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Treatment of Waste Food using Mixed Microorganisms Responsible for the Degradation of Malodor Compounds

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To explore the effective treatment methods for waste food generating malodor compounds, mixed microorganisms (MM) capable of deodorizing of domestic animal waste were applied using household composting box. The mixture of 5 kg whole chips as a bulk agent, 2 kg MM and 1 kg of waste food were input into the compost reactor and agitated. Waste food was supplemented every 24 hours. As the results, the composting volume was stable at 13~14 L for 10 days. In the initial compost process with MM, the pH and temperature were increased more quickly than that of without MM. Also, the conductivity recognized as a barometer of compost was increased from 0.2 to 2.4 mS/cm that was higher than 1.3 mS/cm of without MM, for 10 days. The malodor compounds generated from waste food treatment such as sulfur compounds and volatile fatty acids were effectively reduced about 90~100%, and 70ñ80% for 8 days, respectively. The microorganisms growing under the condition of alkaline phase and higher temperature were dominated during the compost. Moreover, it was demonstrated that inoculated *Bacillus cereus* HY15 dominated during the compost results in responsible to the effective treatment of waste food.

Keywords: *Bacillus cereus*, compost, hydrogen sulfide, malodor, volatile fatty acids, waste food

Pollution generating from human life cycle is serious problem in the world caused by rapid increasing of population and industrial development. Food waste is major issue in both side of waste treatment and odor pollution because the vast amount of waste food is accumulating every day from our daily life cycle.

The amount of food waste is discharged per year is about 5000 million ton in Japan. [3]. Only 1% of them is recycling, and most of the waste food is treating by incineration and landfill methods due to its simplicity and less cost. However this is caused of secondary environmental problem such as contamination of ground water and malodor pollution. Also high moisture content in food waste is accompanying with incomplete combustion in incinerator and caused of generating dioxin, which is strong endorcrine disrupting chemicals (EDC) to human [12, 18]. Moreover, the preservation of food waste is undesired due to its generation of malodor caused by easy spoilage as like animal waste and swage. Therefore, more effective and friendly treatment method is desired.

Many researches have been done on the treatment of waste food [5, 13, 14]. However the malodor generating from the treatment of food waste is still problematic. In addition, the microorganisms capable of compost and deodorization of domestic animal feces and industrial waste within very short time compared to conventional methods were reported [8, 9, 17].

The strategy of this study is to development the effective seed microorganisms for waste food treatment. In this study, the physiological properties and micro-flora changes, and reduction of malodor compounds during the treatment of waste food by inoculation of mixture microorganisms (MM) capable of deodorization of malodor compounds were considered.

Materials and Methods

Mixed microorganisms

The mixed microorganisms (MM) capable of deodorizing of domestic animal waste were kindly provided from

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Prof. Ohta in Hiroshima University. MM was obtained originally from the compost for chicken and pig feces treatment for almost 20 years in Ohta's laboratory and maintained with their dried compost at freezer. MM was acclimatized by the composting of rotten waste food prior to use, and the compost itself was used as seed culture for this experiment.

Preparation of rotted waste food

Waste food was obtained from restaurant at Hiroshima University, Japan. Ten kilograms of fresh waste food was maintained at room temperature for 2 days, and used as a sample of spoiled waste food for analysis. The composition of waste food was approximately 30% of meat (as a source of protein), 30% of rice and noodle (as a source of starch), and 40% of vegetables and fruit shell and etc.

Composting

Compost process was carried out into the composting reactor (Sanyo Electric Comp. Osaka, Japan). The mixture of 5 kg whole chips and 2 kg MM as a seed culture and 1 kg of rotten waste food were input into the compost reactor and agitated with aeration. Every 24 hours, 1 kg of fresh waste food sample was input after taken sample for analysis.

Media used

Volatile fatty acid (VFA) and nutrient agar (Difco, Germany) medium were used for the detection of various microbes during the composting. The VFA medium (g/L) reported as a suitable medium for the microorganisms capable of degrading of malodor compounds by Yun and Ohta [17] and it is composed of sodium butyrate, 10; peptone, 10, KH_2PO_4, 1; $CaCl_2$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $CoCl_26H_2O$, 100 μM; yeast extract, 0.5; agar, 15; water, 1 L and was sterilized at 121°C for 15 min.

Analysis methods

Ten grams sample taken from three different parts of the compost of waste food was diluted with distilled water to be 10% (w/v) and used it for following assay. pH and conductivity of the compost were measured by pH meter (M125 Corning, USA) and conductivity meter (PS17 Corning, USA), respectively. Inside-temperature was monitored by insert of thermometer (MR10 Kuwada, Tokyo, Japan). Moisture content was detected by drying the 2 or 3 grams samples in oven with 110°C until constant weight. Moisture content was obtained difference between wet and dried weight. The volume of compost was measured approximately by mark on the wall of the compost reactor.

Ten grams of rotten waste food obtained before and after compost for 30 days were transferred into each 100 ml Elrenmyer flask stopped up with silicon cap. All samples were incubated at 30°C for 30 min. For hydrogen sulfide analysis, 500 μl of sample from headspace of the flask was taken by airtight syringe and then applied to Gas chromatography (GC~14B, Shimazu, Japan) equipped with flame photometric detector. The column packed with polyphenylether 5 rings (10%, 60~80 mesh) was used. Nitrogen was used as carrier gas and the flow rate was 40 ml min^{-1}.

For volatile fatty acids measurement, 10% (w/v) solution of compost sample was homogenized for 5 min and incubated at 60°C for 30 min with shaking. The rotten waste food was filtered by cheesecloth to remove the solid materials and centrifuged at 4°C at 10,000×*g* for 5 min. The supernatant was used as the sample for volatile fatty acids analysis. Five hundred microliter of the supernatant was applied to GC (GC~14B, Shimazu, Japan) equipped with flame ionization detector. The column packed with Unisole F-200 (2 m, 30~60 mesh) was used. Nitrogen as carrier gas was used at flow rate of 60 ml/min and column temperature was 140°C.

Ten percent of each sample solution was serially diluted and 0.1 ml was inoculated on the nutrient agar and VFA plates adjusted pH 6-10 and incubated at 25, 30, 40, and 50°C. After 48 h, the colonies formed were counted. The counting of coliform bacteria was performed by addition of 100 μl of sample solution on deoxycholate agar (Difco, Germany) and incubation at 37°C for 48 h. The colonies formed red or pink colors were counted as coliform bacteria.

DNA preparation and PCR

The compost extract (10%) was filtered with cheesecloth and centrifuged at 4°C for 5 min. The supernatant was filtered with membrane filter (Ø 5 ìm, Advantec, Tokyo, Japan) and centrifuged at 12,000×g at 4°C for 5 min. The precipitated cells were washed by 10 mM TES buffer composed of 10 mM Tris-HCl, 1 mM EDTA and 0.9% NaCl. The washed cells were reacted with 200 μl Insta-Gene™ Matrix (Bio-Rad, USA) at 56°C for 20 min, and heated at 100°C for 8 min. The solution was centrifuged at

12,000×g for 4 min at 4°C and the supernatant was used as the crude DNA solution.

Single strain counting-PCR (SSC-PCR) method was used for pursuit the target strain [7]. A primer used was 5'-GGCTTCGAAATCG-3' (H81, JapanGene, Japan). The 50 μl PCR reaction was composed of Premix Taq (Takara Taq version, Takarasake Co., Japan), 5 μl of Primer (10 pmol/μl), and 20 μl of crude DNA solution (5 ng/μl). The PCR protocol began with a minute denaturation step at 94°C followed by 35 cycle of denaturation at 94°C for 1 min, annealing at 45°C for 2 min, and extension at 72°C for 3 min. The protocol was concluded with an additional 7 min extension at 72°C. PCR products were electrophoretically separated and visualized in 1.5% agarose gels stained with ethidium bromide.

Results

Physical properties in waste food composting

Fig. 1 shows the physical changes of compost of waste food with MM (a) and without MM (b) for 30 days. The total volume of food waste input into compost reactor was increased from 1 to 30 kg for 30 days. The compost volume with MM was slightly increased from 13 L to 14 L at initial stage. After 7 days, the volume was stabilized at around 13 L for 30 days. However, the volume of compost without MM was increased from 13 L to 15 L and the stabilization of volume was slower than that of the with MM.

The pH in the compost with MM was quickly increased from 7 to 8.8 for 7 days and maintained constant pH. While, the pH in the compost without MM was slightly decreased from 6.7 to 5.7 for 7 days and increased to 8.5 at 14 days. The pH drop to 5.7 might be caused by malodor volatile fatty acids and sulfur compounds produced. However, the constant pH after 15 days was maintained. The pH was stabilized at alkali phase that is around pH 8~9 at the stationary phase.

The temperature during the compost was controlled at around 32~40°C. The temperature changes with MM was fluctuated up and down from 30 to 50°C for 6-10 days and from 40°C to 50°C for 14 days, and gradually decreased until finished experiment. In the case of without MM, the temperature slowly increased from 30 to 43°C for 14 days and gradually decreased.

Moisture content in the compost with MM was more

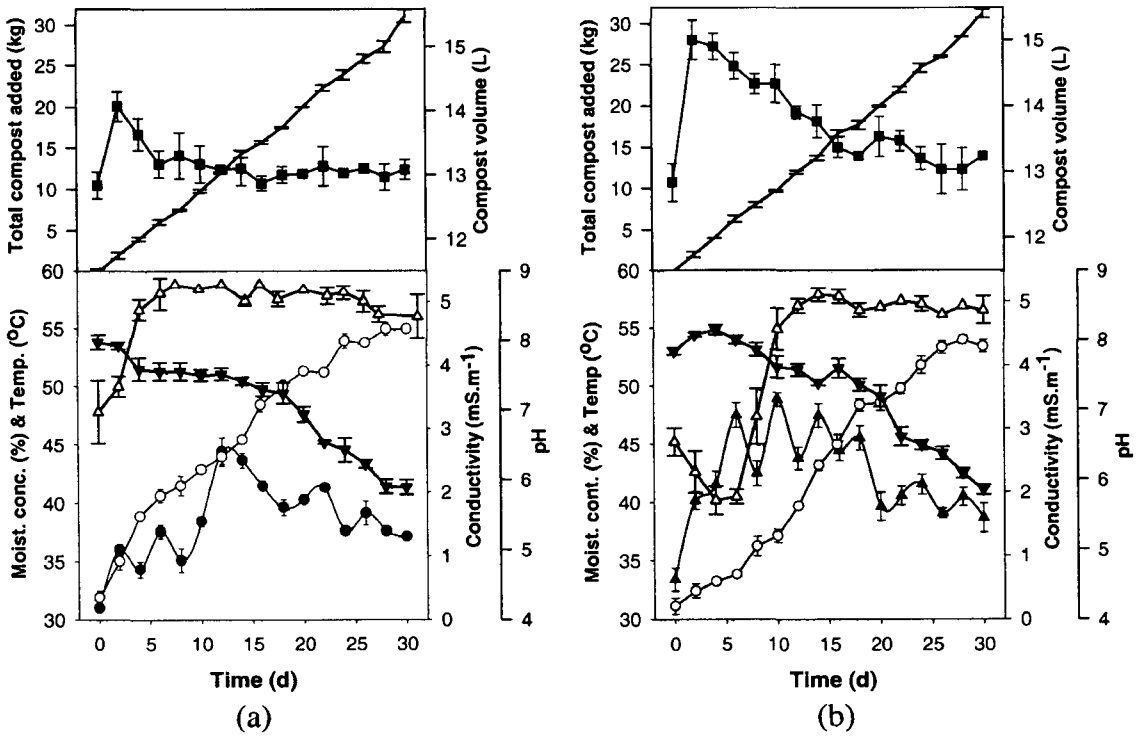

Fig. 1. Comparison of physical changes in the compost with MM and without MM. (a) with MM, (b) without MM. Symbols: (–) Total compost added (kg), (■) compost volume (L), (▼) moisture content (%), (●) temperature (°C), (○) conductivity (mS/cm) (△) pH.

quickly decreased for 10 days than that of without MM. The moisture content was gradually decreased to 40% for 30 days. It was reduced almost 23% compared to about initial moisture content. In the case of without MM, the moisture content decrease was slower than that of with MM. However, the moisture content eventually same as with MM.

The conductivity in the compost with MM was quickly increased from 0.2 to 2.2 mS/cm for 6 days compared to 0.8 mS/cm, without MM and gradually increased to approximately 4.5 mS/cm for 30 days.

Micro-flora changes

The changes in viable cells number during the compost of rotten waste food with MM and without MM were shown in Fig. 2. In the compost without MM, the initial cells number was $2.0\sim6.0\times10^{4-5}$ cfu/g approximately (Fig. 2b). But, in the case of compost with MM, the initial cells number was approximately $\times10^{7-8}$ cfu/g.

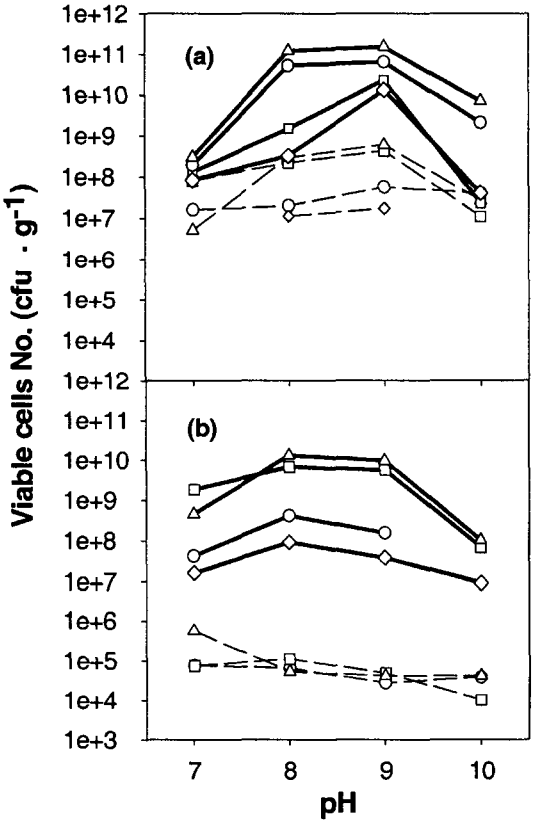

Fig. 2. The changes in micro-flora during the compost for 30 days with MM and without MM. (a) with MM; (b) without MM. Open symbols; initial cells number, Solid symbols; cells number at temperature (○) 20°C; (△) 30°C; (□) 40°C; (◇) 50°C during compost for 15 days. All values represented the mean value of triplicates.

After 30 days processed compost, the cells numbers survived at 30 and 40°C during the compost with MM and without MM were increased from at $\times10^5$ to 10^{10}, and from $\times10^8$ to 10^{11} cfu/g, respectively (Fig. 2a). The cells number that can be survived at 20 and 50°C at was increased below 10^9. Whilst, in the case of with MM, the cells grown at 30 and 40°C and alkali pH, 8~9 were increased from $\times10^8$ to 10^{11}. Overall viable cells numbers in the both compost with and without MM were higher survival at 30 and 40°C than that of 20 and 50°C.

In addition, the numbers of actinomycetes capable of growing under the high temperature, above 50°C during compost were 6.4×10^8 cuf/g at pH 8 (data not shown). Also, the numbers of coliform bacteria obtained from rotten waste food and composted sample were reduced to 1.0×10^8 cfu/g and 7.1×10^2, respectively (data not shown). However, the typical colony of *E. coli*, which has a red or pink color on deoxycholate agar medium, was not detected. This result indicated that the reduction of the growth of coliform bacteria was caused by temperature increase during the waste food compost.

Removal of malodor compounds

Sulfur compounds and VFA: Table 1 shows the malodor compounds such as sulfur compounds and VFA generating from each sources of the waste food. The concentrations of sulfur compounds generated from rotten waste food were 40 ppm of hydrogen sulfide (H_2S), 69 ppm of methanthiol (CH_3SH), and 7 ppm of dimethyl Sulfide (DMS). After 14 days compost, the concentration of hydrogen sulfide, methanthiol and DMS were removed 90, 98, and 100%, respectively. Also, the concentrations of sulfide compounds generated from each sources (meat, fish, others) of waste food were analyzed. As the results, H_2S, CH_3SH and DMS of 32, 53, and 9 ppm respectively were detected from fish. Also, H_2S of 11 ppm, CH_3SH of 9 ppm, and DMS of 2 ppm generated from meat were detected. The small amount, less than 10 ppm of sulfur compounds were emitted. The sulfur compounds were significantly reduced from 116 ppm to 2.5 ppm that is 95% removal efficiency in the compost with MM. While the poor reduction, only 28% of sulfur compounds in the compost without MM have shown.

In the case of VFA which was recognized as a main malodor compounds during the compost, the concentrations of acetic acid, propionic acid, *n*-butyric acid, *iso*-

Table 1. The changes of malodor compounds patters in waste food and compost processed

Malodor compounds		RWF[1)]	Meat	Fish	Others	Compost	
						MM[2)]	Without MM
Sulfur compounds conc. (ppm)	H_2S	40	11	32	6	4	31
	CH_3SH	69	9	53	1	1	51
	DMS	7	2	9	3	–	1
	Total	116	22	97	10	5	83
VFA conc. (ppm)	Acetic acid	125	67	4	28	43	63
	Propionic acid	16	5	2	12	–	7
	n-butyric acid	17	2	–	18	–	12
	iso-butyric acid	26	–	–	23	5	19
	n-valeric acid	12	3	–	20	–	8
	iso-valeric acid	6	3	–	7	–	5
	Total	202	80	6	108	48	114

Note: Ten grams of samples were used to measure the sulfur and VFA compounds. The compost samples were obtained at 14 days processed compost. All values are the mean value of triplicates. Others are the mixture of rice, doodles and vegetables etc.
[1)] rotten waste food; [2)] mixed microorganisms.

butyric acid, *n*-valeric acid, *iso*-valeric aicd emitted from rotten waste food were 125, 16, 17, 26, 12 and 6 ppm, respectively. Also, the concentrations in the variety of sources of the waste food such as meat, fish, and others (vegetables, rice, needles etc.) were detected. The highest concentration of acetic acid was emitted from meat as 67 ppm. The others fatty acids such as propionic, butyric, and valeric acid were more generated most from others sources. The concentrations of the fatty acids were reduced 63% of acetic acid, 80% of *iso*-butyric acid, and 100% of the other compounds for 14 days compost with MM. However, in the compost without MM, less than 60% reduction was showed for all VFA compounds.

Fig. 3a, b shows that the effect of MM on the removal of VFA and methanthiol that is the highest concentration contained during the compost of waste food. The emitted concentration of methanthiol was quickly increased from 2 days and maintained for 6 days. However, in the compost with MM, the concentration of sulfide compounds was reduced almost completely for 8 days compost (Fig. 3a). In the compost without MM, highest VFA was emitted for 4 days and then decreased. Whist, in the case of with MM, VFA emitted was effectively reduced for 8 days (Fig. 3b).

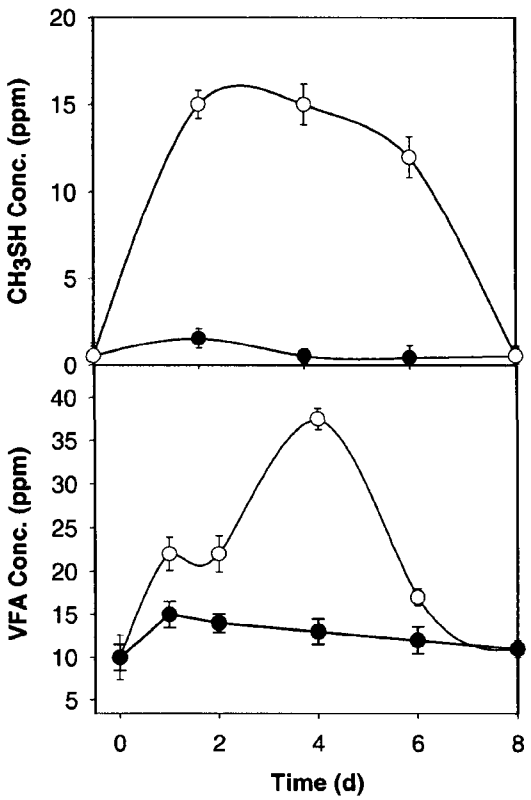

Fig. 3. The changes in sulfur compounds and VFA during the compost with MM and without MM for 8 days. (a) Sulfur compounds content (b) VFA concentration. Symbols: (○) without MM, (●) with MM.

Characteristics of *Bacillus cereus* HY15

The isolate No. 15 was identified phenotypically as *Bacillus cereus* HY15 by using gram staining, physiological properties, chemical test and API20NE kit (BioMerieux, France) test for sugar utilization (Table 2).

PCR

The pattern of PCR product obtained from the extract of microorganisms of compost progressed for one month was

Table 2. General characteristics of *Bacillus cereus* HY15

Items	Characteristics	Items	Characteristics
Morphology	Rod	*N*-Acetyl glucosamine	+
Gram staining	+	Amygdaline	–
Spore forming	+	Arbutine	+
Motility	+	Esculine	+
Catalase	+	Salicin	+
Color of colony	–	Cellobiose	+
O-F test	F	Maltose	+
Utilization of:		Lactose	–
Glycerol	–	Melibiose	–
Erythritol	–	Saccharose	+
D-Arabinose	–	Trehalose	+
L-Arabinos	–	Inuline	–
Ribose	+	Melezitose	–
D-Xylose	–	D-Raffinose	–
L-Xylose	–	Amidon	+
Adonitol	–	Glycogen	+
Â-Methyl-xyloside	–	Xylitol	–
Galactose	–	β-Gentiobiose	–
D-Glucose	+	D-Turanose	–
D-Fructose	+	D-Xyxose	–
D-Mannose	–	D-Tagatose	–
L-Sorbose	–	D-Fructose	–
Rhamnose	–	L-Fructose	–
Dulcitol	–	D-Arabinose	–
Inositol	–	L-Arabinose	–
Mannitol	–	Gluconate	–
Sorbitol	–	2 Aceto-gluconate	–
α Methyl-D-mannoside	–	5 Aceto-gluconate	–
α Methyl-D-glucoside	–		

changed as shown in Fig. 4a, b. The same size band appeared at different time of compost was that of a same strain such as *Bacillus cereus* HY15. Fig. 4a shows that two strong bands, about 660 bp and 2000 bp were appeared for the fist half of the compost. And, the 2000 bp band at the second half stage of compost was more dense compared to the second half (Fig. 4b). In addition, the band of about 4,900 bp was appeared from the sample obtained after 2 weeks.

Table 3. Degradation of malodor compounds by *Bacillus cereus* HY15

Malodor comp	Removal efficiency (%)	Malodor comp.	Removal efficiency (%)
H_2S	18	*n*-butyric acid	75
CH_3SH	13	*iso*-butyric acid	87
Acetic acid	64	*n*-valeric acid	73
Propionic acid	72	*iso*-valeric acid	91

Fig. 4c shows the pattern of PCR product obtained from *B. cereus* HY15 and the microorganisms extracted from rotten waste food, compost without MM as a control. In addition, the patterns for with MM are shown in Fig. 3a, b. The dense band of about 658 and 1,100 bp was showed from extract of rotten waste food and the compost without MM. However, 2000 bp was main band of *Bacillus cereus* HY15.

The degradation of volatile fatty acids and sulfur compounds, which are main odor compounds generating from the treatment of waste food, by *B. cereus* HY15 was examined (Table 3). The removal rate of hydrogen sulfide and methanthiol was 18% and 13% for 3 h, respectively. Acetic, propionic, *n*-butyric, *iso*-butyric, *n*-valeric, iso-valeric acids were significantly removed to 64, 72, 75, 87, 73, and 91% only for three hours. The results are associated to the reducing of odor compounds during the compost of waste food.

Discussion

The study was focused on the effect of MM on the waste

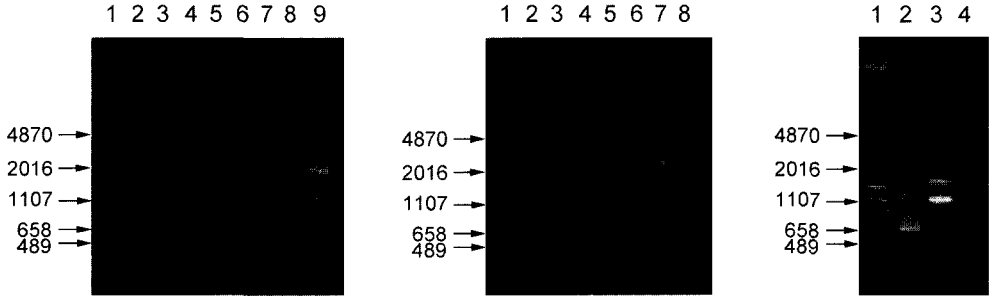

Fig. 4. DNA fragments of time course of microbial flora. (a) Lane 1, marker; Lane 2~8, two days interval (2^{nd}~16^{th}). (b) Lane 1, marker; Lane 2~8, two days interval during 18^{th}~30^{th}, (c) DNA fragments of selected bacteria and micro flora of rotten waste food. Lane 1, marker; Lane 2, waste food; Lane 3, rotten waste food; Lane 4, *Bacillus cereus* HY15.

food treatment. One of the important parameter to evaluate the MM effect, the physical properties was examined. As the results, the total volume (about 30 L) of the waste food input in the compost reactor was maintained approximately 14 L after a month processed. It was suggested that the moisture content evaporated by the increasing of temperature caused by degradation of organic compounds by microorganisms resulted in reduction of the volume. The results are in agreement with the report of composting waste food by control the airflow rate and avoid of heat lost from composting reactor to maintain the temperature high [13]. Also decrease of organic compounds during composting of organic wastes by using slurry was reported [10, 11, 16].

The fluctuation of pH, temperature, and moisture might be caused by difference of food waste composition. In this study, the difference between the compost with and without MM was significant at early stage, 14 days of compost. However their overall tendency during the compost was not shown significant difference by the variation of composition.

Monitoring of moisture content, pH, and temperature are useful assessment of maturity of waste food composting [1, 11, 16]. According to the progressing of compost, heat generating by decomposition of organic compounds of food waste results in the lost of moisture [2]. Fig. 3a, b shows quick increasing of concentration of volatile fatty acids and methanthiol caused by decomposition of food waste or metabolites by microorganisms existed in the waste food [15]. The large amount of VFA and hydrogen sulfide produced from the compost without MM is caused of pH decline and emission of malodor. While, in the case of the run used MM, the volatile fatty acids concentration was not increased during the compost. It is suggesting that the formation of volatile fatty acids and methanthiol were consumed as a carbon source by MM and be caused of avoid of pH drop at early stage of compost. Kown *et al* [6] reported that pH increase is proof of compost progressed. This results indicated that the MM were already dominated at early stages of compost resulted in the effective treatment, minimize the malodor emission and more environmentally friend.

The temperature was controlled at about 30-40°C even thought a linear relationship existed between increase in the efficiency of the process and an increase in temperature [1, 4]. Despite maintaining about 30-37°C, the temperature rising gradually over 50°C might be allowed thermophiles. Also Golueke suggested that optimum range of thermophiles in composting is between 55-60°C and efficiency of composting began to drop rapidly.

The results are clearly showed the change in microbial community of compost by MM (Fig. 4). A 2000 bp dense band as composting progressed is corresponding with the PCR product amplified DNA of *Bacillus cereus* HY15 isolated. The results pointed out that the same or similar microbes of *B. cereus* HY15 are responsible for composting of waste food. Also the strain, recognized as *B. cereus* HY15 has an important role from especially the early stage of the compost. It revealed that *B. cereus* HY15 already acclimatized from initial stage of compost. A dense band of about 1,000 bp and 658 bp obtained from the compost without MM and endogenous microbes were not appeared during the progressing of compost with MM. This result demonstrated that *B. cereus* HY15 grew with sufficient malodor compounds in the waste food during the compost progressing and dominated. Also, the role of microbes existed in rotten food waste are negligible. Also, appearance of a band, about 4900 bp was suggested that the *B. cereus* HY15 prefer alkali pH to acid pH. Also it was proved that the pattern of degradation of volatile fatty acid and sulfur compounds by *B. cereus* HY15 shows closely related with the reduction of malodor compounds during the waste food treatment.

The study is important information for development of seed culture to minimize the malodor problems during the treatment of waste food using conventional compost reactor. In conclusion, the application of MM to treat of waste food are significantly effect on accelerate the process of compost at early stage of compost with less malodor emit as well as more environmentally friendly compare to conventional compost.

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초 록

혼합 미생물에 의한 음식쓰레기 처리와 악취 제거

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축산폐기물의 퇴비화에 사용된 혼합 미생물을 이용해서 음식쓰레기 처리 과정에서 발생되는 악취를 효과적으로 처리 하고자 하였다. 시판되는 가정용 음식쓰레기 처리기를 사용하여 음식쓰레기의 퇴비화와 악취저강 특성을 조사하였다. 퇴비화는 10일 경과후부터 인정화되었으며, 혼합 미생물을 첨가한 경우 온도와 pH 가 혼합 미생물을 첨가하지 않은 것과 비교해 더 빨리 증가하였으며, 퇴비화의 지표 중에 하나인 전기전도도 (Conductivity) 도 1.2 에서 2.4 mS/cm 로 혼합 미생물을 첨가하지 않은 경우의 1.3 mS/cm 보다 급격한 상승을 보였다. 식품쓰레기 처리 중에 발생되는 악취 물질, 즉 황화수소와 저급지방산은 8 일 동안에 각각 90-100%와 70-80% 로 효과적으로 제거되었다. 또한 알칼리 상태와 고온의 조건에서 성장하는 미생물이 퇴비화 과정 중에 우점 하였으며, Bacillus cereus No. 15 균주가 혼합 미생물 중 효과적인 식품쓰레기처리와 악취제거에 관여하고 있다는 사실을 증명하였다.

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