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Performance and Bacterial Communities for Bio-drying with Thermophili Bacteria of Sewage Sludge

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Abstract

This study was conducted to evaluate the effect of the sewage sludge treatment method using bio-drying with Ultra-Thermophilic Aerobic Microorganisms (UTAMs). Twelve specific odorous compounds and various sources of bacteria were tested using the sewage sludge treatment method. Sewage sludge was mixed with a seed material and was composted for 47 days. During composting, the temperature was maintained at 80-90°C. The concentrations of the 12 specific odorous compounds after composting did not exceed the allowable exhaust standard for odor. In terms of the bacterial community number after composting, the thermophile bacterial number was 60% of the total bacterial number. The thermophile bacterial ratio after composting increased by 23% compared to the initial composting. The 16S rRNA gene demonstrated that the change in the bacterial community structure was coupled with shifts in the bio-drying process. Therefore, both stable composting operation and economic benefit can be expected when an ultra-thermophilic composting process is applied to sewage sludge.

Keywords: *bio-drying, sewage sludge, thermophile bacteria, ultra-thermophilic aerobic microorganisms, 16S rRNA*

1. Introduction

The disposal of Waste-Activated Sludge (WAS) in municipal wastewater treatment plants, which can account for up to 50% of current operation costs, is a problem of increasing importance (Tchobanoglous *et al.*, 2004). The Moisture Content (MC) of sewage sludge is about 80%, which causes a series of problems in terms of sludge treatment and disposal. Consequently, reducing the sludge moisture is important in reducing sludge volume and quantity (Zhao *et al.*, 2010).

Bio-drying is an economical and energy-saving method of simplifying thermophilic aerobic fermentation that utilizes the biological energy produced by microbial fermentation to activate bound water and evaporate moisture (Navaee-Ardeh *et al*., 2010), resulting in the rapid reduction of the moisture in the bio-drying material (Zhang *et al.*, 2008). The main drying mechanism in bio-drying is convective evaporation, which utilizes the heat produced from the biodegradation of organic matter and is facilitated by mechanically controlled aeration (Navaee-Ardeh *et al.*, 2006). The process by which the moisture of the bio-drying material is reduced is as follows: the water molecules evaporate from the surface of the material into the air, after which the evaporated water (vapor) is transported and removed by the airflow (Velis *et al*., 2009). Air convection and molecular diffusion are the primary approaches to removing water from the bio-drying material (Frei *et al.*, 2004). During bio-drying, bio-heat is crucial for water evaporation, and it is produced via the microbial degradation of the compounds (e.g., complex carbon, cellulose, hemicelluloses, and proteins) in the material (Storey *et al*., 2015). Fundamentally, the generation of bio-heat for water evaporation is dependent on the degradation of organic matter.

An organic-waste ultra-reduction system employing Ultra-Thermophilic Aerobic Microorganisms (UTAMs) at over 100ºC can properly control the amount of air required for composting inside a fermenter, and can efficiently remove the moisture vaporized at 120ºC, the maximum composting temperature (Poulsen *et al.*, 2010). The duration of the first fermentation process is reduced due to the direct final composting reaction, resulting in the rapid stabilization of the organics at the reaction temperature of 100ºC on average and up to 120ºC. The organic-waste ultra-reduction system with UTAMs offers both the typical concept of organic-waste reduction and the concept of composting (Zhang *et al.*, 2010).

Offensive odor is generally a serious problem not only in the composting process but also in the final compost products (Shen *et al.*, 2012). Exhibiting 25-35% moisture content, the final compost from the organic-waste ultra-reduction system with UTAMs at over 100ºC has been completely composted so that it

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not only has no smell but can also be mixed back with the organic waste in a composting process to remove its offensive odor (Adani *et al*., 2002).

To date, there has almost been no composting method in South Korea that uses UTAMs. In Kagoshima, Japan, from 100 to 110 tons of sewage sludge per day is fermented at an extremely high temperature to produce compost, and in the Niigata Prefecture, 100-ton UTAMs were installed for a demonstration experiment. The characteristics of organic waste in Japan, however, are different from those of organic waste in South Korea, and so are the composting and compost application conditions, such as the climate and soil.

Therefore, to apply the UTAMs that are widely used in Japan to sewage sludge treatment in South Korea, the operating parameters should be acquired by extracting the accurate reaction factors. An ultra-high-temperature aerobic composting technology and a relevant database should be established by conducting a dynamic analysis of the offensive odor and microbes in the composting process. Towards this end, this study analyzed and evaluated the physical changes, offensive odor, and behavioral changes of the microbes in the sewage sludge treatment by applying UTAMs to the bio-drying process.

2. Material and Methods

2.1 Experiment System and Materials

2.1.1 Experiment System

To evaluate the efficiency of the sewage sludge treatment method using the bio-dying process with UTAMs, this study designed, produced, and operated a 10 kg/day lab-scale bio-drying system (Fig. 1). The produced system generally consists of a reaction unit, to which various sensors are installed to monitor the composting device, as well as monitoring and control units, both of which collect and store the data from the PLC and PC and control all the driving units. No pipe diffusers were installed for aeration, but the system allows the natural flow of air through the holes on the upper part and side when the moist air is pulled out by an external pump. The size of the stainless-steel lab-scale bio-drying system is 400 mm (*W*) × 800 mm (*L*) × 300 mm (*H*). The system has a gas sensor for O_2 (0-30%, electrochemical method), CO_2 (0-50,000 ppm, NDIR, semiconductor method), and NH_3 (~ 3,000 ppm). In addition, the hydrometer and pressure gauge of the bio-drying system have 0-95% and -0.1 ~ 2.0 Mpa ranges, respectively. The heating system of the bio-drying system was installed at the bottom of the reactor.

2.1.2 Materials for the Experiment

Shown in Table 1 are the physical properties of the sewage sludge and UTAMs that were used in this study. The moisture contents of the sewage sludge and UTAMs were 85 and 19%, respectively, and the sodium chloride concentration in the sewage sludge was 400 mg/L on average. The optimal mixing test for the sewage sludge and UTAMs during aerobic composting through

Table 1. Characteristics of the Sewage Sludge and UTAMs (dry basis) (average ± standard deviation)

Parameter		Sewage sludge	Ultra thermophilic aerobic microorganisms
Moisture content (%)		82.6 ± 5.3	36.1±9.7
Proximate analysis (wt%, on dry basis)	Volatile matter	71.3 ± 1.3	48.87
	Fixed Carbon	12.3 ± 1.7	7.93
	Ash	3.92	0.22
	NaCl	12.3 ± 1.7	-
	T-N	1.4 ± 0.3	5.0
Ultimate analysis (wt%, on dry basis)	Carbon	44.8	51.6
	Hydrogen	6.5	7.5
	Oxygen	25.1	30.9
	Nitrogen	7.2	3.8
	C/N	6.2	13.6

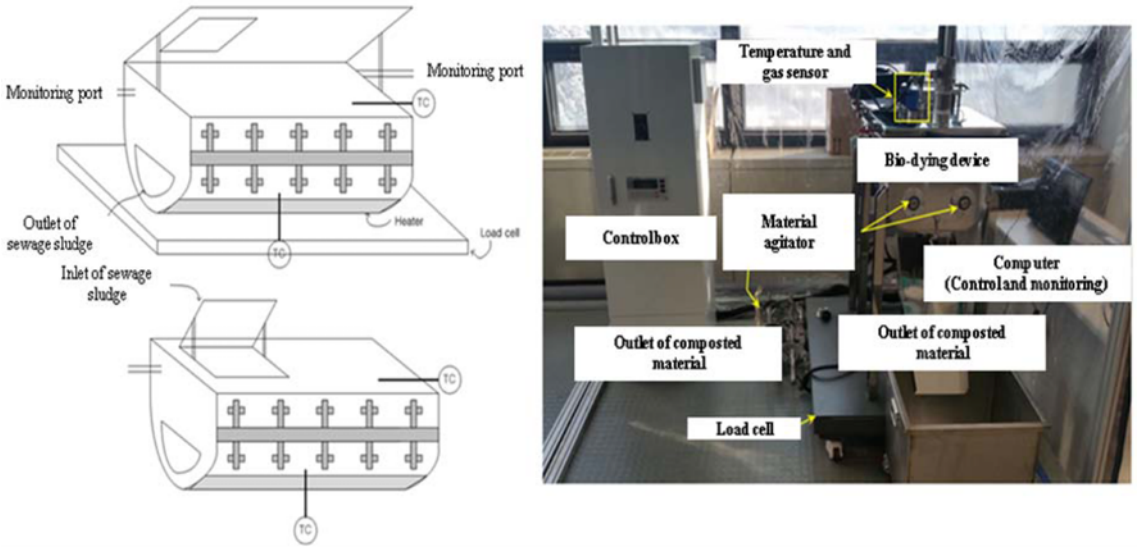

Fig. 1. Schematic Illustration of the Lab-scale Bio-drying Process

fermentation showed that the moisture content and the organic-waste reduction efficiency were highest at the 1:1.5 (sewage sludge:UTAMs) mixing ratio. As such, the sewage sludge and UTAMs were mixed at such ratio for aerobic composting. The fermentation lasted 47 days (experiment duration: May-July 2016). For the UTAMs, the study used the compost produced by Company "S" in Japan based on the high-temperature (100ºC) aerobic composting method. The UTAMs that were used in this study increase the temperature of the fermentation chamber to over 100ºC to remove the moisture content, organic waste, and offensive odor from the sewage sludge.

2.2 Physical Analysis

Factor analysis was conducted to evaluate the changes in the temperature and in the carbon dioxide, ammonia, moisture, and organics contents during the fermentation using the bio-drying process with UTAMs. The specimens were sampled during cultivation of the UTAMs in the sewage sludge, and the temperature was measured in real time at the upper layer of the compost (10 cm). Fermentation gas was monitored using a continuous gas meter (Landfill Gas Monitoring System LM Sxi Infrared) by continuously measuring the carbon dioxide, ammonia, and oxygen concentrations every 2 hours (30 minutes per session). The portable gas meter (tube pump for gas detection) is a vacuum-type gas collector with a highly airtight structure that sucks the sample gas through its gas-detecting tube as the piston reduces the pressure inside the cylinder. Its air suction is initially fast and gradually slows down, a property that most effectively realizes a clear-colored layer. The carbon dioxide and ammonia concentrations were measured at a specific measurement point in the upper part of the fermentation chamber. The pH was measured by creating a suspension and using a supernatant. For the moisture content and organics analysis in the solid matter, the following equations were used (Tambone *et al*., 2011):

2.3 Odor Analysis and Evaluation

For odor analysis of the sewage sludge before and after the fermenting treatment using the bio-drying process with UTAMs, the study conducted an instrumental analysis of the 12 designated offensive odor items (Ministry of Environment of South Korea, 2015): ammonia, hydrogen sulfide, methyl mercaptan, dimethyl sulfide, dimethyl disulfide, trimethylamine, styrene, acetaldehyde, propionaldehyde, n-butyraldehyde, i-valeraldehyde, and n-valeraldehyde. The sulfur compounds were analyzed using TD/GC/FDS (spectrometer system, National Research Council, Canada); the aldehyde compounds, using HPLC (high-performance liquid chromatography, Jasco, Japan); styrene, using TD/GC/MS (thermal desorption gas chromatography mass spectrometer, SCAS, Japan); ammonia, using UV (UV-Vis-NIR 3101PC, Jasco, Japan); and TMA (trimethylamine), using GC/FID (3400-GC, Varian, Japan). A specific number of samples were collected from the pack for the analysis of the UTAMs and the designated offensive odor before the fermentation of sewage sludge, and the offensive odor generated at room temperature was collected and analyzed. Having determined that as the temperature rises the offensive odor would also increase, sampling of an offensive odor for the designated offensive-odor analysis after the sewage sludge fermentation was conducted by collecting gas from the upper part of the fermented sewage sludge and placing it in a Tedlar gas sampling bag right after culturing.

2.4 Measurement of the Total Bacterial Count and Thermophiles

In the composting process of the sewage sludge examined in this study, the starter was inoculated into waste, and the temperature rose to over 95ºC during the bio-drying. The starter, imported from Japan (Sanyu Co.), included *Bacillus* and *Geobacillus,* which grow actively at 70-85ºC, as well as various hyperthermophiles whose culturing may be difficult and which are known to stop growing at 50ºC or below. To survey the changes in the group under a limiting environment, such as a high temperature, the study examined the changes in the total bacterial count and hyperthermophiles as well as the existence of pathogenic indicator microorganisms.

The total bacterial count was measured by mixing 1 g of the sample with 100 ml phosphate-buffered saline (KCl 0.2 g/L, KH_2PO_4 0.2 g/L, NaCl 8 g/L, Na_2HPO 41.14 g/L, pH 7.4) and stirring and diluting the mixture for 5 minutes and then filtering it with a polycarbonate membrane filter (pore size: 0.2 μm; Millipore, USA). The filtered sample was dyed with acridine orange (1 mg/ml) 500μl, and the total bacterial count was measured using a fluorescent microscope (excitation: 500 nm; emission: 520 nm; Nikon, Japan) at 1,500x magnification.

Through the method identical to the measurement of the total bacterial count, the number of thermophiles was measured by leaving 1 ml of the sample diluted with phosphate-buffered saline in a constant-temperature water bath at 80ºC for 1 hour to kill all the microbes, except the thermophiles. The high-temperature-treated sample was filtered with the polycarbonate membrane filter and then dyed with a Live/Dead BacLight™ bacterial viability kit (Invitrogen, USA). The number of thermophiles was measured with a fluorescent microscope (excitation: 470 nm; emission: 540 nm; Nikon, Japan) at 1,500x magnification.

The presence of pathogenic indicator microorganisms was evaluated by measuring the *Escherichia coli* (*E. coli*) and *Salmonella*. The most optimal measurement method proposed by the standard methods was used (APHA, 2005). Identical to the measurement of the total bacterial count, the sample diluted with phosphate-buffered saline was inoculated in Difco™ lauryl tryptose broth at 37ºC for 24 ± 2 hours. The number of *E. coli* in 1 g of the sample was calculated based on the number of test tubes where the production of gas was observed after the cultivation, and on the MPN (most probable number) table. For the measurement of *Salmonella*, the diluted sample was inoculated in Difco™ selenite broth at 37ºC for 18-24 hours. The positive *Salmonella* result was obtained by taking one loop of the culture fluid from all the enrichment broths, plating it on Difco™ bismuth sulfite agar by streaking, and culturing it at 37ºC for 24-48 hours, and

the number of *Salmonella* was calculated using the MPN table.

2.5 Microbial Community

The sludge samples were placed in an ice box following field collection, and were stored at -60ºC until pyrosequencing analysis. Polymerase Chain Reaction (PCR) amplification was performed using primers targeting the hypervariable regions (V1-V3) of the 16S rRNA gene with the extracted DNA. For bacterial amplification, the bar-corded primers of 9F (5'-CCTATCCCCTGTGTGCC-TTGGCAGTC-TCAG-AC-AGAGTTTGATCMTGGCTCAG-3'; the underlined sequence indicates the target region primer) and 541R (5'-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-X-AC-ATTACCGCGGCTGCTGG-3'; "X" indicates the unique barcode for each subject) (http://oklbb.ezbiocloud.net/content/1001) were used. The amplifications were carried out under the following conditions: initial denaturation at 95ºC for 5 minutes, 30 cycles of denaturation at 95ºC for 30 seconds, primer annealing at 55ºC for 30 seconds, extension at 72ºC for 30 seconds, and final elongation at 72ºC for 5 minutes. The PCR product was confirmed using 2% agarose gel electrophoresis, and was visualized under the Gel Doc system (BioRad, Hercules, CA, USA). The amplified products were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). The quality and product size on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) were assessed using a DNA 7500 chip.

The readings obtained from the different samples were sorted based on the unique barcodes of each PCR product. The sequences of the barcode, linker, and primers were removed from the original sequence readings, and any reading with two or more ambiguous nucleotides, a low quality score (average score < 25), or a reading shorter than 300 bp was discarded. The potential chimera sequences were detected using the Bellerophon method, which compares the BLASTN search results between the forward-half and reverse-half sequences (Huber *et al*., 2004). After the removal of the chimeric sequences, the taxonomic classification of each reading was assigned using the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net).

3. Results and Discussion

3.1 Physical-impact Assessment

3.1.1 Temperature

Composting typically occurs at mid-temperature (between 30 and 45ºC) or at a high temperature (between 45 and 65ºC), and the decomposition of organics is known to be promoted at a high temperature (Kuok *et al*., 2012). As such, maintaining the proper temperature is essential in producing good compost. While a high temperature can kill pathogens, decompose organics, and cause moisture to evaporate, too much heat can also kill the microorganisms that contribute to composting. As such, it has been generally agreed upon that composting requires proper temperature control (50-60ºC). As shown in Fig. 2, however, the UTAMs that were used in this study caused fermentation at an

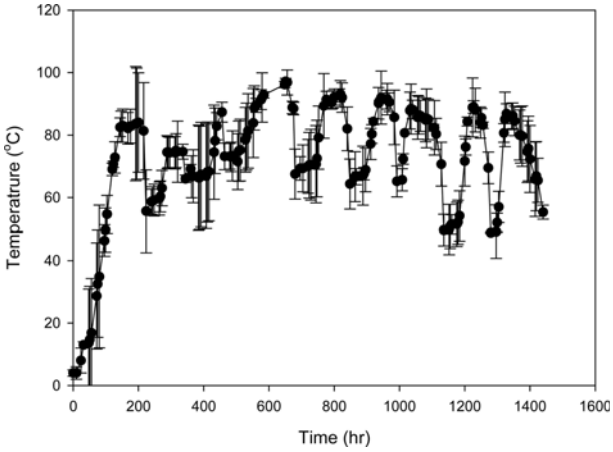

Fig. 2. Variable Temperatures during the Sewage Sludge Treatment using Bio-drying with UTAMs

average temperature of 80ºC or over. After the insertion of sewage sludge, the temperature gradually increased during the fermentation, then rose to over 90ºC, and when the fermentation was nearly completed, it gradually decreased to about 70ºC. Sewage sludge was inserted again when the temperature of the fermenter fell and reached the minimum value, and then the temperature rose again to about 90ºC. During the fermentation, the temperature was maintained at between 80 and 90ºC.

3.1.2 Gas Changes

Shown in Fig. 3 are the changes in the oxygen and carbon dioxide concentrations after the insertion of sewage sludge. As microbes oxidize carbon as their energy source, they consume oxygen and generate carbon dioxide, among others (He *et al*., 2013). It is known that the optimal oxygen concentration required for the microbial decomposition of organics in a composting chamber is between 15 and 20%, and if it drops to below 5%, anaerobic fermentation will take place (Adhikari *et al*., 2009). In this study, the carbon dioxide generation and oxygen concentration reduction showed an inversely proportional relation as the former soared up to about 50,000 ppm in the initial reaction

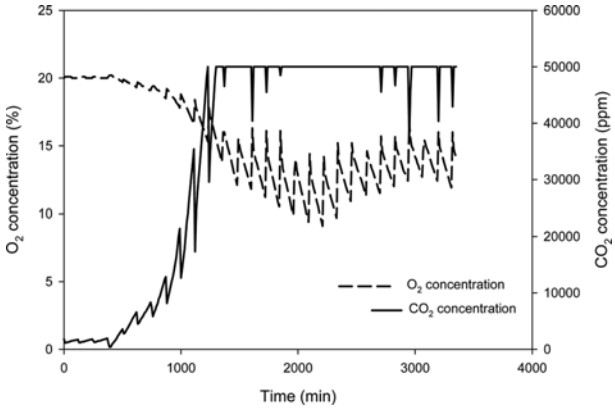

Fig. 3. Concentration Variations of Oxygen and Carbon Dioxide during the Sewage Sludge Treatment using Bio-drying with UTAMs

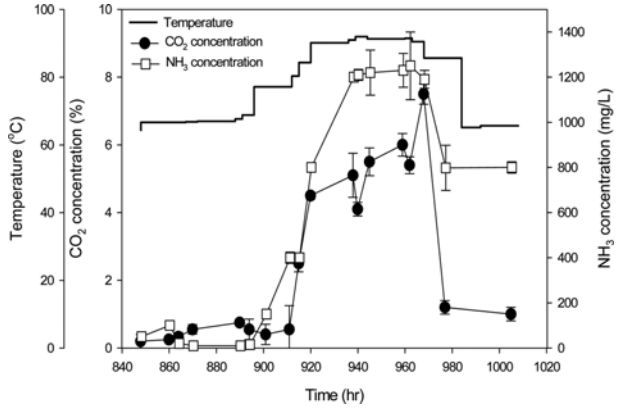

Fig. 4. Concentration Variations of Carbon Dioxide and Ammonia in the Sewage Sludge Treated using Bio-drying with UTAMs

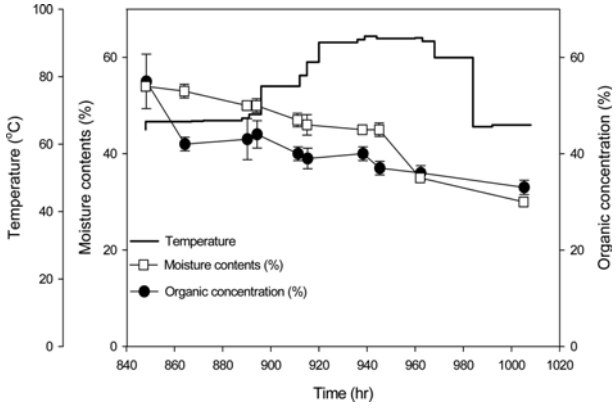

Fig. 5. Variations of the Moisture Contents and Organics Concentrations of the Sewage Sludge using Bio-drying with UTAMs

period while the latter fell to below 15%.

Shown in Fig. 4 are the carbon dioxide and ammonia concentration changes based on the temperature change. The carbon dioxide and ammonia concentrations rose and fell rapidly in the zone where the temperature of the fermenter increased. As shown in Eq. (1), the generation of carbon dioxide, ammonia, and heat occurs at the same time when organics are aerobically decomposed by bacteria (Yamada *et al*., 2006). In particular, ammonia can be generated when organics with high protein contents are decomposed. When protein is hydrolyzed to produce amino acid, the deamination by bacteria produces saturated acid or hydroxy carboxylic acid with one less carbon atom and with ammonia as a byproduct (Zhao *et al.*, 2011). The ammonia concentration was up to 1,200 ppm at the maximum fermentation chamber, and it later tended to show a decline.

$$\text{Organic matter} + O_2 + \text{nutrients} \xrightarrow{\text{Microorganism}} \text{new cells} + \text{resistant organic matter} + CO_2 + H_2O + NH_3 + SO_4^{2-} + \ldots + \text{heat} \quad (1)$$

3.1.3 Moisture and Organics Contents

Figure 5 shows the changes in the moisture and organics contents during the bio-drying process with UTAMs of sewage sludge. The moisture contents of the sewage sludge and seed bacteria in the initial fermentation were 82 and 36%, respectively. It is generally known that the bioactivity is terminated when the moisture content is less than 15% of the weight, and that a 40-65% moisture content should be maintained (Huilinir *et al*., 2015). Accordingly, the moisture content was controlled so that it could be maintained at 55% for the microbial activity after the mixing of the sewage sludge and UTAMs. Moisture control agents like sawdust, chaff, or rice husks were not used to control the optimal moisture content. After fermentation, the moisture content decreased to 30%, which was an about 64% decrease from the initial moisture content of the sewage sludge. Of the 52% moisture reduction, about 27% was due to the dilution effect with the mixture of the UTAMs, and about 25% was due to the microbial metabolic processes and the heat produced by the microbiological oxidation.

The organics content tended to show rapid decomposition in the early stage of fermentation. It is believed that this is because the organics that more easily decompose among those that comprised the sewage sludge were decomposed. Finally, about 50% of the sewage sludge organics were removed by the bio-drying with UTAMs.

Table 2 shows the results of the lab-scale bio-drying experiment in different researches. The composting temperature was higher in this study than in other studies, and the moisture removal efficiency from the UTAMs was also 10-15% higher in this study. This comparison indicates that bio-drying with UTAMs can more effectively remove the moisture in sewage sludge, and that bio-drying will show better performance when performed using UTAMs because in this study, bio-drying with UTAMs maintained a high temperature (80-100°C) during the composting of sewage

Table 2. Results of Lab-scale Bio-drying Experiment in Different Researches

Materials	Initial MC (%)	Residence time	Water removal (%)	Temperature in reactor (°C)	Reference
MSW	40-60	6-12 weeks	66	45	Adani *et al*., 2002
Sludge	40	154 hr	68	55	Zhao *et al*., 2011
Garden waste	68	20 days	51	60	Colomer *et al*., 2013
Dewatered Sludge	78	20 days	-	65	Huilinir *et al*., 2015
Sewage sludge	80	6-10 weeks	80	80-110	Takahiro *et al*., 2013
Sewage sludge	55	47 days	64	80-100	In this study

Table 3. Results of the Analysis of the 12 Specific Odorous Compounds at days 1 and 47

Parameter	Unit	Initial composting[a]	Final composting[b]	Allowable exhaust standard (In Korean) (Ministry of Environment, 2005)
Ammonia	ppm	5.73	0.74	1.0
Hydrogen sulfide	ppb	8.22	5.8	20.0
Methyl mercaptan		16.44	N.D	2.0
Dimethyl sulfide		2.74	0.25	10.0
Dimethyl disulfide		36.99	7.0	9.0
Trimethylamine		20.83	3.52	5.0
Styrene		N.D[c]	N.D	400.0
Acetaldehtyde		13.70	2.55	50.0
Propionaldehyde		N.D	N.D	50.0
n-butyraldehtde		N.D	N.D	29.0
i-valeraldehyde		N.D	N.D	9.0
n-valeraldehyde		N.D	N.D	3.0

a: Analysis at 1 day, b: Analysis at 47 day c: N.D. = Not Detected

sludge. Thus, the moisture decrease can reduce the economic cost of composting due to the reduction of the bio-drying reaction time.

3.2 Odor Analysis Results

Shown in Table 3 are the results of the analysis of the 12-designated offensive-odor materials before and after the bio-drying with UTAMs. The results of the designated offensive-odor materials before fermentation showed that up to eightfold-higher concentrations of ammonia, methyl mercaptan, dimethyl sulfide, and trimethylamine than the regulation standards were detected. After fermentation, however, the detected concentrations of all the designated offensive-odor materials were below the regulation standards. It is believed that a considerable amount of offensive odor was decomposed at a high temperature during the composting process. In the previous studies, sewage sludge treatments using aerobic or anaerobic composting methods at 50-60ºC resulted in a strong odor (offensive-odor level 3) or a severe odor (offensive-odor level 4) (Fraser *et al.*, 2013). Therefore, it was determined that the sewage sludge treatment method proposed in this study will reduce the generation of offensive odor and will minimize its effect on the concerned residents.

3.3 Variation in Microbiology

3.3.1 Decrease in the Total Bacterial Count after Bio-drying

Figure 5 shows the changes in the total bacterial count and

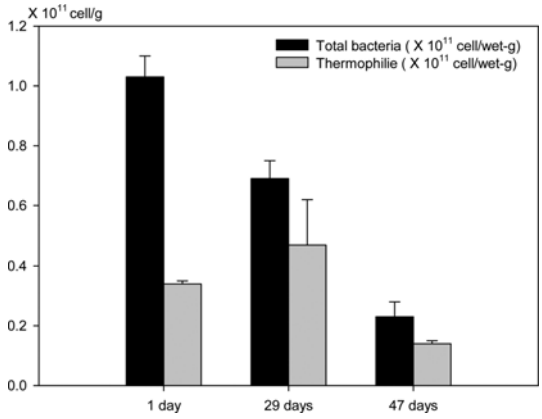

Fig. 6. Numbers of Total and Thermophilic Bacteria

thermophiles during the fermentation in the bio-drying process. A total of three analyses of the total bacterial count and the number of thermophiles were conducted: one on day 1 (beginning of fermentation), another on day 29, and the last on day 47 (completion of fermentation). The results showed that on day 1, the total bacterial count and the number of thermophiles were 1.03×10^{11} cell/g (dry weight) and 0.34×10^{11} cell/g, respectively, and the ratio of thermophiles to the total bacterial count was about 33%. On day 29, the total bacterial count and the number of thermophiles were 0.69×10^{11} and 0.47×10^{11} cell/g, respectively, which resulted in an about 30% decrease of the total bacterial count and an about 38% increase of the number of thermophiles. The ratio of thermophiles to the total bacterial count was about 68%, about twofold higher than on day 1. The total bacterial count and the number of thermophiles on day 47, when the fermentation was completed, were reduced by about 78 and 58%, respectively, compared to those on day 1. It is believed that this is because as the fermentation continued at about over 80ºC, the microbes that were active at a low or mid-temperature were killed, which contributed to the decrease in the total bacterial count. While the total bacterial count decreased after the completion of the fermentation, the ratio of thermophiles to the total bacterial count was about 60%, which was an about 23% increase from day 1. Again, it is believed that this is because the process conducted at an extremely high temperature killed the microbes that were active at a low or mid-temperature.

Shown in Table 4 are the results of the measurement of the *E. coli* and *Salmonella.* The results showed that before the ultra-high-temperature aerobic fermentation, the detected *E. coli* were 542 MPN/wet-g and the detected *Salmonella* were 920 MPN/

Table 4. Detection of *E. coli* and *Salmonella*

Parameter	*E. coli*				*Salmonella*			
	Initial composting		Final composting		Initial composting		Final composting	
	Detecting whether	Detection amount (MPN/wet-g)	Detecting whether	Detection amount (MPN/wet-g)	Detecting whether	Detection amount (MPN/wet-g)	Detecting whether	Detection amount (MPN/wet-g)
Detecting whether and Detection amount	○	Above 542	×	0	○	Above 920	×	0

wet-g. After the fermentation, however, neither *E. coli* nor *Salmonella* was detected. It is believed that this is because they were all killed as the temperature in the composting process rose to over 100ºC.

3.3.2 Investigation of the Bacterial Community

The present study conducted 16S rRNA gene sequence analysis and surveyed the cluster structure by verifying a highly similar strain (Fig. 7). The sequence analysis result of 40-45 clones per sample showed that the starter had 50% *Bacteroidetes* (phylum), 21.4% *Firmicute* (phylum), and 11.9% *y-proteobacteria* (class). In the genus level, *Salesgentibacter*, which belongs to the *Bacteroidetes* (phylum) class, accounted for 16.65%, followed by *Pseudomonas* of the *y-proteobacteria* class (11.9%) and *Bacillus* of the *Firmicute* class (9.5%). Various microorganisms, including *Flavobacterium* of the *Bacteroidetes* class and *Planifilum* of the *Firmicute* class, each accounting for 7.14% of the total, were observed. The sequence analysis result of the samples in the second stage, on day 29, included the highest temperature recorded in the bio-drying process of sewage sludge. These showed that *Bacillus* and *Pseudomonas*, which were between 9 and 11% in the starter sample, increased to 26.6 and 40.0%, respectively. *Firmicute*, which was 21.4%, and *y-proteobacteria*, which was 11.9%, also increased to 46.6 and 42.2%, respectively. *β-proteobacteria*, 4.7% in the starter sample, also increased to 8.8%. *Bacteroidetes*, however, which was dominant with 50.0% in the starter sample, sharply decreased to 2.2%. In the third-stage (stabilization stage) sample, *Firmicute* was recorded at 47.5%, *y-proteobacteria* at 17.5%, *β-proteobacteria* at 15.0%, *Bacteroidetes* at 12.5%, and *Actinobacteria* at 7.5%. At the genus level, *Bacillus*, which belongs to the *Firmicute* class, was dominant at 20%, and *Psychrobacter* of the *y-proteobacteria* class was dominant at 17.5%.

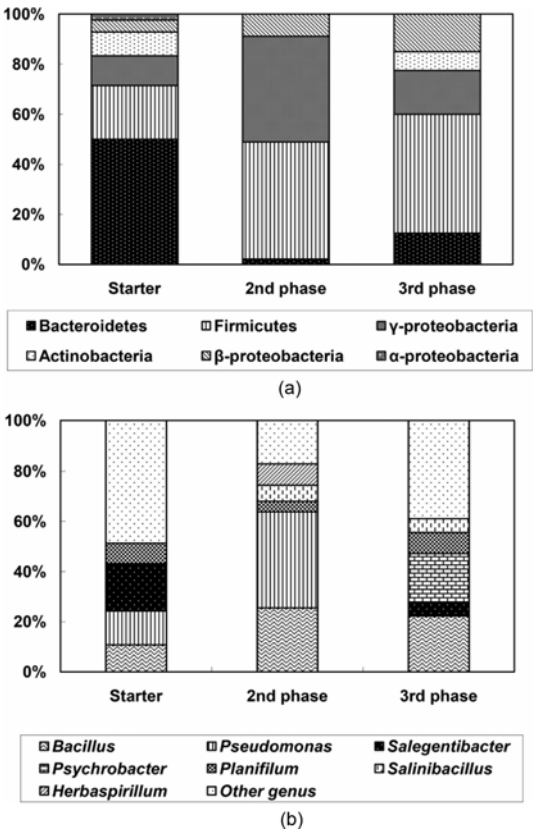

Fig. 7. Variations of the Bacterial Communities in the Composting Reactor (The Proportions of Phylum or Class: (a) and genus, (b) Were Calculated from the 16S rRNA Gene Clone Libraries. Starter: Operating day 1; 2nd Phase: Operating day 29; 3rd phase: Operating day 47)

When the strains observed in the 16S rRNA gene clone library were divided into genus-level samples to calculate the Shannon-Weiner Diversity Index (H) and Simpson Dominance Index (D), the starter had H at 4.07 and D at 0.07, while the sample on day 29, the highest-temperature stage, showed H at 2.43 and D at 0.25. These results indicate an increase in the dominance index of specific clusters. Considering that part of the products of the composting process are supplied to the next compositing process as a starter, it is believed that the starter contained a number of mid-temperature colonies after the stabilization stage. Along with the colonies included in the sewage sludge, the starter contributed to the initial decomposition of organic matter and to the heat generation. The growth of high-temperature bacteria, however, was promoted in the changed environment (Fogarty *et al.*, 1991).

4. Conclusions

This study evaluated the properties of sewage sludge treated using bio-drying with Ultra-thermophilic Aerobic Microorganisms (UTAMs). The temperature was maintained at between 90 and 100ºC, and the changes in the oxygen and carbon dioxide concentrations showed an inversely proportional relation. That is, the carbon dioxide and ammonia concentrations increased when the temperature of the fermentation chamber rose. The moisture content and organics concentrations decreased as the high-temperature aerobic fermentation continued, showing a trend identical to that in the typical aerobic composting process. The study results in relation to the designated offensive-odor materials showed that while there were higher concentrations of ammonia, methyl mercaptan, dimethyl sulfide, and trimethylamine than the regulation standards before fermentation, the concentrations of all these materials were lower than the regulation standards after the high-temperature aerobic composting treatment. The measurement of the total bacterial count and the number of thermophiles during the high-temperature aerobic composting treatment showed that compared to that before the composting treatment, the ratio of the number of thermophiles to the total bacterial count during the composting process increased, indirectly verifying the dominance of the UTAMs. In addition, the results demonstrated that the high-temperature process killed the pathogenic bacteria. The 16S rRNA gene demonstrated that the structure of the bacterial community in the ultra-thermophilic 2nd stage reflected that of a seeding starter. The major decomposers driving the bio-

drying process were identified as phylotypes related to *Bacillus* and *Pseudomonas.* As such, it was determined in this study that sewage sludge treatment using the high-temperature aerobic composting method will safely and economically process sewage sludge and produce quality compost.

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