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Biokinetic Analyses of Adaptation and Succession: Microbial Activity in Composting Municipal Sewage Sludge

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The interactions between temperature and the microbial communities in composting municipal sewage sludge were studied to determine the optimal temperature range for efficient decomposition (stabilization) of the sludge. Information concerning thermophilic successions in such communities was also obtained. Samples were taken from several different temperature areas in a production-scale composting pile throughout the 19-day processing run. Optimum temperatures for microbial activity, determined as the rate of [^{14}C]acetate incorporation into microbial lipids, were determined for each sample. Biomass was determined from the lipid phosphate content of the sample. Maximal activities were generally found in samples coming from lower-temperature areas (25 to 45°C), whereas samples from high temperatures (55 to 74°C) usually had relatively little activity. The temperature giving the optimum activity in samples incubated at a variety of temperatures during the assay tended to increase as the composting time progressed, but never exceeded about 50°C. Many of these temperature response curves were similar in nature to curves reported for purified enzyme systems and pure cultures of bacteria. Comparisons of the apparent energies of activation calculated for different temperature ranges over time also indicated that the overall community was better adapted to higher temperatures during the latter part of the composting run. It was also found that the relationship between the apparent energies of activation and the apparent energies of inactivation (apparent heats of denaturation) consistently changed with sample temperature throughout the composting run, suggesting that the microbial communities from hotter samples were better adapted to high temperatures than those from cooler samples, and vice versa. No evidence for significant activity by extreme thermophiles, having temperature optima of >55°C, could be found at any time during the run. It was concluded that, to obtain efficient and rapid decomposition during composting, temperatures should not be allowed to exceed about 55°C. In addition, information concerning the nature of thermogenic succession in diverse heterotrophic microbial communities was obtained, partially through the analysis of the overall apparent energies of activation for the incorporation of exogenous [^{14}C]acetate into lipids.

Elevated temperature is one of the most extreme environmental stresses to which organisms are exposed, requiring many molecular adaptations for tolerance and growth (3, 8, 10, 15). Temperature is also a fundamental factor affecting the rate and net outcome of chemical and biochemical reactions (24). It has long been known that aggregations of organic-rich matter tend to heat up as the indigenous microbial community rapidly decomposes the utilizable substrates, producing metabolic heat (21, 36). In organic piles of sufficient size and insulation the metabolic heat is trapped and can elevate the temperatures in the pile from the ambient to in excess of 70 or 80°C within a few days (14, 21, 23, 36). Such habitats provide a unique opportunity to study the succession of diverse microbial communities with regard to changing temperature over relatively short periods of time.

The production of composted materials through the manipulation of this process has long been recognized as a valuable means of recycling organic waste products and has recently become an important means of disposing of municipal solid waste and sewage sludge (14, 19, 21, 28, 38). The goals of composting in solid-waste management are to rapidly reduce pathogens, odors, "putrescible" organic matter, moisture, and bulk, producing a biologically "stabilized" material (21, 28, 36). No general agreement has yet been reached as to the best procedures for the optimization of the efficiency of decomposition ("stabilization") in this system, and a wide variety of methods are currently practiced (21). Some researchers have proceeded on the assumption that the production of high temperatures (>60 to 65°C) within the composting mass are necessary to ensure both rapid pasteurization and decomposition (14, 28–29, 30, 33), although a temperature of 55°C for at least 3 days has been found adequate for better-than-significant reduction of pathogens in aerated static pile systems (12, 38). Others have proposed that the accumulation of heat is the critical limiting factor pertaining to microbial decomposition and that aeration systems can be used to dissipate excess heat to optimize stabilization (19, 20, 25). It is generally agreed, however, that given reasonable initial environmental conditions of moisture, pH, air space, and C/N ratios (21, 28–30, 35), and provided that oxygen does not become generally limiting, the most critical parameter influencing the rate of composting and the quality of the product is temperature.

Many of the findings concerning temperature optimization in the composting process have been in conflict (21). This may be due in part to the indirect and incomplete nature of the measurements of microbial activity and biomass associated with the composting material which makes direct comparisons between temperature treatments impossible. Estimations of microbial decomposition activity have typically been made by using such indirect indices as the overall temperature, odor (25), moisture content (20, 25, 35), carbon dioxide evolution (35), or oxygen uptake (33) of the composting pile. In most cases concurrent assessments of microbial biomass were not made or were aimed at quantifying the

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numbers of surviving pathogens (14) or other groups, using only selective isolation techniques (21).

The purpose of this study was to provide a better understanding of the interactions between temperature and the microbial communities in composting sewage sludge, using accurate and consistent measures of microbial activity and biomass so that conclusions concerning an optimal temperature range for rapid decomposition might be made. Information leading to a better understanding of rapid thermogenic microbial successions was also obtained.

MATERIALS AND METHODS

Composting facilities. Composting was performed in a large production-scale composting bin operated by Paygro, Inc., at South Charleston, Ohio. The compost piles measured approximately 6 by 24 by 3 m. Air was forced up through the pile from the perforated base of the composting bin. These facilities have been described in detail previously (32, 39).

Temperatures within the compost pile were continuously monitored at 80 different locations, using thermocouples (type K; Marlin Manufacturing Corp., Cleveland, Ohio) which were connected to a microprocessor (model RP-1D; Kaye Instruments, Bedford, Mass.). These temperature data were used to automatically regulate the operation of the aeration fans so as to keep the mean overall compost temperature below a certain preselected level. This temperature feedback regulation system operates in a manner similar to those previously described for other facilities (20, 25). The mean reading of the 80 temperature sensors is reported as the mean compost pile temperature. This may not be the true mean temperature of the pile, due to limitations associated with the locations of the probes, but should accurately reflect the overall relative changes in temperature which occurred. The entire composting facility is under a roof, alleviating any problems associated with precipitation.

Composting material. Primary raw sewage sludge (undigested and dewatered) from Akron, Ohio, was mixed with bulking agents (hardwood bark chips and sawdust, mostly oak) and previously composted sewage sludge in a ratio of 1.0:0.4:1:2 (vol/vol/vol). Approximately 295,000 kg (wet weight) of this initial mix was composted during each batch-processing run. Experiments performed during two different processing runs will be discussed here, the first beginning 24 April 1982 (run A) and the second beginning 11 June 1982 (run B). The percent dry solids of the initial sludge-bulking agent mixes were approximately 48 and 46% for runs A and B, respectively. The entire compost pile was removed from the composting bin and then replaced (or "turned") twice during each run, at approximately weekly intervals, to provide more air space and a more even exposure of all of the material to elevated temperatures and aeration. Each composting run lasted 19 days, with runs A and B yielding material with approximately 59 and 63% dry solids, respectively.

Sampling procedures. Sampling and microbiological assays were performed on several different days during each composting run. On each sampling day material was taken from several different locations within the compost pile. In this composting system a temperature gradient usually exists from the upper central portion of this pile (the hottest) to the areas near the walls and floor of the composting bin (the coolest) (H. Hoitink and G. Kuter, personal communication), making it possible to sample from a variety of temperature areas on any particular day. An attempt was made to sample from areas similar in other respects (moisture and aeration), so most samples were taken from a depth of about 20 to 40 cm below the upper surface of the pile. As each sample was removed from the pile the temperature was immediately determined by means of a mercury thermometer. The microbiological assays were begun immediately after sampling in a laboratory adjacent to the composting facility. Portions of each sample were homogenized in a high-speed blender for a total of 45 s with enough distilled water to form a 2% (initial wet wt/vol) suspension. During processing each sample was maintained at its ambient sampling temperature in a water bath. Dry-weight determinations were made after quadruplicate subsamples were dried to constant weight at 60°C. Determination of volatile solids content was made from these samples after heating to 500°C for 2 h and correcting for losses of the water of hydration (31).

Experimental design. During run A, microbial activities, both as rates of substrate mineralization and substrate incorporation into lipids, were determined while each sample was incubated in a water bath held at the ambient compost pile temperature from which that sample was taken. In previous experiments it was found that allowing the samples to equilibrate at the incubation temperature for at least 5 min before the addition of labeled substrate resulted in about a 10% increase in measured activity. In the experiments reported here, samples were allowed to equilibrate for 15 min before the beginning of the assay, and all assays were performed in triplicate, except where noted.

During run B, substrate incorporation rates were determined for each sample over a range of incubation temperatures from 25 to 60 or 65°C, including the sampling temperature. In addition, to isolate the effect of temperature and to eliminate any possible effects associated with differences in the chemistry of the particular samples, equal amounts of each sample (volumes of homogenate) taken on a particular day were pooled to form a combined sample for that day. The microbial activity of this pooled sample was determined over a range of temperatures in quadruplicate.

Microbial biomass. Biomass was determined as the lipid phosphate content of the compost, by the method described previously (39, 42). The lipids were extracted in triplicate from each homogenized sample, using chloroform and methanol (7), and digested in 32% perchloric acid for 2 h at 180°C. The inorganic phosphate was then measured colorimetrically at 830 nm, using the molybdate blue reaction (13), and expressed as micromoles of PO_4 per gram (dry weight) of compost.

Microbial substrate incorporation. Compost homogenates (2 ml) were incubated with 0.1 ml of [*U*-^{14}C]acetate (0.2064 to 0.2333 μCi, 0.296 to 0.334 μg) for 1 h at specific temperatures. In separate experiments, it was determined that the incorporation of [^{14}C]acetate into microbial lipids in compost was linear ($r = 0.9999$; $P < 0.01$) for at least 90 min and began to taper off slightly after 120 min ($r = 0.9947$; $P < 0.01$). The rate of incorporation of the label into lipids was determined by extracting the lipids and quantifying the radioactivity by scintillation counting, as previously described (26). Abiotic controls received 0.5 ml of 0.0037 M $HgCl_2$ 15 min before the addition of the labeled substrate.

The resulting data (disintegrations per minute per hour) were then adjusted for biomass (disintegrations per minute per hour per micromole of PO_4) or grams (dry weight) of compost (disintegrations per minute per hour per gram). Since batches of labeled substrate having slightly different activities were sometimes used, the data were also divided by the number of disintegrations per minute of substrate

added before incubation, leaving a unitless numerator (decimal percent incorporation per hour per unit of biomass).

Microbial substrate mineralization. Compost homogenates (5 ml) were incubated with 0.1 ml of L-[*U*-^{14}C]glutamate (0.1911 μCi, 0.00109 μg) in stoppered serum vials for various lengths of time at a specific temperature. Abiotic controls received 1 ml of 0.0037 M $HgCl_2$ in 10% trichloroacetic acid 15 min before the addition of the labeled substrate. The $^{14}CO_2$ evolved was collected and quantified as previously described (17). These data are presented in the same units as the incorporation data (per hour per micromole of PO_4).

RESULTS

Previous experiments (39) revealed a significant negative relationship between temperature and microbial activity in composting municipal sewage sludge, determined as the rates of incorporation of ^{14}C-labeled glucose, acetate, and glutamate into lipids. Microbial biomass of the compost was also negatively correlated with temperature. These results led to the more detailed work reported here concerning the temperature-microbe interactions in this system.

Run A. During run A the mean temperature of the compost pile ranged from 24 to 61°C, the air temperature ranged from 1 to 28°C, and the aeration fans were on 65% of the time. Acetate incorporation rates and glutamate mineralization rates were compared in compost taken from several different temperature regions within the pile. Glutamate mineralization is shown in Fig. 1. On day 5 of composting all of the samples exhibited a lag period of about 0.5 to 1 h, but no lag had been present on day 1 of composting.

Mineralization rates were much higher in samples which were taken from lower-temperature regions of the compost pile (20 to 45°C) than in those from 55 and 65°C. The 65°C sample yielded very low rates of mineralization, but after about 1.5 h of incubation they were significantly greater than those of the abiotic control. The mineralization rates for the sample taken from a 20°C area on day 5 were much lower than those of the 20°C sample taken on day 1. However, the 35 and 45°C samples taken on day 5 exhibited mineralization rates greater than or equal to that of the 20°C sample from day 1 after 2.5 to 3.5 h of incubation.

The rates of acetate incorporation into microbial lipids in the same day 5 samples are shown in Fig. 2 (solid line). The 3.5-h mineralization rates from Fig. 1 are also shown for comparison (dotted line). These two metabolic rates correlated well ($r = 0.8752$; $P < 0.06$). Acetate incorporation rates at 20°C on day 1 were $6.95 \pm 0.42 \times 10^{-2}$ h^{-1} μmol of PO_4^{-1}, not significantly different from those of the 20°C sample taken on day 5.

As seen with glutamate mineralization, acetate incorporation rates at 55 and 65°C were very low, but slightly greater than those of the abiotic controls ($1.46 \pm 0.07 \times 10^{-2}$ h^{-1} μmol of PO_4^{-1}).

Run B. During run B the mean compost pile temperature ranged from 33 to 69°C, the air temperature ranged from 10 to 26°C, and aeration fans were on 89% of the time. Due to packing of sludge at the bottom of the pile, the aeration vents in the floor of the composting bin became partially clogged during the early part of this processing run, resulting in poor aeration and a somewhat slower than normal increase in overall temperature. This problem was remedied during the second turning of the pile (days 10 to 11), and the temperatures subsequently rose more quickly. The fans were kept on

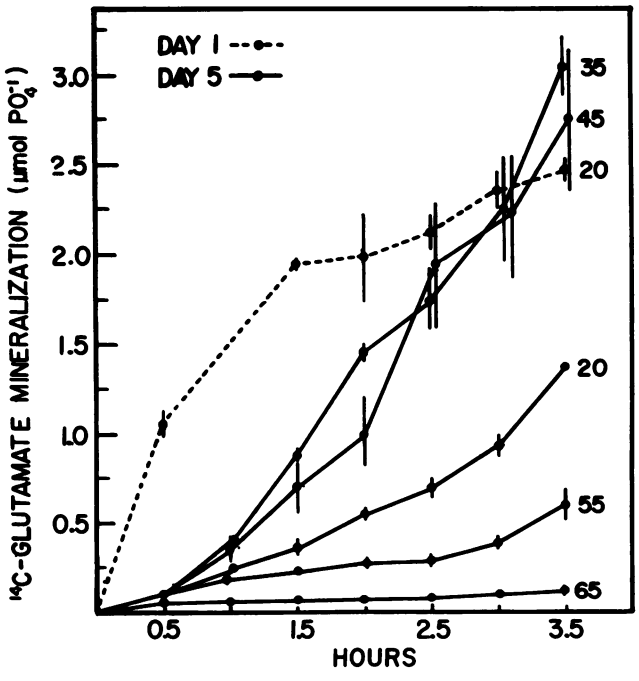

FIG. 1. Microbial activity, determined as the amount of $^{14}CO_2$ produced from [^{14}C]glutamate, in composting municipal sewage sludge 1 and 5 days after the beginning of run A. The sampling temperature (and the incubation temperature of the assay) is indicated to the right of each line. Values shown are means ± 1 standard deviation ($n = 3$).

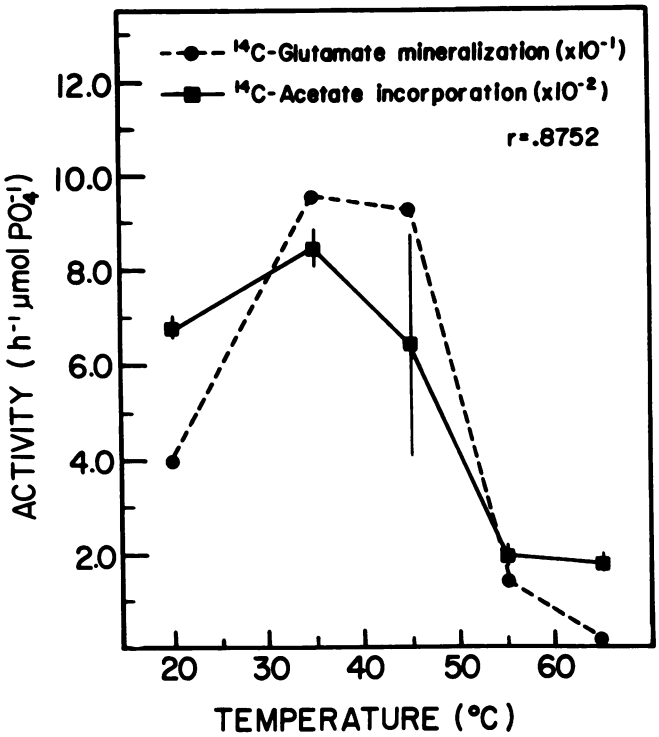

FIG. 2. Comparison of the rates of microbial mineralization of [^{14}C]glutamate (dashed line) and incorporation of [^{14}C]acetate (solid line) at various temperatures in composting municipal sewage sludge on day 5 of run A. Values shown for incorporation are means ± 1 standard deviation ($n = 3$).

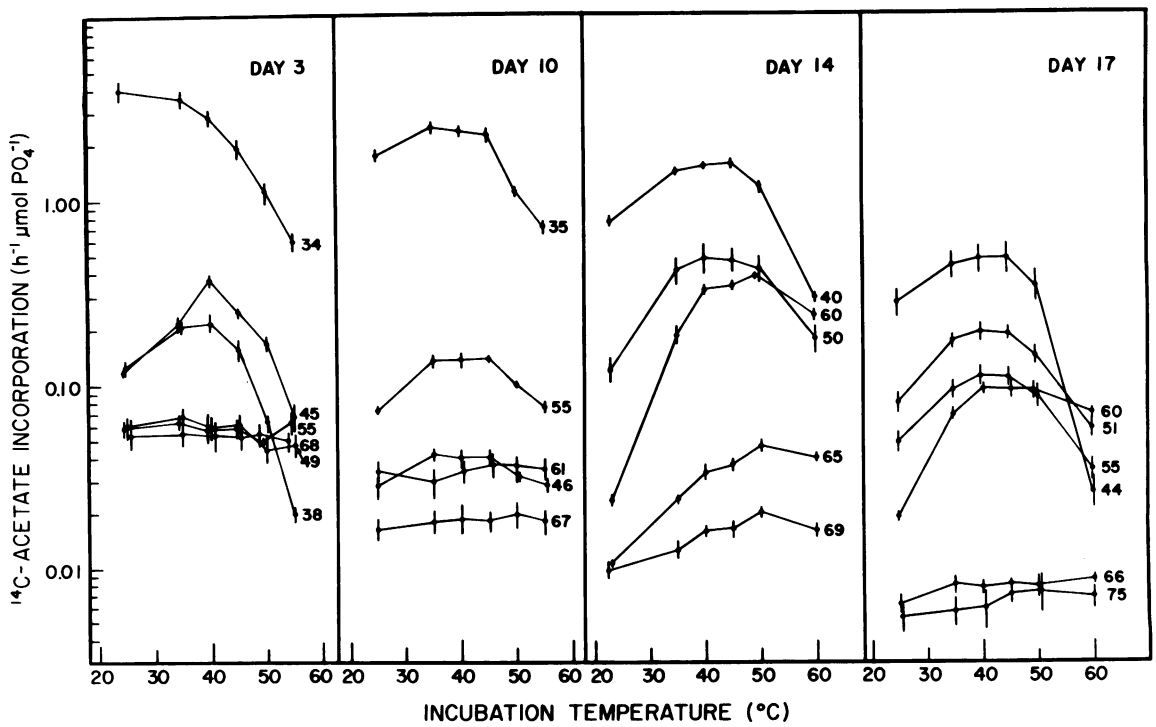

FIG. 3. Microbial activity, determined as the rate of incorporation of [^{14}C]acetate into microbial lipids, in composting municipal sewage sludge incubated at various temperatures (±1.0°C) after 3, 10, 14, and 17 days of composting during run B. Numbers to the right of each line indicate the sampling temperatures (±0.5°C). Values shown are means ± 1 standard deviation (n = 3).

continuously from day 17 until the end of the run to further dry the material. Microbial biomass levels were fairly constant during this run, particularly during the latter part, showing no general pattern with temperature or time. Biomass of individual samples ranged from 5.56 ± 0.14 to 0.54 ± 0.08 µmol of PO_4 g^{-1}. The mean biomass of the pooled samples was 2.03 ± 1.00 µmol of PO_4 g^{-1}. The means of the individual sample biomasses for particular sampling days correlated with the biomasses of the pooled samples for those days (r = 0.7383; $P < 0.05$). The moisture and organic contents of all the samples were also fairly uniform (46.1 ± 4.4% [dry weight] of total weight and 62.1 ± 1.4% [organic] of dry weight).

During run B each sample was assayed for microbial activity at several different temperatures, rather than just at its sampling temperature. Results from four of the sampling days are shown for individual samples in Fig. 3 and for the pooled samples in Fig. 4. Table 1 shows the sampling temperatures and mean compost pile temperatures for each sampling day during run B. Again it is apparent that samples taken from lower-temperature regions in the compost pile (34 to 45°C) generally had much greater microbial activity than higher-temperature samples (65 to 75°C), usually by as much as an order of magnitude, regardless of the temperature of incubation (Fig. 3).

In addition, the samples from high-temperature regions in the compost pile (60 to 75°C) had flatter temperature curves, showing little or no significant response to varied incubation temperature (with the exception of the 60°C sample from day 14). In contrast, the samples from lower-temperature regions of the pile and the pooled samples (Fig. 3 and 4, respectively) generally showed a curvilinear response with temperature, with an optimum temperature for activity within the 35 to 50°C range. The pooled samples from days 0 and 17 did not show significantly different responses to different incubation temperatures. The trends in microbial activity with time and temperature were very similar whether the results were expressed in terms of activity per unit of biomass or as activity per gram (dry weight) of compost for both runs A and B.

The optimal temperatures for [^{14}C]acetate incorporation correlated significantly (r = 0.7128, n = 26; $P < 0.01$) with the sampling temperatures in run B. The temperature optima for microbial activity appeared to shift slightly as the run progressed, with the early (days 3 to 10) optima being at somewhat lower temperatures than the optima later in the run (days 14 to 19). These trends may be more easily examined in terms of the apparent activation energies (or temperature coefficients) for the reaction (acetate incorporation), derived from the Arrhenius equation in the form of $v = Ae^{-E_a/RT}$ or $\ln v = -E_a/RT + \ln A$, where v = rate of acetate incorporation (per hour per micromole of PO_4), E_a = apparent activation energy (calories per mole), R = the gas constant (1.987 cal [ca. 8.314 J] mol^{-1} K^{-1}), T = temperature (degrees Kelvin), and A = an entropy constant.

The apparent activation energy (E_a) can thus be derived by plotting ln v against $1/T$, calculating the slope of the linear portion(s) of the curve and multiplying by the gas constant. Figure 5A shows apparent activation energies calculated from the pooled samples (from Fig. 4). Where possible (when linearity existed), activation energies were calculated from two different temperature ranges, 25, 30, and 35°C (solid line) and 30, 35, and 40°C (dotted line). Early in the run lower activation energies were obtained in the 25 to 35°C range, whereas later in the run these E_a values rose dramatically (Fig. 5A). The reverse was true for the E_a values calculated from the slightly higher 30 to 40°C temperature range. Similar results were obtained when the E_a values were calculated from the individual sample data (from Fig. 3). Figure 5B shows the ratios of the E_a values from the two

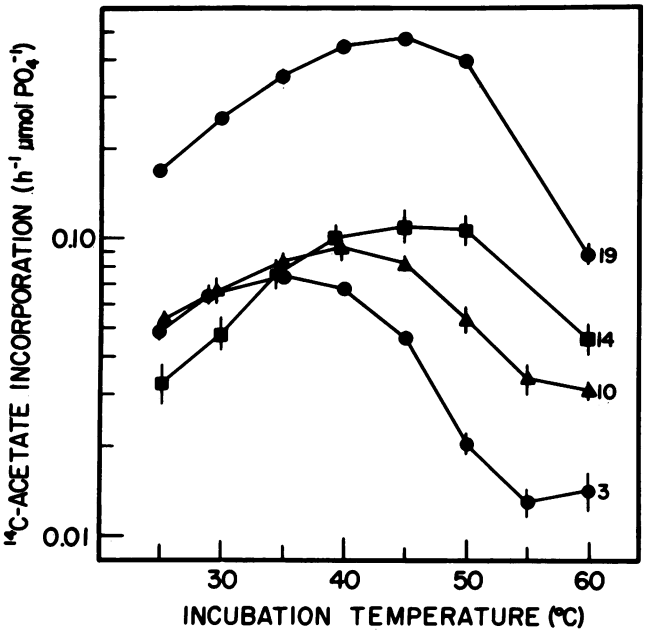

FIG. 4. Microbial activities, determined as the rate of incorporation of [^{14}C]acetate into microbial lipids, over a range of temperatures (±1.0°C) for pooled samples of composting municipal sewage sludge taken from a range of sampling temperatures (Table 1) on a particular day (indicated by the number to the right of each line) during run B. Values shown are means ± 1 standard deviation (n = 4).

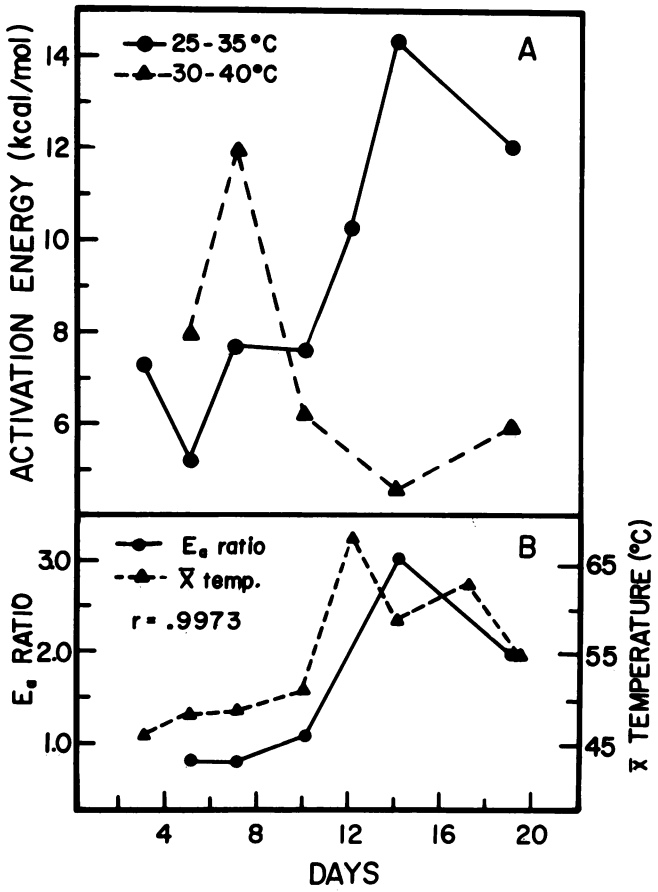

FIG. 5. Apparent energies of activation (E_a) (A) for [^{14}C]acetate incorporation by pooled microbial communities in composting municipal sewage sludge (see Fig. 4) over a low-temperature range (25 to 35°C; solid line) and a higher temperature range (30 to 40°C; dotted line) at various times during the composting process, and the ratio (B) of these two activation energies (E_a at 25 to 35°C/E_a at 30 to 40°C; solid line) compared with the mean temperature of the compost pile (dotted line) over time (for correlation, n = 5).

different temperature ranges shown in Fig. 5A (25 to 30°C/30 to 40°C, solid line) and the mean temperature of the compost pile (dotted line) over time. These two parameters were found to correlate highly significantly (r = 0.9973; $P < 0.01$; n = 5).

Further examination of Fig. 3 indicates that a comparison of the slopes on either side of each temperature optimum might be of interest. The apparent energies of activation, E_a (on the low-temperature side of the curve), and the apparent heats of reversible denaturation, ΔH_i (or inactivation, on the high-temperature side), may be calculated by multiplying each of these slopes by the gas constant (24). At these temperatures the sum of E_a and the apparent ΔH_i is approximately equal to (within the limits of experimental error) the heat of denaturation, E_i (24), which will be referred to as the energy of inactivation.

TABLE 1. Temperatures from which compost samples were taken on various days after the beginning of composting run B and the mean compost pile temperature at the time of sampling

Sampling day	Sample temp (°C)	Mean sample temp (°C)	Mean compost pile temp (°C)
0	33, 34, 29, 35	33	33
3	34, 38, 45, 49, 55, 68	48	46
7	40, 45, 50, 54.5, 60, 68	53	49
10	35, 46, 55, 61, 67	53	51
12	48, 55, 66, 72	60	68
14	40, 50, 60, 65, 69	57	59
17	44, 51, 55, 60, 66, 75	58.5	63
19	40, 45, 50, 55	48	55

Table 2 shows the apparent energies of activation, the apparent heats of inactivation, and the energies of inactivation for the individual samples from run B, along with the temperature ranges from which each slope was calculated. To eliminate inconsistencies due to the leveling off of some of the curves at either temperature extreme and near the optimum, the values were calculated from the steepest parts of the slopes. In addition, Johnson et al. (24) recommend calculating the apparent ΔH_i from the slope in the temperature range giving 20 to 40% of the maximal activity, to avoid the influence of large E_a values near the temperature optimum and possible contributions of irreversible denaturation or inactivation at very high temperatures. Most of the calculations presented here for the ΔH_i were made within this range.

Figure 6 shows the relationship between the ratios of apparent $\Delta H_i/E_a$ and the sampling temperatures for the samples in Table 2. The log transformation of the ordinate allowed for a correlation (r = −0.8727; $P < 0.01$; n = 21; slope = −0.1006; y-intercept = 5.41; standard error of the estimate = 0.4570) which was somewhat better than that obtained with the linear, square root, arcsine, or double-reciprocal transforms. The log of the apparent energies of

TABLE 2. Apparent energies of activation (E_a), apparent heats of inactivation (ΔH_i), and the corresponding energies of inactivation (E_i), along with the incubation temperatures used to calculate these values, for individual compost samples taken on various days after the beginning of composting run B

Sampling day	Sampling temp (°C, ±1°C)	Cal mol^{-1}[a]			E_a temp (°C, ±1°C)[b]	ΔH_i temp (°C, ±1°C)[c]
		Apparent E_a	Apparent H_i	Apparent E_i		
3	38	8,152	26,368	34,520	24, 35	50, 55
	45	12,545	38,742	51,287	24, 35, 40	50, 55
7	45	13,723	40,464	54,187	25, 35, 40	50, 55
	50	12,921	29,528	42,449	35, 40	50, 55
	54.5	8,625	13,156	21,781	25, 35, 40	50, 55
10	35	6,176	18,836	25,021	25, 35	50, 55
	46	7,115	10,725	17,840	25, 35	45, 50
	55	11,374	12,201	23,575	25, 35	50, 55
12	55	23,129	18,393	41,522	24, 35	50, 60
14	40	9,494	30,627	40,121	23, 35	50, 60
	50	18,435	18,677	37,112	23, 35	50, 60
	60	30,983	10,833	41,816	23, 35	50, 60
	65	12,252	3,050	15,302	23, 35	50, 60
17	44	8,279	55,565	63,844	25, 35	50, 60
	51	13,884	20,100	33,984	25, 35	50, 60
	55	12,191	20,160	32,351	25, 35	50, 60
	60	23,439	5,779	29,218	25, 35	50, 60
19	40	12,436	55,246	67,682	25, 35	50, 60
	45	12,678	37,186	49,864	25, 35	50, 60
	50	15,723	30,603	46,326	25, 35	50, 60
	55	20,719	16,915	37,634	25, 35	50, 60

[a] See text for methods of calculation (1 cal = 4.184 J).
[b] The incubation temperatures of the activity assay used to calculate the apparent E_a.
[c] The incubation temperatures of the activity assay used to calculate the apparent ΔH_i.

activation (E_a) and the apparent heats of inactivation (apparent ΔH_i) individually correlated ($P < 0.01$) with the sampling temperatures, with $r = 0.6360$ and -0.7340, respectively ($n = 21$). The apparent $\Delta H_i/E_a$ ratio also correlated with the optimum temperature for acetate incorporation for these samples ($r = -0.4975$, $n = 21$; $P < 0.05$).

DISCUSSION

Temperature has been shown to affect rates of microbial decomposition in many systems (16, 36). Most of the detailed work on this environmental parameter has been done over comparatively narrow temperature ranges and usually not under conditions of temperature-driven succession. In the rapid decomposition of organic matter typical of composting, responses of initially diverse (21) microbial communities to rapid temperature increases induced by their own metabolic heat production can be examined. Temperature variations, as well as variations in pH and moisture (4), usually exist within a composting pile. During these experiments, communities were sampled from a wide temperature gradient on each sampling day.

Results of previous studies on the relationship between temperature and microbial activity in composting systems were not in agreement, nor were they comprehensive. Schulze (33) reported that oxygen uptake was directly related to temperature up to 70°C, and several composting systems are apparently operated on this basis (14, 21, 28–30). However, Waksman et al. (40), Wiley (43), Jeris and Regan (23), and Finstein's group (20, 25, 35) report temperature optima for activity in the 55 to 60°C range. Much of these data are, however, difficult to interpret due to variations in composting materials and practices, as well as differences or inadequacies in methods of quantifying microbial activity and biomass. During the present study microbial activities were primarily determined by measuring the rate of incorporation of [^{14}C]acetate into microbial lipids. This method has previously been shown to correlate well with weight loss (18, 27), respiration (41), α-D-mannosidase activity (41), and heterotrophic CO_2 fixation (18) in decomposing plant litter and was shown during these studies to correlate with the rates of incorporation of [^{14}C]glucose or [^{14}C]glutamate into lipids and with the mineralization rates of [^{14}C]glutamate in composting sewage sludge. Due to the nature of the assay, the incorporation rates measured may reflect primarily bacterial rather than fungal activity. The role of thermophilic or thermotolerant fungi in the composting process has not yet been established (21). However, the upper temperature limit for fungi is 55 to 60°C (8, 10), and it has been found that, in composting systems undergoing temperature increases, the numbers of viable fungi rapidly decline as the temperatures approach 60°C and disappear completely at 65°C or less (21, 40). Bacteria tend to predominate over fungi throughout the temperature ascent when adequate moisture is present (21). Thus, it is unlikely that thermophilic fungi would contribute significantly to the overall microbial activity, particularly at temperatures greater than 55°C.

The shapes of many of the temperature response curves for the microbial communities found in composting sludge (Fig. 3 and 4) are typical of those for purified enzyme

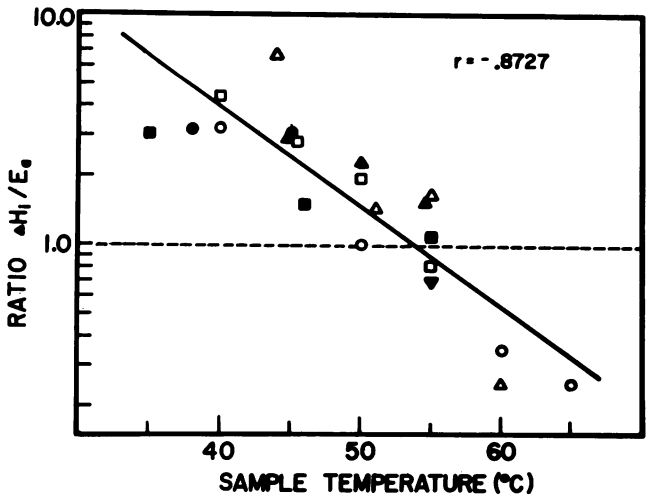

FIG. 6. Ratio of the apparent heat of inactivation (ΔH_i) to the apparent energy of activation (E_a; log scale) for the rate of [^{14}C]acetate incorporation into microbial lipids by microbial communities in composting municipal sewage sludge (see Fig. 3) with respect to sampling temperatures. Samples were taken after 3 (●), 7 (▲), 10 (■), 12 (▼), 14 (○), 17 (△), and 19 (□) days of composting during run B. The solid line was determined by computer to be the best linear fit for the 21 points. The dotted line indicates a $\Delta H_i/E_a$ ratio of 1.0, which intersects the calculated line at a point corresponding to 54°C.

systems and pure cultures of bacteria (22, 24, 37). Jeris and Regan (23) report similar curves for their composting system, finding that the effect of temperature on oxygen consumption in all of the different materials which they composted could be expressed in the form of $y = ax^2 + bx + c$, where y = the rate of composting, x = temperature (degrees Celsius), and a, b, and c are constants. The data presented here fit this form best when a log transformation was used, with the exception of those curves which were flat.

The presence of some flat curves (Fig. 3), showing little or no significant response to various incubation temperatures, may indicate that the organisms in these samples were so stressed or inactivated that they could not respond. These samples typically had very low levels of activity compared with other samples taken at the same time. This level of inactivation most often occurred in samples taken from very high-temperature (65 to 75°C) areas in the compost pile, although a few such samples came from lower temperatures (49 and 55°C on day 3; 46°C on day 10). The inactivity of these samples could be due to lack of sufficient oxygen, particularly since they occurred early in the run when the aeration levels were low.

Most of the other temperature response curves look very similar to the type I curves described by Han (22), which are typically associated with the heat inactivation of enzymes. This is the most common type of nonlinear Arrhenius plot and is seen when the reaction rate is modified by a single-equilibrium process (22). The placement of the optimum and the slopes on either side may be influenced by many different chemical properties of the environment (24). However, the shape of the curve is primarily dependent on the relative rates of two processes having different temperature efficiencies: (i) the rate of the limiting enzyme reaction, and (ii) the equilibrium rate between the active and the inactive forms of the enzyme (22, 24).

Activation energies may be calculated for the reaction on either side of the temperature optimum. On the low-temperature side of the optimum, the rate of the reaction increases with temperature at a particular rate, whereas the rate of inactivation or denaturation is relatively low. At the optimum temperature these two rates balance each other out, and as the temperature is increased further the rapidly increasing rate of inactivation overshadows the still increasing rate of the reaction, such that a net decline in the apparent reaction rate is observed. When the slope on the low-temperature side of the optimum (or the energy of activation, E_a) is much less than the slope on the high-temperature side (or the apparent heat of inactivation, ΔH_i), high rates of activity are possible over a wider range of the lower temperatures, whereas relatively rapid inactivation occurs at temperatures just above the optimum. This type of curve was reported (37) for the growth of *Aerobacter aerogenes* in continuous culture with a temperature optimum of 35°C and having an E_a of 14 kcal (ca. 58 kJ) mol^{-1} and a ΔH_i of 33 kcal (ca. 138 kJ) mol^{-1}. However, when the E_a is much larger than the apparent ΔH_i, the opposite is true; inactivation rates on the low side of the optimum are greater than those on the high side. It might, therefore, be expected that a ratio of $\Delta H_i/E_a$ (Fig. 6) would offer some information concerning the energetic adaptations of the communities to different temperatures. Results from this study indicated that the $\Delta H_i/E_a$ ratio decreased with increasing sampling temperature as a log function (Fig. 6). The point at which the ratio of $\Delta H_i/E_a$ equaled 1.0 on the fitted line corresponded to a temperature of about 54°C. This temperature is just above the temperature range (44 to 52°C) reported for the transition from mesophilism to thermophilism by facultative thermophiles (6).

One would expect that organisms having lower activation energies at a particular temperature would be better adapted to that temperature than organisms having higher activation energies. If this is the case, the ratio of the E_a for a low-temperature range (25 to 35°C) to the E_a for a slightly higher temperature range (30 to 45°C; Fig. 5A) also seems to indicate that a succession with respect to thermal efficiency occurred as the mean temperature of the system increased over time (Fig. 5B). However, overall adaptations of the communities to extremely high temperatures were not indicated. From the data presented here, it is not apparent whether this functional succession is primarily the result of changes in the species composition of the community or within-species enzymatic adaptation or a combination of both. Similar results could be obtained by calculating changes in Q_{10} values, although these have no real thermodynamic basis (24). It has been suggested that log-log plots of temperature-dependent growth rates of organisms in soils are preferable to the standard Arrhenius plots in that better linearity is achieved (34). The slopes of such curves for the pooled samples were found to correlate with the mean pile temperature ($r = 0.8610$; $P < 0.01$), as did the changes in activation energy ratios ($r = 0.9973$; $P < 0.01$; Fig. 5B).

The temperature optima of many of the temperature response curves (Fig. 3 and 4) tended to shift toward higher temperatures as the composting run progressed, but they never exceeded about 50°C. This appears to also indicate that, although extreme thermophiles with temperature optima of >55°C may have been present, they did not exhibit enough activity (with regard to this assay) at these temperatures to show it. The temperature optima early in the run ranged from 23°C (ambient) to about 40°C. This seems reasonable, since it has been shown that the intestinal microbial communities of mammals are optimally adapted to the body temperature of the host (2).

Allen and Brock (1) found that heterotrophic microcosms seeded with a variety of inocula, including raw sewage, sewage sludge, and hot soils, could be readily optimally adapted to temperatures from 2 to 55°C within a period of 2 weeks. However, growth from 60 to 75°C was not established until hotsprings bacteria were added, and even with this treatment no growth was seen at 80 to 85°C. It has also been found that, even when organisms are optimally adapted to a very high temperature, often their efficiency of metabolism and growth are less than those for organisms adapted to lower temperatures (1, 8, 9, 11). Hence, it seems unlikely that rapid rates of growth or decomposition should be expected in composts exceeding 55°C, and even seeding with extremely thermophilic microorganisms would probably not increase the overall efficiency significantly.

It should be noted that, for the samples taken from different temperature areas within the compost pile, changes in the shapes of their temperature response curves, their temperature optima, and their degree of activity could also be influenced in part to some indirect consequences of the changes in temperature, such as variations in the partial pressure of oxygen, the pH, or some other chemical or physical property of the microenvironment. The moisture and volatile solids contents of these samples were fairly uniform, however, as was the biomass. Also, it should be cautioned that the estimates of microbial activities made during this study should be viewed as potential activities, rather than actual activities, since the homogenization of the samples during processing could disrupt the natural associations between various members of the microbial community, as well as change the immediate chemical environment of the cells. It has been shown that disturbance of estuarine sediments may cause an increase in the rate of acetate incorporation into microbial lipids (R. H. Findlay and D. C. White, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I171, p. 168). The rates of [^{14}C]acetate incorporation into the lipids of plant litter decomposers from an arctic lake were found to be about 0.015 ± 0.005 h^{-1} μmol of PO_4^{-1} (at 15°C; estimated by using literature values [42] to convert the ATP biomass of the litter to micromoles of PO_4). This value lies at the lower end of the range of activities found for compost during this study (Fig. 3).

The major findings of this study include the following. Both respiration and lipid synthesis from exogenous glutamate and acetate, respectively, were similarly affected by temperature during composting (Fig. 2). Temperatures exceeding 55 to 60°C severely inhibited microbial activity. Incubation of samples at different temperatures revealed that (i) samples from high temperatures (65 to 75°C) usually had very low microbial activity, even at the temperatures from which they were sampled; (ii) the optimum temperatures for activity shifted to somewhat higher temperatures as the composting run progressed, but at no time exceeded about 55°C; (iii) thermal succession was further indicated by changes in the activation energies of samples from various temperatures over time; and (iv) comparisons of activation energies and apparent heats of inactivation seemed to indicate that the microbial communities in samples taken from higher temperatures were better adapted to high temperatures than the communities from lower temperatures. These findings suggest that mesophiles and facultative thermophiles play important roles in the composting process. Evidence that obligate thermophiles become important in the latter part of the process, when samples from 60°C exhibit sharp declines in activity below about 40°C, was also found. No evidence that extreme thermophiles, having temperature optima of >55 to 60°C, become important in the decomposition process could be found, however. It can be concluded that temperature control during the composting process is necessary to obtain maximal rates of decomposition and that temperatures should not be allowed to exceed approximately 55 to 60°C. In addition, the calculation of activation energies for rate processes may provide a useful means of following functional changes in populations and successions of communities with respect to temperature and perhaps other environmental factors as well.

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