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Change in Microbial Numbers during Thermophilic Composting of Sewage Sludge with Reference to CO_2 Evolution Rate

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Dewatered sewage sludge was composted in a laboratory-scale autothermal reactor in which a constant temperature of 60°C was kept as long as possible by regulating the air feed rate. The change in CO_2 evolution rate was measured continuously from the start up through the cessation of composting. The succession of mesophilic bacteria, thermophilic bacteria, and thermophilic actinomycetes was also observed during the composting. Specific CO_2 evolution rates of thermophilic bacteria and actinomycetes in the constant-temperature region of 60°C were assessed quantitatively. It was found that the CO_2 evolution rate was attributed to thermophilic bacteria at the initial stage of 60°C and to thermophilic actinomycetes at the later stage of 60°C.

A serious and complex problem in cities today is the disposal of huge amounts of sewage sludge produced from wastewater treatment facilities. Conversion of the sludge into stable organic material by composting is of great interest recently as one method of sludge treatment. As composting is a microbial decomposition process of organic matter, much microbiological research has been done in this area. The primary concern, so far, has focused on the pathogenic problems (2, 7, 12, 14) and on microbial populations in municipal waste compost (6, 10) and in agricultural and manure composting (4, 5, 8, 9, 12, 13). No information was available on microbial research of sewage sludge composting. However, it is difficult to assess real factors which affect the microbial succession unless microbial analysis is done under carefully controlled conditions of composting.

In this paper, isolation of microbial groups from a sewage sludge compost was conducted at several stages of the composting process in a laboratory-scale autothermal reactor in which a constant temperature of 60°C was maintained as long as possible by controlling the air feed rate to the reactor. During the composting, the microbial activity was monitored by measuring CO_2 evolution rate, and the specific activities of isolated groups of microorganisms were assessed quantitatively.

MATERIALS AND METHODS

Apparatus. The composting reactor used is cylindrical (300 mm in diameter, 400 mm in depth) and made of thermoresistant polyvinyl chloride resin with a perforated plate at the bottom to distribute the air supplied from outside. This reactor was surrounded with a cubic Styrofoam insulator (1.2 by 1.2 by 1.2 m^3) to maintain minimum heat loss from the wall of the reactor. Temperature was detected with three thermocouples which were placed at the bottom, center, and upper parts of the reactor. The temperature difference between the upper and bottom parts of the reactor was ±2°C at most. Air from a compressor was split into two streams: one for a lower flow rate, 400 ml/min; and the other for a higher flow rate, 4,000 ml/min. When the experiment started, the air was supplied at the lower flow rate. Once the signal from the thermocouple in the center of the reactor indicated a temperature higher than the set point of 60°C, a temperature controller changed the lower flow rate to the higher one and temperature was maintained autothermally at 60 ± 1°C by the on-off control. A temperature of 60°C has already been found to be optimal for sewage sludge composting by our research (1). A schematic diagram of the apparatus is shown in Fig. 1.

Composting material and seed. Dewatered sewage sludge cake from the Minamitama Wastewater Treatment Plant (Inagi City, Tokyo, Japan) was used as the composting material. The sludge was a mixture of raw settled sludge and excess sludge from the activated sludge process, containing approximately 60% water, 7.5% slaked lime, and 2.5% ferric chloride as dewatering agents. The average composition of the dried sludge was 50% volatile matter (VM), in which C, H, and N were 25, 4, and 3%, respectively, and 50% ash. Fresh sludge was collected from the plant just before each run of the composting operation. Compost product, produced in our laboratory-scale composting reactor by the procedure described herein and cured for 3 to 4 weeks at room temperature, was used as an inoculating seed. It was also ascertained that CO_2 evolution from the seed was negligible (1). The sludge cake and the seed, which were ground and sieved to <5 mesh, were mixed in the following ratios of seed/sludge (percent [dry weight]): 0% (run A), 10% (run B), 24% (run C), 133% (run D).

No bulking agent was added to simplify the reaction system and delete additional effects derived from such agents. The initial weight of the sludge-seed mixture packed into the reactor was 3,300 to 5,000 g (wet weight).

Operation of the reactor. As the sludge contained lime, the initial pH value of the material was around 11, and a long lag period (about 3 days) was observed before microbial degradation of the raw material started. To shorten the lag time, CO_2 gas from a cylinder was supplied to the reactor at the start of each run at a rate of 500 ml/min to neutralize the lime. The supply of CO_2 was continued until the pH of the material decreased to around 8. About 10 h later, after neutralization, a sharp rise in temperature and CO_2 evolution by microbial degradation of the sludge were observed.

Turning of the compost material in the reactor was conducted by hand every 24 h after the temperature reached 60°C. The sampling times of the materials during the composting were as follows: at the time when the CO_2 supply was stopped, when the temperature reached around 43 and

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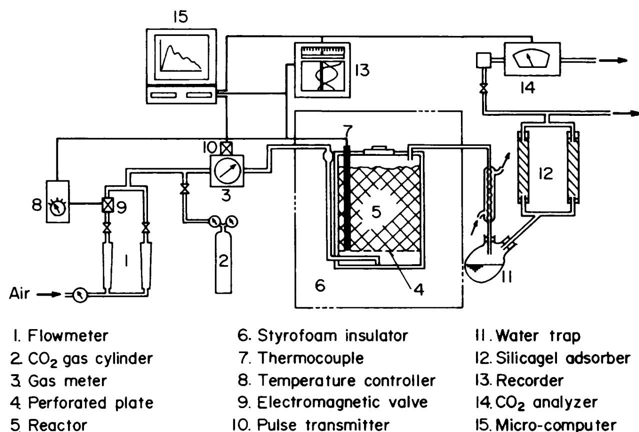

FIG. 1. Schematic diagram of composting reaction system.

60°C, when turning was done, and when the operation was stopped. The composting operation was stopped when temperature control with varying air flow was no longer available and when the temperature decreased to 30°C even at a lower air flow rate. The moisture content was adjusted to around 60% at the start of the experiments and was not controlled during the reaction.

Isolation of microorganisms. Bacteria, actinomycetes, and fungi, both mesophilic and thermophilic, were isolated. As preliminary experiments, several media for bacteria, actinomycetes, and fungi were tried, and the media in which the largest number of isolates appeared were adopted as the isolating media. They are as follows: Trypticase soy agar (BBL Microbiology Systems) medium for bacteria (Trypticase peptone, 17 g; phytone peptone, 3 g; NaCl, 5 g; K_2HPO_4, 2.5 g; glucose, 2.5 g; agar, 20 g; distilled water, 1 liter [pH 7.3]); malt-yeast extract agar medium for actinomycetes (yeast extract [Difco Laboratories], 4 g; malt extract [Difco], 8 g; glucose, 4 g; agar, 20 g; distilled water, 1 liter [pH 7.3]); and potato-dextrose agar medium for fungi (potato-dextrose agar, 39 g; distilled water, 1 liter [pH 5.2]). Other media which were tried but not used were as follows: for bacteria, nutrient medium (Difco), PGY medium (peptone, glucose, yeast extract), and brain heart infusion medium (Difco); for actinomycetes, ISP no. 4 medium (inorganic salts-starch agar) and PGY medium; and for fungi, Czapek solution agar (Difco). Several media supplemented with water extract of the sludge were also tried. However, no remarkable increase in the number of isolated microbes was observed.

Ten grams (wet weight) of a sample was suspended into 90 ml of sterile water in a sterile homogenizer cup and dispersed at 10,000 rpm for 10 min with a homogenizer (type EX-3, Nihon Seiki Ltd). After serial dilution in sterile water, the suspension was spread onto the three types of medium plates defined above. The incubation temperatures were 30°C for isolation of mesophiles and 60°C for thermophiles. The incubation time was 3 days for mesophilic bacteria, 2 days for thermophilic bacteria, and 7 days for actinomycetes and fungi, mesophilic and thermophilic, respectively. The average number of microorganisms isolated on three of six plates was considered the viable cell number. The deviation of the numbers was <10%.

Analysis. The activity of microorganisms was continuously monitored by measuring the CO_2 concentration of the off-gas from the reactor by means of a CO_2 analyzer (type ZFP4, Fuji-Denki Co., Ltd.). The off-gas was passed through both a condensor and a silica gel column to remove moisture before it entered the analyzer (see Fig. 1). The water content of the solid sample was determined from the loss of weight after drying at 80°C for 24 h. The dried solids were ground in a mortar and heated at 600°C for 30 min in an electric oven. After cooling, a few drops of ammonium carbonate solution (25%, wt/vol) were added to the residue followed by heating at 200°C for 1 h. The loss of weight was calculated as VM. As it was found from preliminary experiments that the amount of dry-weight reduction of the compost has a linear relationship with the cumulative amount of CO_2 evolved, the value of VM at a certain time was estimated from this relationship (see equations 2 and 3).

RESULTS

Change in microbial population during composting process. The changes in the number of mesophilic bacteria, thermophilic bacteria, and thermophilic actinomycetes from runs A, C, and D are shown in Fig. 2 to 4, respectively. As the number of mesophilic actinomycetes and fungi were of the order of 10^2 cells per g of dry compost, their contribution was considered to be small in this composting process. The change in the number of mesophilic bacteria was not remarkable compared with those of thermophilic bacteria and actinomycetes. It is interesting that appreciable numbers of them existed during the thermophilic stage at 60°C. Their

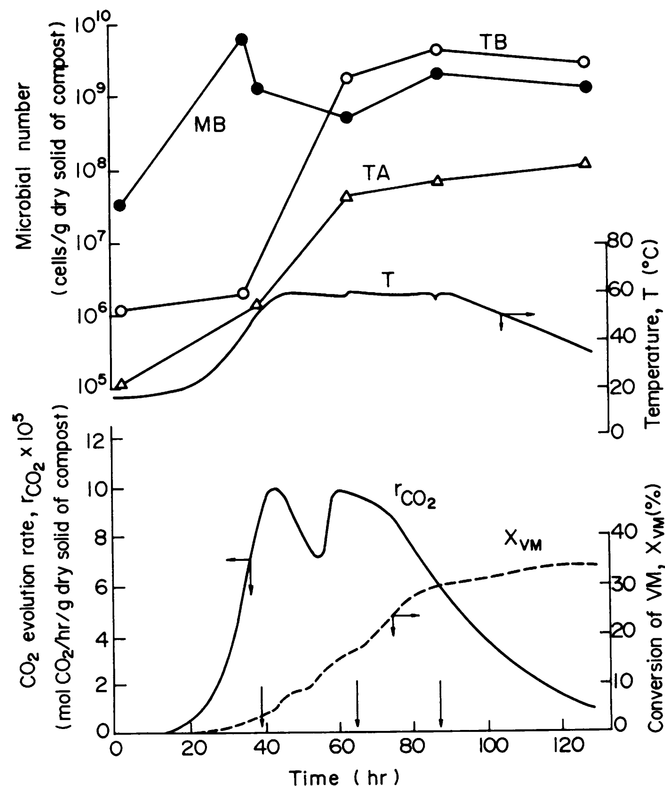

FIG. 2. Time courses of temperature (T), CO_2 evolution rate (r_{CO_2}), the cell number of isolated microorganisms, and conversion of VM, X_{VM}, during composting in run A (no seed added). Symbols: ●, mesophilic bacteria (MB); ○, thermophilic bacteria (TB); △, thermophilic actinomycetes (TA). Arrows on the abscissa indicate the points at which compost was turned.

survival was due to their thermotolerance property, details of which are explained in the accompanying paper (11).

The average numbers of microorganisms isolated from the raw sludge and the seed are shown in Table 1. The effect of increasing seed was clearly reflected in the increase in initial numbers of thermophilic bacteria and thermophilic actinomycetes (see Fig. 2 to 4).

Pattern of CO_2 evolution rate. Time courses of CO_2 evolution rate, r_{CO_2}, are also shown in Fig. 2 to 4. The r_{CO_2} is defined as moles of CO_2 evolved per hour per gram (dry weight) of compost. It seemed that the seeding ratio affected both the pattern and the values of the CO_2 evolution rate. As the seed ratio was increased, the second peak of CO_2 evolution appeared more closely to the first one and eventually only one peak was seen in run D (Fig. 4). As described below, the peak CO_2 evolution rate was closely related to specific groups of microorganisms.

Estimation of metabolic activity for isolated groups. Although a large number of mesophilic bacteria were isolated from the compost materials even at the thermophilic stage of 60°C, their respiratory activity at 60°C was found to be negligible, as described in the accompanying paper (11). Therefore, the contribution of both thermophilic bacteria and thermophilic actinomycetes to the overall CO_2 evolution rate during a constant temperature of 60°C was estimated. It was shown by Bach et al. (1) that the CO_2 evolution rate is mostly associated with degradation of VM of the raw sludge in the composting material, and the amount of degradable VM in the inoculating seed is negligible compared with that of the raw sludge. During the thermophilic stage, the two groups of microorganisms (i.e., thermophilic bacteria and thermophilic actinomycetes) were assumed to have their

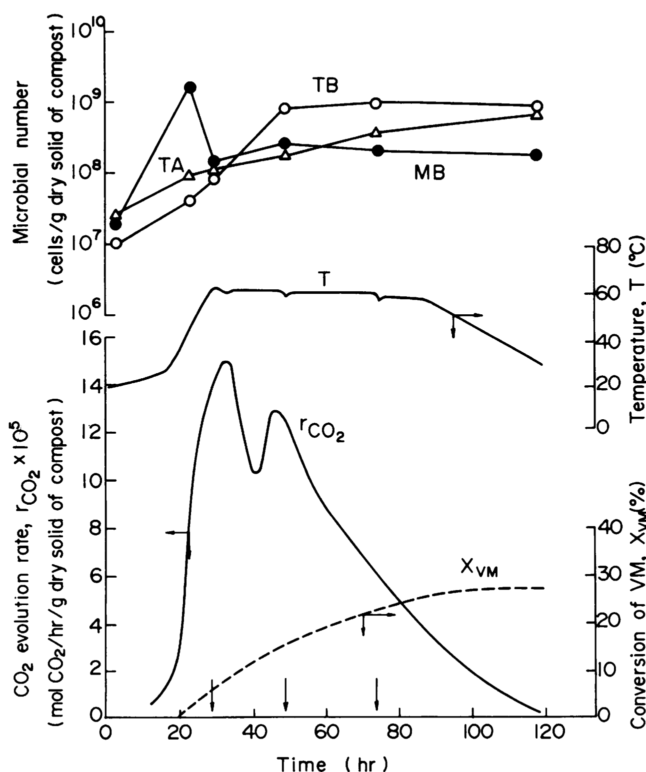

FIG. 3. Time courses of composting in run C (24% seed). For symbols and details, see legend to Fig. 2.

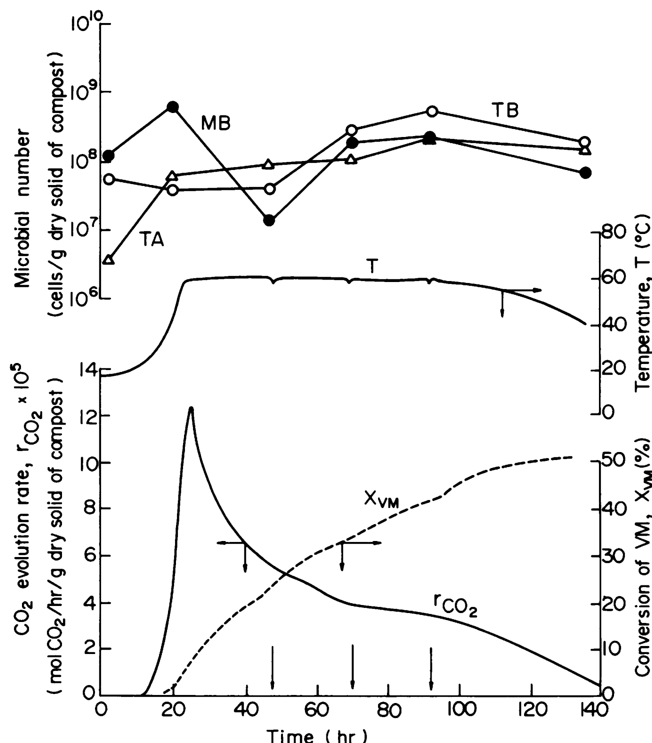

FIG. 4. Time courses of composting in run D (133% seed). For symbols and details, see legend to Fig. 2.

own specific CO_2 evolution rates which are the function of only the VM conversion of the raw sludge. Based on that assumption, the following linear equation in the thermophilic region of 60°C was introduced:

$$n_a R_a + n_b R_b = r_{CO_2} \quad (1)$$

where R_a is the CO_2 evolution rate per cell of thermophilic actinomycetes (moles of CO_2 per hour per cell); R_b is the CO_2 evolution rate per cell of thermophilic bacteria (moles of CO_2 per hour per cell); n_a is the cell number of thermophilic actinomycetes (cell per gram [dry solid] of compost); n_b is the cell number of thermophilic bacteria (cells per gram [dry solid] of compost); and r_{CO_2} is the CO_2 evolution rate (moles of CO_2 per hour per gram [dry solid] of compost).

The conversion of VM in the raw sludge, X_{VM}, is defined and calculated in the following way:

TABLE 1. Number of microorganisms isolated in raw sludge and in seed

Microorganisms	Cells/g (dry wt) of material	
	Raw sludge	Seed
Mesophilic		
Bacteria	3.6×10^7	1.2×10^8
Actinomycetes	$<10^3$	$<10^3$
Fungi	4.2×10^2	1.0×10^2
Thermophilic		
Bacteria	1.4×10^6	6.8×10^7
Actinomycetes	1.4×10^5	3.5×10^8
Fungi	<10	<10

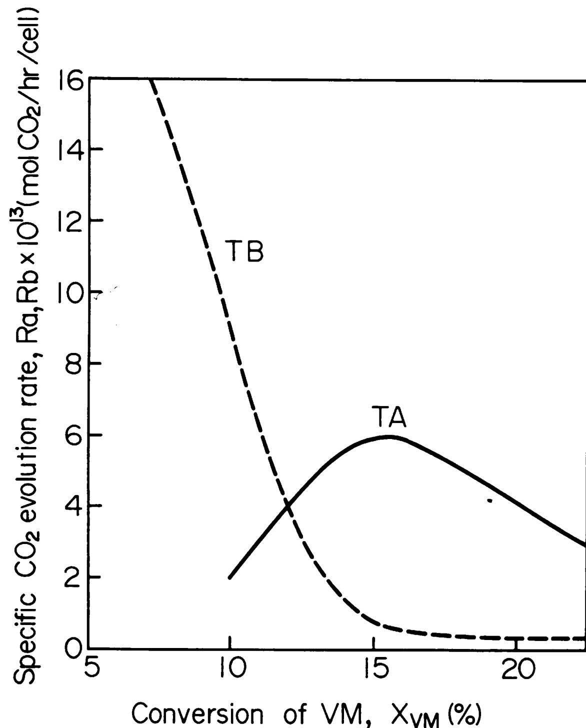

FIG. 5. Estimated specific CO_2 evolution rates of thermophilic bacteria, R_b, and thermophilic actinomycetes, R_a, as a function of conversion of VM. Symbols: - - - - -, thermophilic bacteria (TB); ——, thermophilic actinomycetes (TA).

$$X_{VM} = (VM_0 - VM_t)/VM_0 = \frac{Y}{VM_0} \int_0^t R_{CO_2}\, dt \quad (2)$$

where VM_0 is the initial content of VM (grams); VM_t is the content of VM at time t (grams); R_{CO_2} is the CO_2 evolution rate at time t (moles of CO_2 per hour); and Y is the conversion factor of VM degraded to CO_2 produced (grams per mole of CO_2). Y was obtained from the experiments by using the following equation:

$$Y = (DS_0 - DS_f) \Big/ \int_0^{t_f} R_{CO_2}\, dt \quad (3)$$

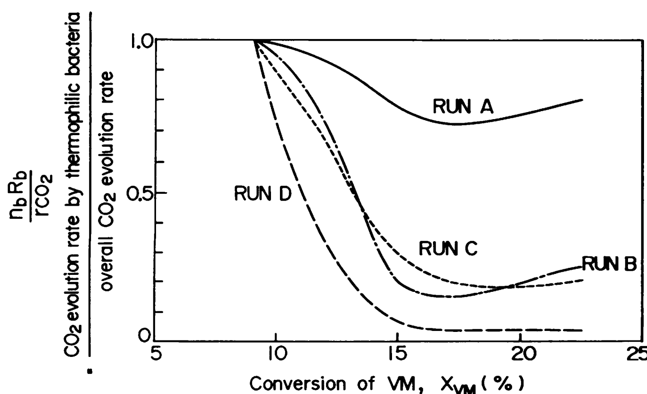

FIG. 6. Estimated CO_2 evolution rate attributed to thermophilic bacteria/overall CO_2 evolution rate in the four composting runs.

where DS_0 is the weight of dry solid in the initial composting material (grams); DS_f is the weight of dry solid at the time of termination of composting (grams); and t_f is the time when composting was terminated.

Each term in the left-hand side of equation 1 defines the CO_2 evolution rate by either thermophilic bacteria or actinomycetes. If the experimental results of the cell numbers and CO_2 evolution rates from four runs are plotted against the conversion of VM in the sludge, four sets of values for n_a, n_b, and r_{CO_2} are available for a given conversion of VM. Therefore, R_a and R_b for each conversion of VM can be calculated by the method of least squares. Figure 5 shows the result. The temperature reached 60°C after about 7% VM conversion was attained (Fig. 2 to 4). The value for R_a estimated from the least-squares method was negative between 7 and 10% VM conversion. This is because the activity of thermophilic actinomycetes is so small compared with that of thermophilic bacteria that the values of the first and second terms in the left side of equation 1 were different in their order. Therefore, the value for R_b in the range of 7 to 10% was estimated by discounting the term of $n_a R_a$ in equation 1. By using the calculated values of R_a and R_b in Fig. 5, the contribution of CO_2 evolved by each group of microorganisms to the total CO_2 evolved was assessed (Fig. 6). The ordinate represents the ratio of CO_2 evolution rate attributed to thermophilic bacteria/overall CO_2 evolution rate. It is obvious that CO_2 evolution is attributed mainly to thermophilic bacteria at the initial stage of 60°C constant temperature and later to thermophilic actinomycetes. When the seed increased, the contribution of thermophilic actinomycetes became more pronounced. In Fig. 7, the contribution of the two groups of thermophiles to the CO_2 evolution rate for run C is depicted by solid lines.

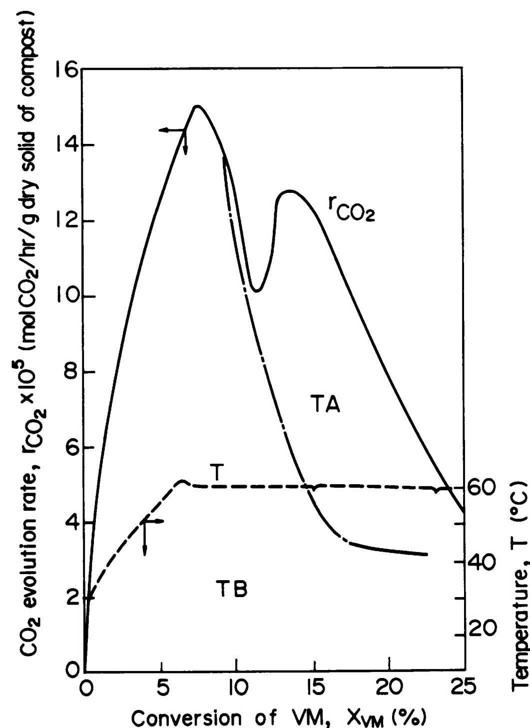

FIG. 7. Contribution of CO_2 evolution rates by both thermophilic bacteria (TB) and thermophilic actinomycetes (TA) to the overall CO_2 evolution rate measured during composting, run C.

DISCUSSION

Appropriateness of assumption in estimating specific CO_2 evolution rate. When the specific activities of thermophilic bacteria and actinomycetes were estimated by equation 1, the assumption was made that the activities were only a function of VM conversion. If specific CO_2 evolution rate depends on VM concentration as well as VM conversion, R_a and R_b in equation 1 can be replaced by $R_a'z$ and $R_b'z$, respectively. Here z is an initial VM concentration of raw material and R_a' and R_b' are CO_2 evolution rate coefficients for actinomycetes and bacteria, respectively. Equation 1 will therefore be modified to:

$$n_a R_a' z + n_b R_b' z = r_{CO_2} \quad (4)$$

The same method of least squares used to assess the values R_a and R_b from equation 1 was applied to obtain the values R_a' and R_b'. The value of z was taken as the weight fraction of initial VM concentration in the raw sludge/initial dry solid concentration in the raw material. To assess the adaptabilityof equations 1 and 4, the correlation factors between the measured r_{CO_2} and calculated r_{CO_2} from the values R_a and R_b (or R_a' and R_b') were compared in two cases. They were 88% for equation 1 and 44% for equation 4. This result suggests that the assumption made to use equation 1 is more reasonable. Since the compost reaction proceeds on a solid surface which microorganisms inhabit, the specific activity of CO_2 evolution of a microorganism will depend solely on the availability of VM of the raw sludge. On the other hand, if the growth rate of a microorganism in the compost material is considered, the specific activity of the microorganism might depend not only on the VM conversion, which corresponds to quality of the substrate, but also on the initial VM concentration, which corresponds to quantity of the substrate. In the present study, however, the experimental data obtained were not enough to determine the growth rate of microorganisms accurately.

Specific activity of isolated microorganisms. No quantitative analysis has been made about CO_2 evolution rate with reference to cell numbers during composting. The CO_2 evolution rate of common mesophilic bacteria such as *Bacillus* sp. and *Azotobacter* sp. was estimated to be on the order of 10^{-16} to 10^{-14} mol of CO_2/cell per h (3). When only the mesophilic bacteria were assumed to be responsible for the CO_2 evolution at the early stage of the composting, when the temperature was <40°C, the specific CO_2 evolution rate for the mesophilic bacteria was estimated to be on the order of 10^{-14} mol of CO_2/cell per h. No data, however, have been reported for corresponding values for thermophilic bacteria or actinomycetes. The specific CO_2 evolution rate estimated based on equation 1 was on the order of 10^{-13} mol of CO_2/cell per h (Fig. 5). The values seem to be rather high compared with those of common mesophilic bacteria. However, *Pseudomonas* sp. was reported to have a high O_2 uptake rate (3), corresponding to the order of 10^{-13} when converted to the CO_2 evolution rate.

Microbial succession and CO_2 evolution rate. At the initial stage of the composting, mesophilic bacteria inherent to the raw sludge mainly contributed CO_2 evolution. As temperatures rose, the leading group of microorganisms which contributed to CO_2 evolution changed from mesophilic to thermophilic microorganisms. From the result shown in Fig. 7, it is apparent that the first peak in the CO_2 evolution rate in Fig. 2 and 3 was also mostly associated with the activity of thermophilic bacteria, whereas the second one was associated with that of thermophilic actinomycetes. Figure 4 shows, however, only one peak in CO_2 evolution rate. This can be interpreted as meaning that larger inoculation of seed gave a higher concentration of actinomycetes and the peak of CO_2 evolution of actinomycetes coincided with that of thermophilic bacteria. The contribution of the actinomycetes depended strongly on the seeding ratio, as shown in Fig. 6 at the region of high conversion of VM.

Actinomycetes develop far more slowly than most bacteria or fungi and are rather ineffective competitors when nutrient levels are high. Moreover, as they are generally more tolerant to high temperature, they become more active in a later period at 60°C, when the level of nutrients becomes lower. The estimated values for specific CO_2 evolution rates in Fig. 5 indicate that the thermophilic actinomycetes were more competitive when the nutrient level became lower. The dominance of actinomycetes in the experiments was noticed by the white color on the surface of the compost at a later period in the composting.

Fungi could barely be detected throughout the experiments. The fact that few fungi were detected is partly due to the high moisture content of the composts and partly because the optimal temperature for thermophilic fungi is 45 to 50°C; high temperature is thus unfavorable to fungi. Fungi were reported to be essentially absent at 65°C for straw composting (6). That only restricted groups of microorganisms appeared in the composting and the small deviation in the numbers of isolated microorganisms can be attributed to the high pH value of the lime-containing raw sludge used in the experiments.

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