermentation stages and increased again in the finishing stages (Fig. 3) accompanied by the change of the number of aerobes (Fig. 2). Such a pattern was commonly observed for mesophilic bacteria in composts¹⁵⁾. These results indicate that skatole- or indole-degrading microbes exist constantly in the mesophilic microbial flora in the composting process.

Skatole- or indole-degrading microbes were larger in number in the culture at 37°C, compared with those

at room temperature at the fermentation and finishing stages in the pig waste composting process. The temperature at the fermentation stage in the pig waste composting process was 75°C, which was relatively high in common composting systems. This results may suggest the selection of microbes which can survive under the higher temperature. Although no thermophilic microbes which anaerobically degrade skatole or indole were detected in the composting processes⁹⁾, we should investigate the thermophilic microbes which aerobically degrade these indolic compounds in the future work.

Facultatively anaerobic gram-positive cocci were dominant at the pretreatment and fermentation stages in both composting systems and also at the finishing stage in the pig waste composting system. Our previous work⁹⁾ showed that 67 to 91% were facultative anaerobes among microbes which anaerobically degrade skatole or indole in the composting systems. Neither of gram-negative bacteria, actinomycetes or fungi were detected among microbes degrading skatole or indole under the aerobic condition in this study and the anaerobic condition⁹⁾. Therefore, facultatively anaerobic bacteria may dominantly degrade skatole and indole aerobically and anaerobically at the early stages of the composting processes. Composts are generally in aerobic conditions; however, anaerobic conditions prevail in the interior of composts under some circumstances^{8, 14)}. Therefore, facultatively anaerobic bacteria may have the advantage over aerobes or anaerobes in the composting process.

In the chicken waste composting, aerobic gram positive microbes instead of facultative anaerobes were dominant at the finishing stage. There are many factors affecting microbial dominancy, e.g. water components, air flow, fermentation temperature or contents of waste. The mechanisms of the selection of microbes should be determined under the laboratory condition in the future study.

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Table 3. Enzyme activity of crude extract of cultures of isolates

Isolate	Substrate	Substrate degradation rate (%)
SC-54	Skatole	11.7
SP-06	Skatole	20.4
SC-53	Skatole	9.0
IC-55	Indole	34.1
IP-03	Indole	19.6
IC-53	Indole	20.7

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