Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 19(2007) 1014–1019
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Microbial safety control of compost material with cow dung by heat treatment

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Received 13 October 2006; revised 11 December 2006; accepted 5 January 2007

Abstract

Various kinds of pathogenic bacteria derived from the intestinal tract of animals exist in compost material like cow dung. In order to sterilize the pathogenic bacteria completely in compost material, the cow dung was put into a heat treatment machine in pilot plan, and harmless condition in short time was examined. The results indicated, pathogenic indicator bacteria such as coliform bacteria, fecal coliform, *Escherichia coli* and salmonella were all 10^6 cfu/g dw at the beginning, died rapidly when cow dung temperature rose to above 50°C, and not detected at 54–68°C for 6–24 h heat treatment. Coliform bacteria and salmonella in heated cow dung were not detected by re-growth culture and enrichment culture examination. Moreover, it was hardly influenced on the fermentation ability of composting microbe, organic decomposition bacteria. During heat treatment, the mesophile decreased rapidly and the thermophile stabilized or increased, and the most of composting microbe were bacillus in cow dung by fluorescence microscope, this indicated that bacillus was dominator and composting microbe in composting process.

Key words: microbial safety control; pathogenic bacteria; compost; cow dung; heat treatment

Introduction

Increasing public interests have been concerned on safety foods and the environmental aspects recently, especially on the so-called organic foods, which are made from crops grown with organic fertilizers or compost but not chemical fertilizers. Public concerns on organic products have accelerated the use of compost even to vegetables that are usually consumed without heat treatment. Compost made from animal waste and other organic refuse can serve as a valuable nutrient resource to the agricultural fields, and decrease environmental load if they are treated properly.

However, various kinds of pathogenic bacteria such as coliform bacteria and salmonella exist in the compost materials (Deportes *et al*., 1995). In cow dung, sewage sludge and foodresidues, the number of coliform bacteria was detected up to 10^5–10^9 cfu/g dw (Greenberg *et al*., 1986; Pereira-Neto *et al*., 1986; Pera *et al*., 1991; Vuorinen and Saharinen, 1997; Deportes *et al*., 1998). The detected rate of coliform bacteria was higher in organic vegetables than that in commercial vegetables (Ueda and Kuwabara, 2002). It has been reported that several foodborne outbreaks of bacterial infections were associated with the consumption of raw fruits and vegetables contaminated by manure (Cieslak *et al*., 1993; Chapman *et al*., 1997; Itoh *et al*., 1998; Little *et al*., 1999). Among human pathogens associated with compost, *Escherichia coli*, especially that of serogroup O157:H7, could be the most serious menace.

It is considered that detrimental microbe in compost mostly become extinct with fermentation heat. However, the temperatures of the inside and outside compost pile are not uniform and the outside temperature is lower than that of the inside one. It could have been 60°C inside, but still be 30–40°C outside. Therefore, it is possible that some pathogenic bacteria are survived in the compost. Moreover, these survived pathogenic bacteria were also detected in the compost product (Pera *et al*., 1991; Soares *et al*., 1995; Sciancalepore *et al*., 1996; Gong *et al*., 2005a, b).

In this research, compost material was put in the large-size sealing container in pilot plan, and investigated the microbial safety control conditions at about 60°C during a short time. In addition, in order to check whether pathogenic bacteria were survival or damage state in heated compost material (Mote *et al*., 1988; Deportes *et al*., 1998; Someya *et al*., 2003), and whether the organic decomposition bacteria were destructed by heat treatment, re-growth culture, enrichment culture experiment and fermentation ability were examined.

1 Materials and methods

1.1 Microbial safety experiment by heat treatment

1.1.1 Microbial safety equipment

Fig.1 is the assumption diagram of the drum rotation machine (diameter: 1.36 m; length: 2.17 m; capacity 2.2 m^3). This machine can process about 1 t compost materials (cow dung) every time, and it was attached with three

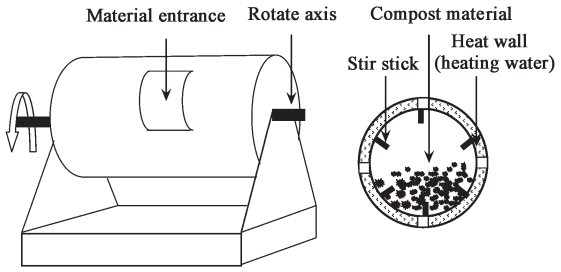

Fig. 1 Assumption diagram of drum rotation heat-treatment machine.

heaters (1 kW).

1.1.2 Compost materials and heat treatment method

The experiment was performed at Shiroishi-chou of Saga, Japan. Compost materials were the raw cow dung with moisture content of 82% (cow dung 1), and the raw cow dung with moisture content of 51% (cow dung 2, a part of the cow dung 1 was ventilated at room temperature using an electric fan, until the moisture content was decreased to 51%). Both of cow dung 250 kg was put into the heat treatment machine, respectively. Heat treatment was at about 60°C for 24 h, the rotating speed of the machine was maintained at 1 r/min.

1.1.3 Sampling, measurement of temperature and moisture

Cow dung samples (each 500 g) were collected at five different places after heat treated for 0, 1, 3, 6 and 24 h, respectively. These samples were well mixed and about 500 g of the mixture was taken and sealed in a plastic bag, which was kept in a cooler and carried to the laboratory. All samples were analyzed in laboratory within 2 d after collection. Temperature was measured at five different places by temperature meter (Sansho, Japan) and then got the mean values at the same time with collecting samples. Moisture content was measured by the water meter (Halogen HG53, Japan) by taken about 1g from the well mixed sample.

1.1.4 Determination of various bacteria

Each sample (10 g wet weight) was added to sterile saline solution (95 ml, 0.85% NaCl), and homogenized at 15000 r/min for 15 min with homogenizer (Nissei AM-3, Japan), then 10-fold serial dilution was made with sterile saline solution, to obtain dilutions of 10^{-1}–10^{-7}. The population density was determined by the direct count methods and the culture methods. The total direct count (TDC) by ethidium bromide (EB) fluorescence dyeing method (Someya, 1995), and the number of viable bacteria by 6-carboxy fluorescein diacetate (CFDA) fluorescence dyeing method (Yamamoto *et al*., 1996) were occupied in a sample with direct count by fluorescence microscope (Nikon EFD-3, Japan), respectively.

The number of mesophile, thermophile, the organic decomposition bacteria (cellulolytic bacteria, lipolytic bacteria, amylolytic bacteria and proteolytic bacteria), coliform bacteria, fecal coliform, *E. coli* and salmonella were determined by the dilute plate counts method (DPC) or the most probable number method (MPN), their media and cultural conditions are shown in Table 1.

For DPC methods, three aliquots (100 μl) of each diluted suspension (the mesophile, thermophile and the organic decomposition bacteria by 10^{-5}, 10^{-6} and 10^{-7}, or coliform bacteria, *E. coli*, and salmonella by 10^{-1}, 10^{-3} and 10^{-5}) were inoculated on triplicate plates. Plates containing 25 to 250 colonies were enumerated and recorded as colony forming units (CFU) per gram of the dry weight, after incubation at 30°C or 37°C for 1–5 d. For MPN method, five aliquots (1 ml) of each dilution (fecal coliform by 10^{-1} to 10^{-5}) were inoculated into the medium (10 ml) in quintuplicate test tubes. The growth of bacteria was observed after incubation at 45°C for 24 h, and the number of bacteria was calculated.

1.2 Re-growth culture examination of pathogenic bacteria

Put 150 g of heat treated cow dung into the sterilized jar and sealed, and incubated at 30°C for 7, 14 and 21 d, respectively. Then, 10 g sample was homogenized from 10^{-1} to 10^{-5} diluted suspension, and 100 μl suspension was inoculated into DCA or MLCB culture medium, respectively, incubated at 37°C for 24 h. Finally, it would be decided whether there were colony of coliform bacteria or salmonella.

1.3 Enrichment culture examination of pathogenic bacteria

Heat treated cow dung (25 g) was put into 225 ml of lactose broth medium (Eiken, Japan) or EEM broth medi-

Table 1 Media and cultural conditions of microbe

Microbe	Medium	Method	Condition	Reference
Mesophile	NA	DPC	30°C, 5 d	Sugiyama *et al*., 1999
Thermophile	NA	DPC	60°C, 5 d	Sugiyama *et al*., 1999
Cellulolytic bacteria	CMC	DPC	30°C, 3 d	Suyama *et al*., 1993
Lipolytic bacteria	PSM	DPC	30°C, 5 d	Sierra, 1956
Amylolytic bacteria	SS	DPC	30°C, 3 d	Cowan, 1979
Proteolytic bacteria	CS	DPC	30°C, 1 d	Martley *et al*., 1970
Coliform bacteria	DCA	DPC	37°C, 1 d	Japan Pharmaceutical Association, 2000
Fecal coliform	ECB	MPN	45°C, 1 d	Japan Pharmaceutical Association, 2000
Escherichia coli	CCA	DPC	37°C, 1 d	Frampton *et al*., 1988
Salmonella	MLCB	DPC	37°C, 1 d	Japan Pharmaceutical Association, 2000

NA: nutrient agar (Eiken, Japan); CMC: carboxymethyl cellulose (Sigma, USA); PSM: polyoxyethylene sorbitan monolaurate (Nacalaitescue, Japan); SS: soluble starch (Katayama, Japan); CS: casein sodium (Katayama, Japan); DCA: desoxycholate agar (Eiken, Japan); EC: *Escherichia coli* broth (Eiken, Japan); CCA: chromocult coliform agar (Merck, Germany); MLCB: mannitol L-lysine crystalviolet brilliantgreen agar (Nissui, Japan).

um (Eiken, Japan), respectively, incubated at 36°C for 24 h. After that, every incubation suspension was inoculated into DCA and MLCB culture medium, respectively to observe whether there were colony of coliform bacteria or salmonella.

1.4 Fermentation ability examination

After heat treated for 24 h, the cow dung 2 was incubated at 30°C for 7 d, and analyzed the number of TDC, total number of viable bacteria, mesophile, thermophile, the organic decomposition bacteria (cellulolytic bacteria, lipolytic bacteria, amylolytic bacteria and proteolytic bacteria), coliform bacteria, fecal coliform, *E. coli* and salmonella. The changes were compared before and behind various bacteria, especially, the organic decomposition bacteria useful for making composting.

2 Results and discussion

2.1 Temperature change of cow dung during heat treatment

Fig.2 shows the temperature change of machine wall and the cow dungs. In the case of cow dung 1, temperature of machine wall and cow dung was 62 and 34°C at the beginning, and the cow dung became 50 and 54°C after 3 and 6 h heat treatment, respectively, finally reached 66°C after 24 h. To the case of cow dung 2, temperature of cow dung was 32°C when it was put into the machine of 63°C, then got to 52 and 59°C after 3 and 6 h heat treatment, and reached to 64°C when the heat treatment was over after 24 h. As shown in Fig.2, the temperature rose quickly during 0–6 h, and then the increased speed fell down but increased continually, during 6–24 h heat treatment, and temperature of cow dung 1 rose slowly than cow dung 2.

The results showed that the temperature of the whole cow dung can be equably heated to about 60°C very quickly in 24 h by this heat treatment machine.

2.2 Change of bacteria number in cow dung during heat treatment

2.2.1 Change of pathogenic indicator bacteria

Fig.3a shows the change of pathogenic indicator bacteria for cow dung 1. The number of coliform bacteria, fecal

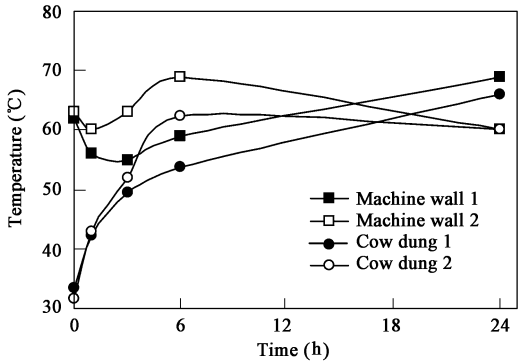

Fig. 2 Temperature change of the machine wall and cow dung during heat treatment.

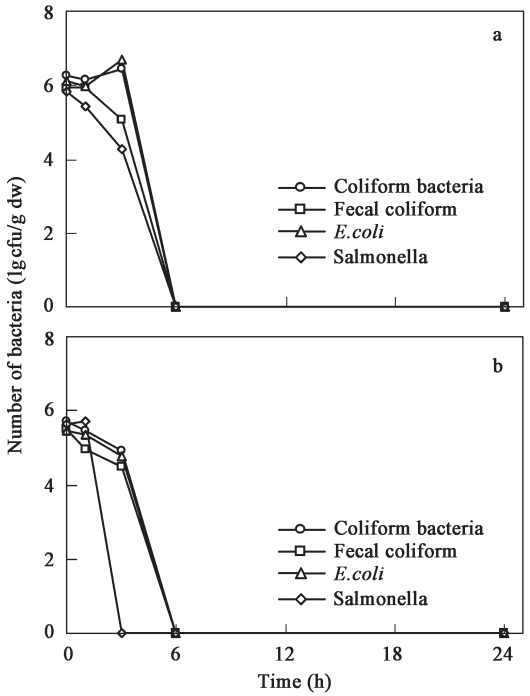

Fig. 3 Number change of pathogenic indicator bacteria during heat treatment. (a) cow dung 1; (b) cow dung 2. not detected ($<10^2$ cfu/g dw) to coliform bacteria, *E. coli* and salmonella; not detected ($<10^1$ MPN/g dw) to fecal coliform.

coliform, *E. coli* and salmonella were about 10^6 cfu or MPN/g dw at the beginning, decreased quickly after 3 h heat treatment and were not detected after 6 h heat treatment. However, the number of bacteria kept almost unchanged except that of salmonella decreased after 1 h heat treatment, when the temperature increased from 34 to 42°C, which meant that the multiplication and death of pathogenic indicator bacteria were almost kept dynamic balance at this temperature. When temperature was risen from 42 to 50°C during 1–3 h heat treatment, coliform bacteria and *E. coli* were increased in cow dung 1, which suggested that a part of high temperature-resistant pathogenic bacteria existed in cow dung materials with the higher moisture content.

The number change of pathogenic indicator bacteria for cow dung 2 is shown in Fig.3b. All of them were about 10^6 cfu or MPN/g dw at the beginning, and decreased slowly except that of salmonella after 3 h heat treatment, and then all of them were not detected after 6 h heat treatment. The number change of salmonella was almost the same as that in cow dung 1 after 1 h heat treatment, when the tempterature increased from 43°C to 52°C, while it was not detected after 3 h heat treatment in cow dung 2. The phenomena mean that the salmonella is more sensitive to high temperature than the other pathogenic bacteria.

All of pathogenic indicator bacteria in cow dung 1 and 2 were died rapidly when temperature was risen to above 50°C after 3 h heat treatment, and not detected after 6 h. This result indicated that the fatal temperature of pathogenic bacteria in compost materials can be gotten in a short time by the heat treatment machine.

Until now, when the temperature and the period of pathogenic bacterial destruction in composting process

were described, it is usually reported as "above 55°C for more than 3 d" (Greenberg *et al*., 1986), "55°C to 60°C for more than 3 d" (Pereira-Neto *et al*., 1986), or "above 65°C for more than 2 to 3 d" (Japan Greenhouse Horticulture Association, 2003), etc. In this study, it is above 50°C for 6–24 h of the detection limit, which is much shorter than the previous reported data.

2.2.2 Change of other various bacteria

Fig.4 shows the number change of TDC and the total viable bacteria during the heat treatment. In cow dung 1, TDC reduced from 2.1×10^{11} to 9.3×10^{10} cells/g dw, and total viable bacteria decreased from 4.5×10^{10} to 9.0×10^{9} cells/g dw after heat treatment for 6 h, only a half and 1/5 of the original numbers. Similarly, in cow dung 2, TDC reduced by half from 3.4×10^{11} to 1.4×10^{11} cells/g dw after 6 h from the beginning, total viable bacteria decreased to about 1/14 of the original number, 8.5×10^{10} to 5.9×10^{9} cells/g dw for 6 h.

The number of total viable bacteria in cow dung 2 decreased the fastest when temperature of cow dung was risen to above 50°C at 3–6 h heat treatment, then it became slowly under rising temperature continually. It may be considered that, when the microbe died rapidly, and a part of high temperature bacterium grew in heat treated cow dung simultaneously.

The number change of heterotrophic microorganisms is shown in Fig.5. In cow dung 1, the number of thermophile changed in the range of 1.0×10^{6}–6.6×10^{6} cfu/g dw. While the mesophile decreased rapidly to 1.8×10^{7} and 1.3×10^{6} cfu/g dw after 6 and 24 h from the original number of 1.8×10^{10} cfu/g dw at the beginning. In cow dung 2, thermophile increased from 3.9×10^{5} to 1.0×10^{7} cfu/g dw after heat treated for 6 h, about 24 times was increased. While the mesophile decreased from 1.8×10^{10} to 9.3×10^{8}, 5.5×10^{7} and 4.6×10^{10} cfu/g dw after 3, 6, and 24 h, respectively.

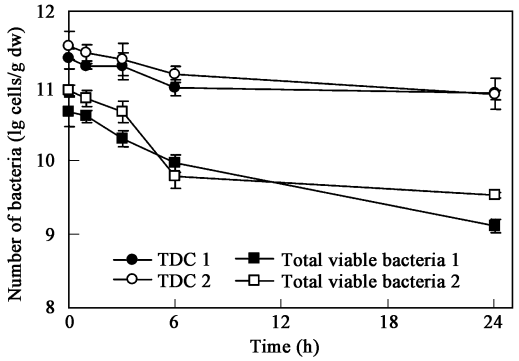

Fig. 4 Change of total direct count (TDC) and total viable bacteria during heat treatment.

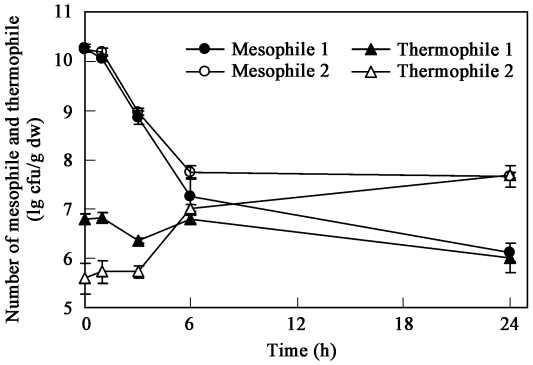

Fig. 5 Change of mesophile and thermophile in cow dung during heat treatment.

After heat treatment, the thermophile in cow dung kept almost unchanged (cow dung 1) or increased (cow dung 2), while the mesophile decreased very quickly during the period of 0–6 h heat treatment, and the death became slower during the period of 6–24 h of heat treatment. The more important one is that, the number of mesophile and the thermophile became almost the same after heat treated for 24 h, both for cow dung 1 and cow dung 2, which suggested that thermophile is from some kinds of high temperature-resistant mesophile. In the experiment, it could be also observed that the most of thermophile composting microbe were bacillus in cow dung by fluorescence microscope, which indicated that bacillus was dominator and composting microbe in future composting process.

2.3 Effect of re-growth culture on pathogenic bacteria

It can not be detected if the number was below the detection limit, it was possible that slightly survival pathogenic bacteria can be re-growth (Mote *et al*., 1988; Deportes *et al*., 1998; Someya *et al*., 2003) when temperature fell in late period of heat treatment. Therefore, re-growth culture was used to examine whether coliform bacterial and salmonella pathogenic bacteria were survival in heated cow dung. After 6 and 24 h heat treated cow dung were incubated at 30°C for 7, 14 and 21 d, respectively. The experimental results show that neither coliform bacterial nor salmonella were detected in both of cow dung 1 and 2.

2.4 Effect of enrichment culture on pathogenic bacteria

It is possible that some pathogenic bacteria changed into the damage state and undetectable in the selection culture medium (Someya *et al*., 2003). Therefore, enrichment culture was performed in this research. Table 2 lists the detection result of pathogenic bacteria enrichment culture in heat treated cow dung. Both coliform bacteria and salmonella was not detected after 6 h in cow dung 1, coliform bacterial was not detected in cow dung 2, but salmonella was re-detected in 6 h heat treatment. It indicates that there is a possibility of the pathogenic bacteria existence in undetected compost materials.

On the other hand, these results indicate that, when moisture content is low, bacteria destruction is slower than

Table 2 Examination of enrichment culture on pathogenic bacteria in heat treated cow dung

Sample	Heating time (h)	Coliform bacteria (cfu/g dw)	Salmonella (cfu/g dw)
Cow dung 1	6	ND	ND
	24	ND	ND
Cow dung 2	6	ND	3.3×10^{3}
	24	ND	ND

ND: not detected ($<10^{2}$ cfu/g dw).

Table 3 Number of bacteria in raw cow dung, heated and incubated cow dung*

Bacteria	Number of bacteria (cells or cfu/g dw)		
	Not heat treated cow dung	60°C, 24 h heat treated cow dung	60°C, 24 h heat treated cow dung was incubated 7 d, 30°C
TDC	$(3.4\pm1.2)\times10^{11}$	$(7.3\pm1.0)\times10^{10}$	$(1.8\pm0.3)\times10^{11}$
Total viable bacteria	$(8.5\pm1.4)\times10^{10}$	$(3.3\pm0.2)\times10^{9}$	$(4.7\pm0.9)\times10^{10}$
Mesophile	$(1.7\pm0.2)\times10^{10}$	$(4.6\pm1.8)\times10^{7}$	$(1.9\pm0.2)\times10^{10}$
Thermophile	$(3.9\pm2.0)\times10^{5}$	$(4.8\pm0.7)\times10^{7}$	$(3.1\pm0.4)\times10^{7}$
Cellulolytic bacteria	$(1.2\pm0.1)\times10^{9}$	$(3.0\pm0.4)\times10^{8}$	$(3.8\pm0.2)\times10^{8}$
Lipolytic bacteria	$(9.5\pm0.7)\times10^{8}$	$(1.9\pm0.2)\times10^{7}$	$(4.5\pm0.5)\times10^{9}$
Amylolytic bacteria	$(6.0\pm0.6)\times10^{8}$	$(4.5\pm2.6)\times10^{7}$	$(6.1\pm0.4)\times10^{8}$
Proteolytic bacteria	$(6.7\pm3.1)\times10^{7}$	$(4.6\pm1.4)\times10^{6}$	$(3.2\pm0.4)\times10^{8}$

*Mean values±standard deviations from 10 microscopic observations were shown.

the higher moisture content, even at the same temperature. And this is the same as the result of author's research on survival of *E. coli* in compost (Gong *et al*., 2005a, b).

2.5 Heat treatment influence on the composting bacteria

In order to check whether the organic decomposition bacteria were destructed when the pathogenic bacteria in cow dung were destructed by heat treatment, the fermentation ability examination was preformed. The number of bacteria recovered by incubated for 7 d, although TDC, total viable bacteria, and mesophile decreased by heat treatment after 24 h. On the other hand, the number of thermophile increased by heat treatment, and there was almost no influence after incubation. As for the case of the organic decomposition bacteria, which is useful to composting, such as cellulolytic bacteria, lipolytic bacteria, amylolytic bacteria and proteolytic bacteria were decreased largely after heat treated for 24 h (Table 3). However, after incubated at 30°C for 7 d, all the numbers except cellulolytic bacteria recovered to almost the same as that in unheated cow dung, and this result indicates that heat treatment has almost no influence on composting bacteria in cow dung.

3 Conclusions

The main conclusions obtained are as follows:

The fatal temperature of pathogenic bacteria in compost material can be reached in a short time by the heat treatment machine.

During the heat treatment, coliform bacteria, fecal coliform, *E. coli* and salmonella in cow dung decrease rapidly and are hardly detected after 24 h heat treatment in both of cow dung with 82% and 51% moisture content. They are undetected after the re-growth and enrichment culture examination.

The most of composting microbe are bacillus in cow dung, and bacillus is dominator and composting microbe in composting process.

Heat treatment has almost no influence on composting bacteria in cow dung.

Acknowledgements

The author thanks Gu Wei-bing for his assistance, and Ph.D T. Someya for his technical guidance.

References

Betoldi M, Zucconi F, Civilini M, 1988. Temperature, Pathogen control and product quality[J]. Biocycle, 29: 43–50.

Chapman P A, Siddons C A, Manning J, 1997. An outbreak of due to verocytotoxin–producing *Escherichia coli* O157 in four families: the influence of laboratory methods on the outcome of the investigation[J]. Epidemiol Infect, 119: 113–119.

Cieslak P R, Barrett T J, Griffin P M, 1993. *Escherichia coli* O157:H7 infection from a manured garden[J]. The Lancet, 342: 367.

Cowan S T, 1979. Manual for the identification of medical bacteria[M]. Tokyo: Kindai Press. 335.

Deportes I, Benoit-Gyuod J L, Zmirou D *et al*., 1995. Hazard to man and the environments posed by the use of urban waste compost: a review[J]. The Science of the Total Environment, 172: 197–222.

Deportes I, Benoit-Gyuod J L, Zmirou D *et al*., 1998. Microbial disinfection capacity of municipal solid waste (MSW) composting[J]. Applied Microbiology, 85: 238–246.

Frampton E W, Restaino L, Blaszko L, 1988. Evaluation of β-glucuronidase substrate 5-bromo-4-chloro-3-indol-β-D-glucuronide (X–GLUC) in a 24 hour direct plating method for *Escherichia coli*[J]. Journal of Food Protection, 51: 402–404.

Gong C M, Inoue K, Inanaga S *et al*., 2005a. Survival of pathogenic bacteria in compost with special reference to *Escherichia coli*[J]. J Environ Sci, 17: 770–774.

Gong C M, Koshida J, Moriyama N *et al*., 2005b. Occurrence and survival of coliform bacteria, *Escherichia coli* and *Salmonella* in various manure and compost[J]. Jpn J Soil Sci, 76: 865–874.

Greenberg A E, Shastid T B, Ellgas W M, 1986. Quanity control monitoning[J]. Biocycle, 27: 36–38.

Itoh Y, Sugita-Konishi Y, Kasuga F, 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts[J]. Appl Environ Microbiol, 64: 1532–1535.

Japan Greenhouse Horticulture Association, 2003. Raw vegetable hygiene management guide[M]. Tokyo: Japan. 35–39.

Japan Pharmaceutical Association, 2000. Methods of analysis in health science[S]. Tokyo: Kingen Press. 69–89.

Little C, Roberts D, Youngs E, 1999. Microbiological quality of retail imported unprepared whole lettuces: a PHLS food working group study[J]. Food Prot, 62: 325–328.

Martley F G, Jayashankar S R, Lawrence R C, 1970. An improved agar medium for the detection of proteolytic organisms in total bacterial counts[J]. Appl Bact, 33: 363–370.

Mote C R, Emerton B L, Sallison J *et al*., 1988. Survival of coliform bacteria in static compost pilis of dairy waste

solids intended for free stall bedding[J]. Dairy Sci, 71: 1676–1681.

Pera A, Vallini G, Frassinetti S *et al*., 1991. Co-composting for managing effluent from themophilic anaerobic digestion of municipal solid waste[J]. Environmental Technology, 12: 1137–1145.

Pereira-Neto J T, Stentiford E I, Smith D V, 1986. Survival of fecal indicator micro-organisms in refuse/sludge composting using the aerated static pile system[J]. Waste Management & Research, 4: 397–406.

Sciancalepore V, Colangelo M, Sorlini C *et al*., 1996. Composting of effluent from a new two-phases centrifuge olive mill microbial characterization of the compost toxicol[J]. Environ Chem, 55: 145–158.

Sierra G, 1956. A simple method for the detection of lipolyticactivity of microorganisms and some observations on the influence of the contact between cells and fatty substrates[J]. Antonie van Leeuwenhoek, 23: 15–22.

Soares H M, Cardenas B, Weir D *et al*., 1995. Evaluating pathogenre-multiplication in biosolids compost[J]. Biocycle, 36: 70–72.

Someya T, 1995. Three-dimensional observation of bacteria inorganic debris with a confocal laser scanning microscope[J]. Jpn Soil Microorganisms, 46: 61–69.

Someya T, Gong C M, Inoue K, 2003. Recent topics on the biosafety control of composting[C]. In: Proceedins of the Japan/Taiwan Symposium on Molecular Biology of Functional Regulation in Plant and Microbe. 187–194.

Sugiyama J, Watanabe M, Ohwada K *et al*., 1999. Methods of analysis in microbiology[S]. Tokyo: Yokendo. 82–83.

Suyama K, Yamamoto H, Naganawa T *et al*., 1993. A plate count method for aerobic cellulose decomposers in soil by congo red staining[J]. Soil Sci Plant Nutr, 39: 361–365.

Ueda S, Kuwabara Y, 2002. Bacteriological evaluations of the produce and organic fertilizer from organic farming fields[J]. Jpn Bokin Bobai, 30: 145–152.

Vuorinen A H, Saharinen M H, 1997. Evolution of microbiological and chemical parameters during manure and straw co-composting drum composting system[J]. Agriculture Ecosystems and Environment, 66: 19–29.

Yamamoto H, Hashimoto Y, Ezaki T, 1996. Study of non-culturable *Legionella pneumophilla* cells during multiple–nutrient starvation[J]. FEMS Microbiol Ecol, 20: 149–154.

Correction Note

Published in Vol.18, No.7, pp. 854–858, 2007.

Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods

ZHOU Shi-ping, DUAN Chang-qun, FU Hui, CHEN Yu-hui, WANG Xue-hua, YU Ze-fen

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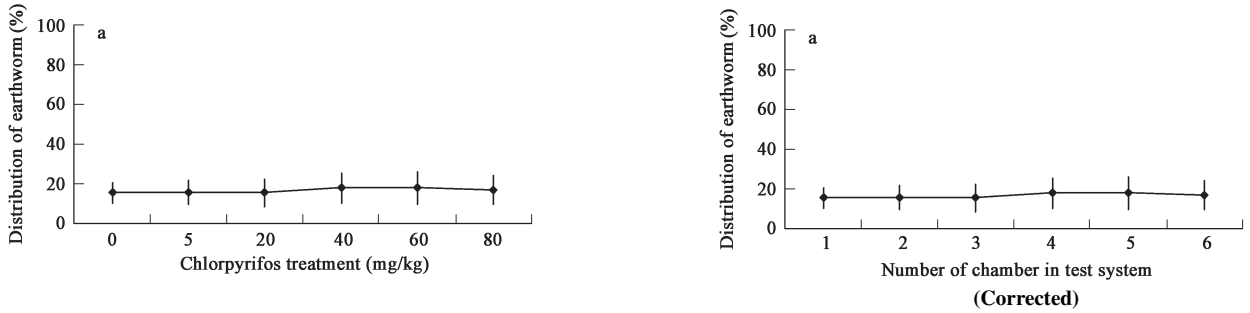

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