

## GLOBAL JOURNAL OF ENGINEERING SCIENCE AND RESEARCHES DETERMINATION OF THERMAL MICROORGANISMS FOR COMPOSTING OF HIGH-SALT AND –OIL FOOD WASTE

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### ABSTRACT

The purpose of this study was to select microbial strains suitable for the composting of Korean food wastes with high salinity and oil content. For this purpose, enzyme activity, salt tolerance and temperature-dependent growth ability were investigated for various *Bacillus sp.* and *Actinomycetes*, and composting efficiency and odor reduction efficiency were evaluated by applying the selected strains to real-scale treatment device. The analysis results showed that, in the *Bacillus sp.*, *Geobacillus thermoglucosidasi* has highest activity for amylase, lipase, and cellulose, and in the *Actinomycetes*, *Thermomonospora fusca* has highest activity. These two strains were found to have high growth ability under high salinity and high temperature conditions and also have high culture yield for both forms of food wastes, effluent and solid. These two strains, accordingly, selected as eligible microorganisms and field applicability test for them showed high extinction rate up to 88% and excellent reduction effect for odor.

**Keywords:** *Thermal microorganism, food waste, composting, application test.*

### I. INTRODUCTION

The amount of food waste per day in Korea is 12-20,000 tons, corresponding to about 23% of municipal waste. The methods of disposing food waste include landfill, incineration, drying, fermentation-extinction, composting, and feed conversion[1,2]. The incineration has weakness of air pollution resulting from incomplete combustion due to the low amount of heat generation and high water contents, while the landfill method has problems of generation of a large amount of leachate to pollute groundwater and soil, bad odors due to corruption, and shortening the available period of used landfill site[2,3].

The fermentation-extinction is a method in which the food wastes are fermented thoroughly by feeding food for a long time, leading to evaporation of moisture in the food and decomposition of organic matters into carbon dioxide and water[4]. This method produces very low amount of food residuals after treatment thus ensures high user satisfaction. This method, however, requires a precise understanding of the process, water-regulating agents up to 20 times amount of input food, large construction area, and high maintenance cost. In addition, the period of time taken to treat food waste is relatively long compared to other processes, and sometimes deterioration of performance and odor problem due to contamination by germs may occur[5,6].

The most preferred method for treating food waste has been composting process. Composting refers to the treatment of food waste in the form of semi-dry solid organic matter through biological action using various aerobic microorganisms under aerobic conditions[7]. Since the composting proceeds under high temperature, the removal rate of the organic matter is high and the stability of treatment efficiency is remained even under the condition of rapid fluctuation in organic matter load. In addition, since the nitrification reaction does not occur under such high temperature, the supplied oxygen can be efficiently used, which is another merit of this process[8].

Considerations in applying the composting process include operating techniques to improve process efficiency, development of microbiological formulations containing degradation carrier, and selection of microorganisms with high enzyme activity. Although this process also has problem of generating odor, this problem can be solved

through the complete degradation of the food waste by the microorganism. The most prominent cause of odor incomplete decomposition of proteins and fats in food wastes, meaning that complete decomposition using high activity of protease and lipase is a solution to minimize the occurrence of odor[9].

The characteristics of food waste in Korea compared to Western one include high salinity due to miso, soy sauce, and kimchi, and high fat content due to various stew, pot, and fry. High salinity and fat content inhibit microbial activity thus lower composting efficiency. In order to increase composting efficiency and reduce odor, therefore, it is important to select a microbial strain suitable for the characteristics of food waste. The most important factors for the efficient composting of food wastes with high salinity and fat content such as Korean one are the potency of amylase, cellulase, protease, and lipase, while the potency of protease and lipase are important one for suppressing odor generation. Although many microorganisms have been reported as food fermentation strains, conventional technologies have not been able to show equal decolorizing activity on starch, fat, protein, and cellulose, resulting in a problem of low fermentation-extinction effect and deodorizing effect, ultimately low composting efficiency[10,11].

The purpose of this study was to determine suitable microbial strains of composting method considering the characteristics of Korean food wastes. For this purpose, enzyme activity, salt tolerance, and temperature-dependent growth were tested based on various *Bacillus sp.* and *Actinomycetes*. The selected strains were applied to a real-scale treatment device and composting efficiency and odor reduction efficiency were analyzed. The field applicability of the microbial strains selected based on the analysis results were evaluated.

## II. MATERIAL & METHODS

A composting strain was developed by selecting *Bacillus sp.* and *Actinomycetes* that has high growth temperature of 40-60 °C and suitable for composting as a strain suitable for characteristics of Korean food wastes. The selected *Bacillus sp.* were *Geobacillus thermoglucosidasius* (KCCM 41418, ATCC 43742, DSM 2542), *Bacillus licheniformis* (KCCM 11560, ATCC 27811), and *Annerini Bacillus subtilisophilus* (Aneurinibacillus thermoaerophilus, KCCM 41604, ATCC 700303), while the selected *Actinomycetes* were *Thermomonospora fusca* (KCCM 12398, ATCC 27730, IFO 14071, DSM 43792) and *Thermoactinomyces vulgaris* (KCCM 41014, ATCC 27868, IFO 15851, DSM 43796). These microorganisms were obtained from Korean Microorganism Conservation Center (KCCM).

### Enzyme Activity Test

The enzyme activities of microorganisms were examined to select ones with the highest degradability to main components of food wastes such as carbohydrates, proteins, fats and fibrin components.

For the starch degradability test, the culture medium was formulated adding starch of 1% to LB agar medium (Tryptone; 10g, Yeast extract; 5g, NaCl; 5g, Agar; 15g, distilled water; 10). The starch activity was measured by observing the size of clear zone generated from staining the strains inoculated and cultured for 24 hours with iodine solution (iodine 1g, potassium iodide 2g, distilled water 300ml) under 50°C. For the protein degradability test, the culture medium was formulated adding nonfat milk of 1% to LB agar medium. The protein activity was measured by observing the size of clear zone generated after inoculating and culturing for 24 hours under 50°C. For the fat degradability test, the culture medium was formulated adding tributyrin of 0.1% to LB agar medium. The fat activity was measured by observing the size of clear zone generated after inoculating and culturing for 24 hours under 50°C. For the cellulose degradability test, the culture medium was formulated adding carboxymethyl cellulose (CMC) of 0.5% to LB agar medium. The cellulose activity was measured by observing the size of clear zone generated after inoculating strain, culturing for 24 hours under 50°C, staining for 30 minutes with Congo red of 0.2%, and washing 15 minutes with 1M NaCl.

**Growth Ability by Change in Temperate and Salinity**

For the determination of growth temperature range of microorganisms, the presence of growth was evaluated after inoculating strains to LB agar and culturing for 48 hours under 20, 30, 40, 50, 60, and 70°C. The growth ability was determined by sensibly evaluating the size of strain colony.

For the growth ability test by change in salinity, the strains were inoculated to the LB agar supplemented with NaCl of 1%, 2%, 4%, 6%, and 8% (w/v) and cultured for 24 hours under 50°C. The growth ability was determined by sensibly evaluating the size of strain colony.

**Culture Yield in Food Effluent and Solid**

The culture yield was evaluated by counting the number of population colonies after culturing microorganisms at LB medium for 24 hours under 50°C, transferring to and culturing at agar plain medium for 48 hours. The food wastes are divided into effluent components containing most of moisture and remaining solid component (including absorbed components) based on the physical characteristics. During composting process of food waste, the earlier and later phases are for degradation of effluent and solid components, respectively. For the evaluation of the degradation ability of the effluent components in the microorganism, after adding half of the weight of the food waste to the food waste and gently stirring to obtain effluent, into which the agar was added to produce solid medium for microorganism culture, this culture was sterilized. After inoculating each medium and culturing for 48 hours under 50°C, the number of strain colonies was counted. For the evaluation of the degradation ability of the solid components in the microorganism, the dried and washed solid food wastes was high-speed pulverized into a slurry state, and distilled water, which weights five times solid state, and agar were added and sterilized. After inoculating each medium and culturing for 48 hours under 50°C, the number of strain colonies was counted.

**Extinction Rate**

A microbial formulation was developed by using strains selected as suitable for the composting of Korean food wastes. Each strain was inoculated into each 10 L culture medium containing nutrient broth medium (Tryptone 3g, yeast extract 3g, glucose 3g, NaCl 5g, K<sub>2</sub>HPO<sub>4</sub> 1g, H<sub>2</sub>O 1ℓ). and cultured for 48 hours under 50°C to obtain culture solution. The culture was mixed with sawdust as a carrier in a volume ratio of 2:10 (culture solution: sawdust) and dried to produce a dried microbial body. This body was mixed with nutrients nutrients of the same ratio of glucose, molasses, soybean meal, fish meal in a volume ratio of 0.5: 10 (nutrient:dried microbial body) to produce microorganism formulation for food waste composting.

The prepared microorganism formulation was put into device for food waste composting having a total capacity of 100 L equipped with a stirrer, a temperature controller, a deodorizer and a fan. During the fermentation period of 30 days, the internal temperature of the device was maintained as 50°C, the stirring period five minute/hour, and the stirring speed five revolutions/minute. Adding 10kg of food wastes (average moisture content 80%) daily, the rate of extinction, viable cell count and moisture content were measured.

**Odor Intensity Index**

For the evaluation of the degree of odor generated during the composting of food wastes, five sensory testers measured odor levels using direct olfactory method during above extinction rate experiment. The measured values were quantified as 1-5 levels and averaged. The food waste without the microbial agent was used as control group.

**III. RESULTS & DISCUSSION****Result of Enzyme Activity Test**

The enzyme activities, as shown in Table 1, varied greatly depending on the strains. Of the *Bacillus sp.*, KCCM 41418 showed the highest activity for amylase, lipase, and cellulose while KCCM 41604 showed the highest activity for protease. In the case of *Actinomycetes*, KCCM 12398 showed highest activity for all enzymes, and especially

showed higher activity for cellulose than those for *Bacillus sp.*. These results showed that there is a large difference in enzyme productivity and activity thus in composting efficiency, depending on microorganism strains,

**Table 1. Enzyme activity of each species**

Species	Name	Enzyme Activity (Diameter of clear-zone, mm)			
		Amylase	Protease	Lipase	Cellulase
Bacillus sp	KCCM 41418	29	18	14	22
	KCCM 11560	16	13	11	19
	KCCM 41604	15	21	0	14
Actinomycetes	KCCM 12398	18	12	5	27
	KCCM 41014	13	7	0	13

### Growth Ability by Change in Temperature

For the determination of growth temperature range of microorganisms, the strains were inoculated to LB agar medium and the presence of growth was evaluated varying the temperature in the range of 20-70°C, the results of which are shown in Table 2.

**Table 2. Growth ability of microorganisms by change in temperature**

Species	Name	Growth Ability by Change in Temperature					
		20°C	30°C	40°C	50°C	60°C	70°C
Bacillus sp	KCCM 41418	-	+	+++	+++	++	-
	KCCM 11560	++	++	+++	+	-	-
	KCCM 41604	-	+	++	+++	++	-
Actinomycetes	KCCM 12398	+	++	+++	+++	+	-
	KCCM 41014	+	++	+++	++	-	-

(+++ : Good, ++ : Normal, + : Weak, - : Growth impossible)

KCCM 41418 and 41604 showed good growth ability at 40-60 °C and KCCM 12398 and 41014 showed good growth ability at 40-50 °C. On the other hand, KCCM 11560 showed weak growth ability at 50 °C and good growth ability at 30-40 °C. It is considered, therefore, that selected microorganisms except KCCM 11560 are good candidates for composting microbial formulations because they showed high growth ability at high temperature up to 40-60 °C

### Growth Ability by Change in Salinity

For the determination of growth ability of microorganisms by change in salinity, the strains were inoculated to LB agar medium supplemented with NaCl of 1, 2, 4, 6, and 8% (w/v) and presence of growth was evaluated, the results of which are shown in Table 3.

**Table 3. Growth ability of microorganisms by change in salinity**

Species	Name	Growth Ability by Change in Salinity				
		1%	2%	4%	6%	8%
Bacillus sp	KCCM 41418	+++	+++	++	+	-
	KCCM 11560	+++	+++	++	+	-
	KCCM 41604	+++	+++	+	-	-
Actinomycetes	KCCM 12398	+++	+++	+	-	-
	KCCM 41014	+++	++	+	-	-

(+++ : Good, ++ : Normal, + : Weak, - : Growth impossible)

KCCM 41418 and 11560 maintained at salinity of 1%-6% and showed strong resistance to salt, thus being proposed as candidates for composting microbial formulation of food waste with high salinity. On the other hand, KCCM 41604, 12398, and 41014 showed growth ability at salinity of 1-4%.

**Culture Yield in Food Effluent and Solid**

As shown in Table 4, In the *Bacillus sp.*, KCCM 41418 showed the highest activity for both food effluent component (578 CFU/mL) and solid component (216 CFU/mL), thus highest culture yield. In the case of *Actinomyces*, the highest culture yields were observed in KCCM 12398 (82 CFU/mL) for food effluent components and KCCM 41014 (29 CFU/mL) for food solid components.

Table 4. Culture Yield in Food Effluent and Solid

Species	Name	Culture Yield (×100,000CFU/ml)	
		Effluent	Solid
Bacillus sp	KCCM 41418	578	216
	KCCM 11560	225	78
	KCCM 41604	362	107
Actinomyces	KCCM 12398	82	29
	KCCM 41014	47	22

**Extinction Rate**

The tests for enzyme activity, growth ability by temperature, salt tolerance, and culture yield for each microbial strain showed that *Geobacillus thermoglucosidarius* and *Thermomonospora fusca* were found to be most excellent strains for composting food wastes in Korea. The microorganism formulations were prepared using above two strains which were put to 100 L-capacity composting device and evaluated for characteristics such as extinction rate, moisture content. Figure 1 shows the results.

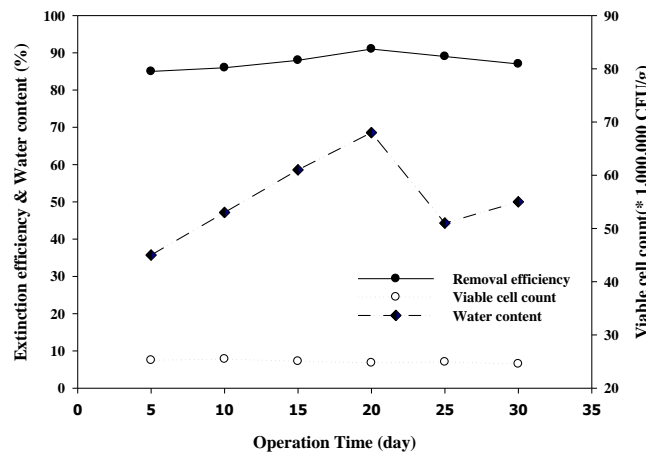


Figure 1. Extinction efficiencies changes of food waste

The extinction rate of food wastes ranged from 85 to 91%, resulting in 30-days average 88%. In *Actinomyces*, the average value was  $5.55 \times 10^7$  CFU/g maintained for 30 days and stably. The water content maintained 7.1%, which satisfying the criteria of extinction rate specified in guideline for food waste reducer published by Ministry of Environment

### Odor Intensity Index

The odor level was found to be normal representing odor level 1-2 even after composting process, shown in Figure 2. In the control group, the odor was very strong and its level reached up to 5.0 after the first five days maybe because the selected microorganism had excellent treatment rate and thus produced complete degradation.

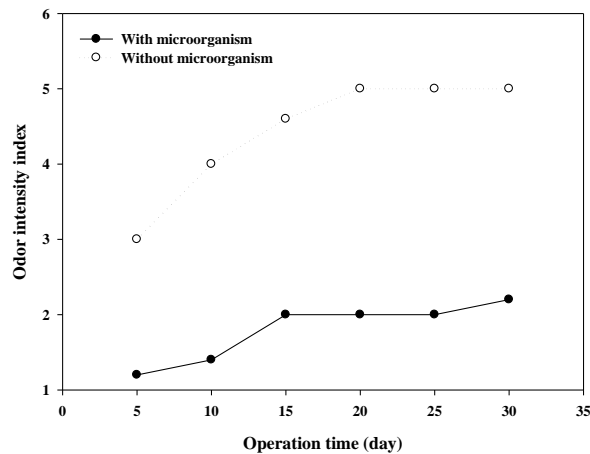


Figure2. Odor intensity changes of food waste composting

(Odor level 1: No smell; Odor level 2: Little level; Odor level 3: Clear perception of odor; Odor level 4: Discomfortable odor; Odor level 5: Very strong discomfortable odor)

In addition, the results of analysis, carried out as a field application test, of the characteristics of the compost produced showed that the ratio of organic matter in the compost was 83.4%, which was much higher than the standard (over 25%), that the organic matter/nitrogen ratio was 24.5, satisfying the standard, and that no heavy metals such as arsenic, cadmium, mercury, lead, chromium, copper, nickel, and zinc were detected. Although the salt content is varied depending on the type of food waste, in the case of the above experiment, the salt content (standard: less than 1%) was 0.0134%, confirming excellent compost. However, if the compost is accumulated over a long period of time, the salt content may exceed the allowable standard, 1.0%. It is necessary, therefore, to collect the compost early to keep the accumulation of salt from reaching standard for salinity.

## IV. CONCLUSION

The purpose of this study was to select microbial strains suitable for the composting of Korean food wastes with high salinity and oil content. For this purpose, enzyme activity, salt tolerance and temperature-dependent growth ability were investigated for various *Bacillus sp.* and *Actinomycetes*, and composting efficiency and odor reduction efficiency were evaluated by applying the selected strains to real-scale treatment device. The analysis results showed that, in the *Bacillus sp.*, *Geobacillus thermoglucosidasiu* has highest activity for amylase, lipase, and cellulose, and in the *Actinomycetes*, *Thermomonospora fusca* has highest activity. These two strains were found to have high growth ability under high salinity and high temperature conditions and also have high culture yield for both forms of food wastes, effluent and solid. These two strains, accordingly, selected as eligible microorganisms and field applicability test for them showed high extinction rate up to 88% and excellent reduction effect for odor.

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