A Critical Evaluation of Inoculums in Composting

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The practice of producing organic fertilizer through the biological decomposition of organic wastes has been carried on for centuries as an art generally known as composting. The widest application of the practice has occurred in the Orient and in Europe where dense populations have for generations placed a great burden on soil fertility. Until recently, interest in composting in the United States has been limited to the amateur gardener and the organic farming enthusiast. At present, two important factors are contributing to a broadening interest in composting in this country. One is the possibility of composting as an answer to the increasingly complex and serious problem of municipal refuse disposal. The other is the growing concern over the depletion of our agricultural soils by our present intensive farming practices which fail to return organic humus to the earth. The especial importance of both of these factors to the state of California prompted the Sanitary Engineering Research Laboratory of the University of California to begin a study of the application of composting to the reclamation of municipal refuse. It soon became apparent that in spite of the antiquity of composting there existed very little scientific knowledge of the process.

Because there is a widespread conviction that an "inoculum" of some sort is needed for successful and rapid composting, one of the problems studied in the investigation was the effect of such an additive on the process. Since composting is a biological process, its course should be determined by the magnitude and nature of the microbial population present. It is easy, therefore, to assume that by artificially increasing the numbers of organisms, the process should be accelerated.

Refuse is a mixture of relatively undecomposed heterogenous material. During the composting of such material there appears a succession of environments, especially with regard to temperature and substrate, with a corresponding series of microbial populations. Since the environmental conditions at any one time are suitable to a limited variety of bacteria, it would seem doubtful that organisms added as an inoculum could be any more effective than similar organisms indigenous to mixed refuse. To determine the validity of this reasoning a series of experiments were conducted concurrently with a broader investigation of composting to learn what benefit, if any, would accrue to the process from the use of an inoculum. Materials which have often been considered as inoculums essential to the composting process include: animal manures, garden soil, decomposing material, and a variety of proprietary bacterial cultures. In the experiments, inoculums consisting of soil, of horse manure, and of partially composted material, as well as a commercial preparation of bacterial cultures were tested.

MATERIALS AND METHODS

Inasmuch as the experiments with inoculums paralleled other studies on composting, they varied in size from laboratory to field scale. Laboratory studies were conducted in a special room in which temperatures were maintained at 50 C ± 5 C. Material was composted both in 19 L glass jars holding approximately 3 kg, and in 208 L steel drums having a capacity of approximately 70 kg. Pilot scale studies were made in bins approximately 1 m square and 1.5 m high holding approximately 450 kg of material. These bins were constructed of 1-inch lumber upon the wooden floor of an unheated sheet metal warehouse. The bins were provided with removable front boards to facilitate handling the compost. Material composted in the field was stacked in open piles approximately 1.5 m high and containing about 2700 kg of material.

Three different mixtures of material were used in the studies. Laboratory investigations were made on a mixture of vegetable trimmings (non-marketable green vegetables and partially spoiled fruits from grocery stores) and chopped straw. Experiments having shown that vegetable trimmings would not compost readily unless the moisture content was reduced and the original porosity of the mass maintained (University of California, Technical Bulletin 9, 1953), the straw was added both to control moisture and to maintain structure of the material. To approximate municipal refuse more closely, paper was substituted for straw in the pilot studies, although it did little to reduce the moisture content and added nothing to the structure of the mass. Field studies were made on mixed municipal refuse from which noncompostable material had been removed.

Raw material consisting of vegetable trimmings, straw and paper was used in all of the studies, and was shredded to a size of approximately 2.5 cm prior to composting.

After shredding, the material to be composted was

placed in jars, drums, bins, or in open stacks, depending upon the size of the experiment. Aerobic conditions were maintained by periodically turning the composting material. Strictly speaking, "partially aerobic" is a more accurate description of the conditions because only a limited amount of atmospheric oxygen is supplied by turning. This is sufficient, however, to prevent the onset of anaerobic putrefaction which is characterized by objectionable odors and a slow rate of decomposition. Such an aerobic method makes possible the accumulation of sufficient heat from bacterial metabolism to raise the temperature of the mass to the thermophilic range. Waksman (1931) in his studies on decomposition has shown that in this range the biological breakdown of organic matter is most rapid. In turning the composting material, care was taken to loosen the mass and to turn the outer layers into the center. This was done with jar, drum, and bin samples by removing the material and shaking it back into the container. Open piles were reconstructed in a new location by forking. During the turning process water was added by spraying when necessary to maintain an optimum moisture content.

A rather complete analysis was made of all material from the raw material to the finished product. Determinations included moisture content, pH, carbon, nitrogen, phosphorus, and potassium. Carbon was determined as CO_2 by means of a standard C and H microcombustion apparatus using about 25 mg of sample. It would seem next to impossible to obtain a representative sample in so small a portion. Nevertheless, replication of results sufficiently consistent for the purpose of the investigation was obtained by the following procedure: Grab samples were taken from several different parts of the pile of material and thoroughly mixed. Three 100 g portions were removed for carbon and nitrogen analyses and three for ash determinations. Those to be used in carbon and nitrogen analyses were slurried in a Waring Blendor with three to five times their weight of distilled water. A portion of the slurry was then dried to constant weight, ground through a screen, and the proper amount weighed out for the carbon analysis. A nitrogen determination was made from each of the three slurries by the Kjeldahl method (Official Methods, A.O.A.C., 1950) using a 5 g portion. Percentage moisture was determined on the 100 g samples to be used in ash determinations by observing the weight loss during a 24-hour period of drying at 105 C. The samples were then ignited in a muffle furnace and percentage ash calculated. Phosphorus and potassium were determined by a subsequent analysis of the ash. The Truog-Mayer modification of the Deniges method was used for phosphorus (Official Methods A.O.A.C., 1950) and a standard colorimetric test for potassium (Snell and Snell, 1949).

Measure of rate and extent of decomposition. In his studies on the decomposition of stable manure, straw, alfalfa, and similar plant residues, Waksman measured both rate and extent of decomposition by change in per cent total nitrogen, water soluble organic matter, hemicelluloses, cellulose, lignin, protein, and ash (Waksman *et al.*, 1939a, 1939b, 1939c). Although such tests give an excellent indication of rate and extent of decomposition, the difficulty of making the necessary chemical analyses of heterogeneous material such as municipal refuse makes simpler criteria desirable in a practical composting operation. It was necessary, therefore, to establish criteria for judging the rate and extent of decomposition, and to devise some standard by which the material could be judged adequately stabilized.

It was found that the course of temperature in a compost pile was indicative of the progress of the process from its beginning to its completion. Normally, the temperature inside a composting mass begins to rise immediately after grinding. If the mass is large enough to be self-insulating, the temperature increases rapidly to 45–50 C, remains at this level for 24–48 hours, and then continues to rise to a maximum of 70–75 C, which persists until active decomposition is over. Thereafter the temperature slowly decreases. The material may be considered sufficiently stabilized when the declining temperature reaches 45–50 C. Although temperature is affected by the carbon-nitrogen ratio, by moisture content, and by the insulating qualities of the material, it proved to be a useful criterion in the studies on inoculation. Other useful indices of rate and extent of decomposition included increase in per cent ash and decrease in per cent carbon. Since the original ash content of a material remains unchanged even though mass is lost through oxidation of some organic constituents of the material, increase in percentage of ash is proportional to extent of decomposition. Loss in carbon, likewise, represents decomposition of organic matter because in the process the carbon of organic compounds is oxidized to CO_2. When no loss of nitrogen occurs the change in C/N ratio is also indicative of degree of decomposition. Even though nitrogen is lost, the C/N ratio is valuable in estimating the effect of a compost on soil nitrogen.

Experiments

Horse manure as an inoculum. Horse manure was selected as the first additive to be studied, because many advocates of composting consider it or other animal manure essential as a device for introducing a large microbial population. Waksman has shown that the high bacterial count in horse manure includes a large actinomycete population approximating 15×10^7 per g (Waksman, 1950). Actinomycetes are especially active in breaking down cellulosic material.

In this experiment a series of four 19 L jars and four 208 L drums were filled with a mixture of shredded vegetable trimmings and straw. The jars were divided into two groups. One group was left uninoculated and the other group was inoculated with 2 per cent by weight of horse manure. To counteract any possible inhibition of bacterial activity resulting from increase in hydrogen ion concentration, two jars received a dosage of 8 per cent by weight of industrial $CaCO_3$. Similarly, the drums were divided into two pairs, inoculated and uninoculated. One of each pair received a dosage of 4 per cent of $CaCO_3$. All units were incubated in the high temperature laboratory and turned daily. The experiment continued for 9 days and all samples produced a satisfactory compost.

No significant difference in temperature was noted between the various samples. The temperature in the jar samples followed a somewhat erratic course because the poor insulating qualities of small masses of material made them responsive to fluctuations in the ambient temperature. The upper layers also showed a tendency towards excessive drying because of the high ambient temperatures. The behavior of compost in the drums was less erratic.

Temperature readings and pH values of the drum samples during the 9 days of the experiment are shown in figure 1. A rapid rise in temperature of all samples is notable during the first 24 hours of incubation. This rise was accompanied by a significant decline in pH which could have occurred only as a result of bacterial activity. Such activity is further evidenced by the fact that temperature curves remained appreciably above ambient temperature during a 3-day period. An acid condition occurred in all samples, and disappeared on the fourth day. Thereafter a rapid rise in temperature was paralleled by a corresponding increase in pH, until finally a normal decline in temperature marked the completion of the process. The jar samples showed the same general trend observed in the less sensitive drum samples.

The results of the analyses made during the course of the experiment are listed in table 1. Some discrepancies appear in the data as a result of some variability in small masses of composting material and difficulties in obtaining representative samples. Nevertheless, the data demonstrate clearly that rate and extent of decomposition, as indicated by increase in per cent ash and decrease in per cent carbon, was approximately the same for all samples whether inoculated or uninoculated, buffered or unbuffered.

There appeared to be a typical succession of microorganisms, each of which played an important role.

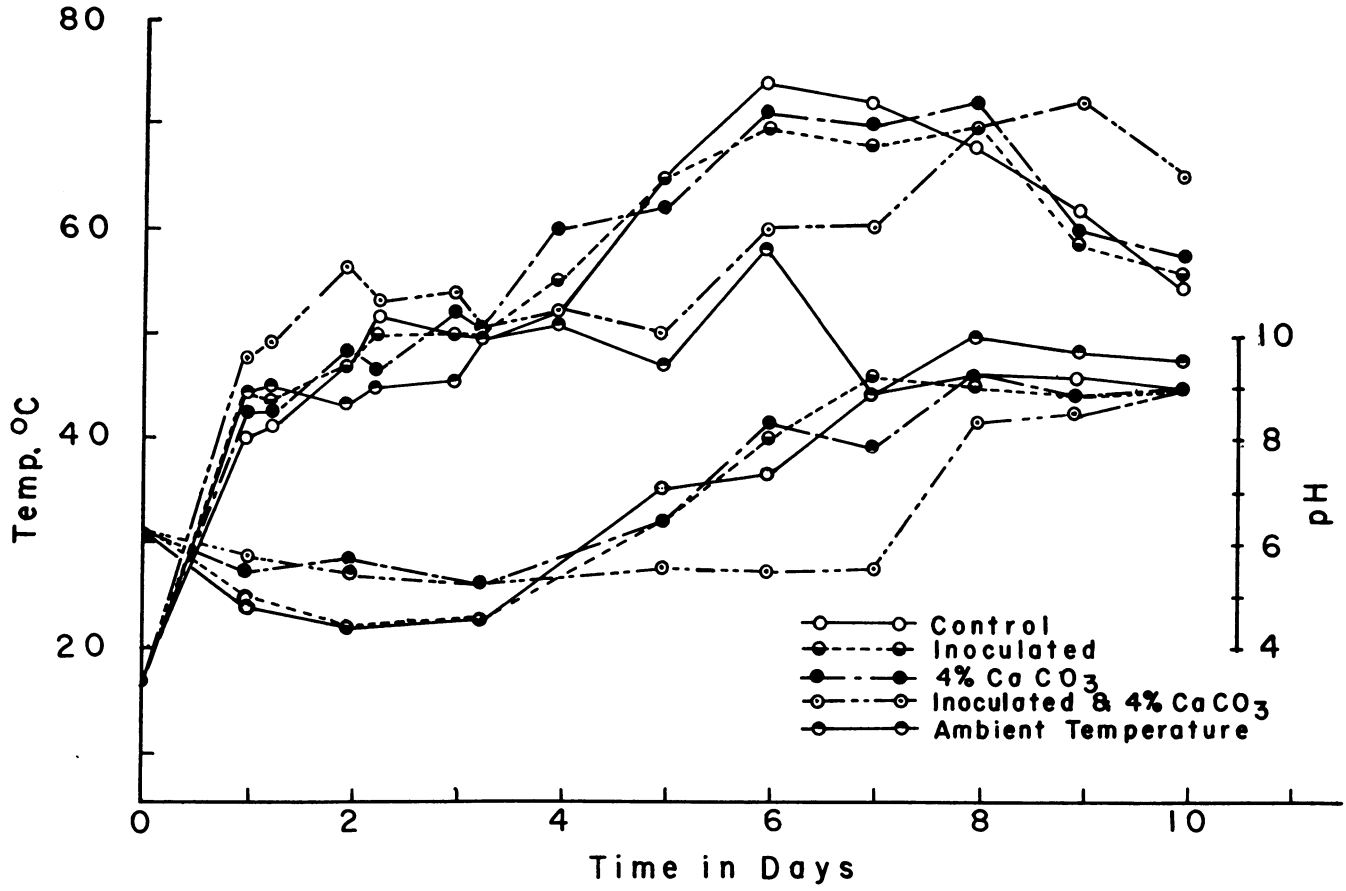

FIG. 1. The effect on the temperature of a composting mass resulting from the addition of 2 per cent horse manure to a mixture of vegetable trimmings and straw.

Acid-producing bacteria were the first to appear, as was evidenced by a strong acetic acid odor and less pronounced odors of lactic and butyric acids. Thereafter, thermophilic bacteria together with the fungi, more or less sparse in number, made their appearance. The thermophilic fungi were limited to three species: *Thermomyces sp.*, *Penicillium dupontii*, and *Aspergillus fumigatus*. In the final stages, when the temperature began to decline, members of the Actinomycetaceae became the dominant group. Species in the genera *Streptomyces* and *Micromonospora* were observed, with those of *Micromonospora* being the more numerous in all samples. The sequence of organisms and size of populations seemed to be independent of inoculation.

The temperature curves for "seeded" and "non-seeded" materials (fig. 1) are so nearly identical as to support the conclusion that inoculating with horse manure had no effect on either temperature or pH. Increase in per cent ash and decrease in per cent carbon demonstrated that the degree of decomposition was not affected by the addition of horse manure.

Inoculating with composting material. The effect of seeding fresh material with actively decomposing material was studied on a pilot scale experiment involving vegetable trimmings and paper. Prior to the start of the experiment a 208 L drum was filled with approximately 54 kg of shredded vegetable trimmings and paper in the proportion of 9 to 1, and placed in the high temperature laboratory. Decomposition progressed with great rapidity and within 4 days reached the stage

TABLE 1. *Chemical analyses of composts produced from vegetable trimmings and straw.*

SAMPLE	DAY	ASH*	MOISTURE†	CARBON*	NITROGEN*	PHOSPHORUS*	POTASSIUM*	C/N	pH
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Jar sample, not inoculated	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	11.5	78.8	38.4	2.3	0.25	0.77	17	4.5
	6	13.0	74.1	38.8	2.0	—	—	18	5.6
	9	13.4	63.1	40.3	2.2	0.33	1.38	18	7.7
Jar sample, inoculated‡	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	9.3	78.8	38.5	1.8	0.17	1.22	18	4.7
	6	16.5	73.1	35.2	1.7	—	—	20	8.5
	9	—	68.3	37.4	2.0	0.43	1.89	16	8.8
Jar sample, not inoculated, buffered with 8% $CaCO_3$§	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	19.3	71.9	26.0	1.6	0.25	0.47	13	7.0
	6	36.9	63.7	17.0	1.8	—	—	9	8.7
	9	37.6	45.0	14.3	1.1	0.25	0.67	13	9.2
Jar sample, inoculated,‡ buffered with 8% $CaCO_3$§	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	31.2	64.2	19.8	1.7	0.27	0.84	12	8.4
	6	34.7	41.5	18.3	1.5	—	—	12	8.7
	9	36.7	63.4	10.9	1.4	0.25	0.83	8	8.9
Drum sample, not inoculated	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	14.1	79.8	21.8	1.9	0.17	1.18	11	5.2
	6	11.4	76.8	36.0	—	—	—	—	8.9
	9	17.8	76.7	35.5	2.35	0.32	2.41	11	9.1
Drum sample, inoculated‡	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	12.9	79.3	38.4	1.8	0.17	1.22	21	4.6
	6	11.9	75.7	35.2	1.7	—	—	21	9.2
	9	20.1	68.5	37.4	2.0	0.47	1.89	18	8.8
Drum sample, not inoculated buffered with 4% $CaCO_3$§	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	28.1	76.8	27.2	1.7	0.19	0.73	17	5.2
	6	31.9	71.1	28.2	1.7	—	—	16	7.9
	9	36.6	72.6	25.8	1.4	0.29	1.60	17	9.0
Drum sample, inoculated,‡ buffered with 4% $CaCO_3$§	1	10.9	81.1	42.0	2.1	0.16	1.38	20	6.2
	3	30.3	75.0	27.8	1.4	0.19	1.04	20	5.4
	6	—	72.4	23.4	1.4	—	—	16	5.5
	9	30.2	68.5	26.1	1.4	0.38	1.34	19	8.5

* Values expressed as per cent dry weight.
† Values expressed as per cent wet weight.
‡ "Seeded" with 2 per cent horse manure.
§ Includes corrections made for $CaCO_3$.

where actinomycetes became the predominant group. These organisms were so abundant that the whole mass appeared gray in color. At that time the experiment was begun by thoroughly mixing and placing in a bin the partially composted contents of the drum and 525 kg of freshly shredded material consisting of roughly 90 per cent vegetable trimmings and 10 per cent paper. For comparison, a second bin was filled with 372 kg of the shredded mixture. The material in both bins was turned once each 24 hours. By the end of the tenth day, the material in the second bin had shrunk from its original depth of 142 cm to a depth of 36 cm, thus exposing so great a surface area in proportion to volume that heat was lost at an excessive rate. To remedy this, the front boards of the bin were moved inward so as to reduce the area of the surface to half and thus double the depth of the pile.

Temperature observations of the piles were made daily with a mercury thermometer at a depth of 5 cm below the surface and at mid-depth. At each depth nine readings were taken. Temperature readings observed in the center of the pile, both near the surface and at mid-depth are plotted in figure 2. The rise in temperature at all points in the pile during the first 24 hours was similar in both piles. The general rate of increase in both was slower than previously observed with vegetable trimmings and straw, probably because less adequate aeration resulted from the greater compaction characteristic of a mixture containing paper.

The extensive development of actinomycetes in the seeding material at the time the experiment was begun furnished an opportunity to observe the reactions of a group of organisms introduced into a mixture of refuse not yet decomposed to a suitable degree. All visible microscopic evidence of the original population of actinomycetes disappeared within the first 24–36 hours. Actinomycetes appeared again, however, as usual near the end of the experiment. The likelihood that other organisms transplanted from the drum contributed anything to speed up the process is precluded by the absence of any unusual rate of initial temperature rise (fig. 2) or any significant difference in initial and final percentages of ash and carbon as is shown in table 2.

Bacterial cultures as an inoculum. A typical commercial preparation reported to contain mixed cultures of organisms effective in accelerating the composting process was tested in a pilot scale experiment. Inasmuch

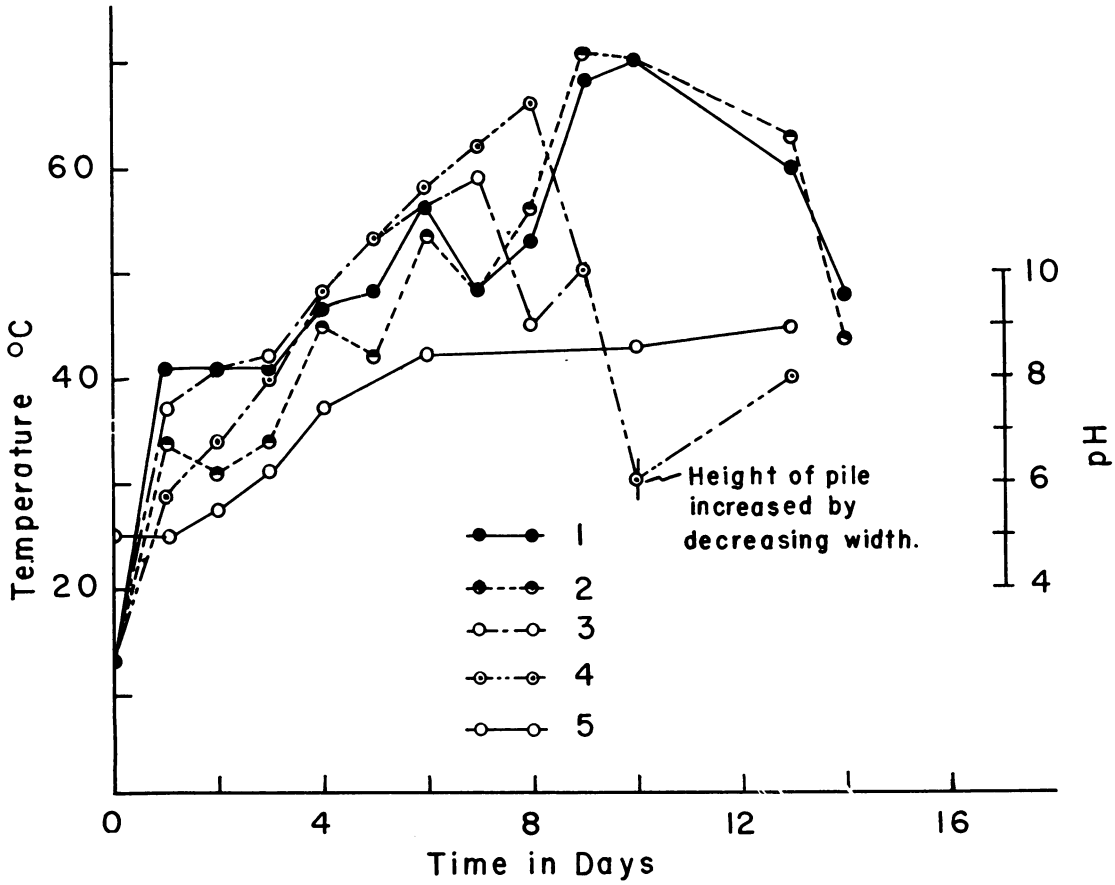

FIG. 2. A comparison of temperature curves of two composts, one of which was recirculated with partially decomposed material. Curve 1: Recirculated material, temperature at mid-depth, center of pile. Curve 2: Recirculated material, temperature at 2″ below surface, center of pile. Curve 3: Control, temperature at mid-depth, center of pile. Curve 4: Control, temperature at 2″ below surface, center of pile. Curve 5: pH.

as one of the features claimed for the inoculum is an abundance of actinomycetes, a preliminary study was made of the comparative number of actinomycetes in a sample of the preparation, of rich garden soil, and of poor soil. One gram of each material was diluted $1:10^3$ in sterile distilled water. From each dilution 1 ml portions were transferred to sterile Petri dishes and poured with starch-asparagine agar (starch, 10.0 g; KH_2PO_4, 0.5 g; asparagine, 0.5 g; agar, 15 g; H_2O to 1 L). One-ml portions were also poured on the surface of starch-asparagine agar contained in sterile Petri dishes. The plates were incubated at a temperature of 50 C for 5 days to simulate the temperature range suited to thermophilic actinomycetes. The counts obtained were as follows: inoculum, 15.8×10^7 colonies per g; "rich" soil, 13.4×10^7 colonies per g; and "poor" soil, 15.8×10^6 colonies per g. These figures compare with the 15.0×10^7 colonies of actinomycetes found by Waksman to be present in 1 g of horse manure (Waksman, 1950).

TABLE 2. *Chemical analyses of two composts produced from vegetable trimmings and paper*

SAMPLE	DAY	ASH*	MOISTURE†	CARBON*	NITROGEN*	PHOSPHORUS*	POTASSIUM*	C/N	pH
		%	%	%	%	%	%		
Not recirculated	1	11.2	81.9	40.8	1.5	1.46	0.47	27	
	3	13.6	78.5	42.2	1.6	—	—	26	
	8	18.9	80.1	37.6	1.6	—	—	24	
	14	24.1	79.9	32.2	1.6	—	—	20	
Inoculated‡	1	6.8	80.9	43.2	1.3	0.34	2.03	33	5.0
	3	13.3	79.1	43.6	1.3	—	—	33	6.2
	6	14.9	79.3	40.8	1.6	—	—	26	8.5
	10	20.1	74.0	33.4	1.2	0.28	0.62	27	8.6
	14	26.4	73.7	30.7	1.3	—	—	24	9.2

* Values expressed as per cent dry weight.
† Values expressed as per cent wet weight.
‡ Inoculated with partially decomposed material.

To test the effect of the preparation on composting refuse, two bins were filled with 480 kg each of an identical mixture of shredded vegetable trimmings and paper. The material in one bin was not inoculated and that in the second was sprayed and thoroughly mixed with a suspension of the inoculum prepared in accordance with the directions of the manufacturer. Both piles were turned daily.

No significant difference in appearance and odor could be observed in either of the piles during the entire process. As is shown in figure 3, temperatures of both heaps were closely parallel. It is noteworthy that the incidence of actinomycetes was similar in both piles. The compost in both bins had a satisfactory appearance

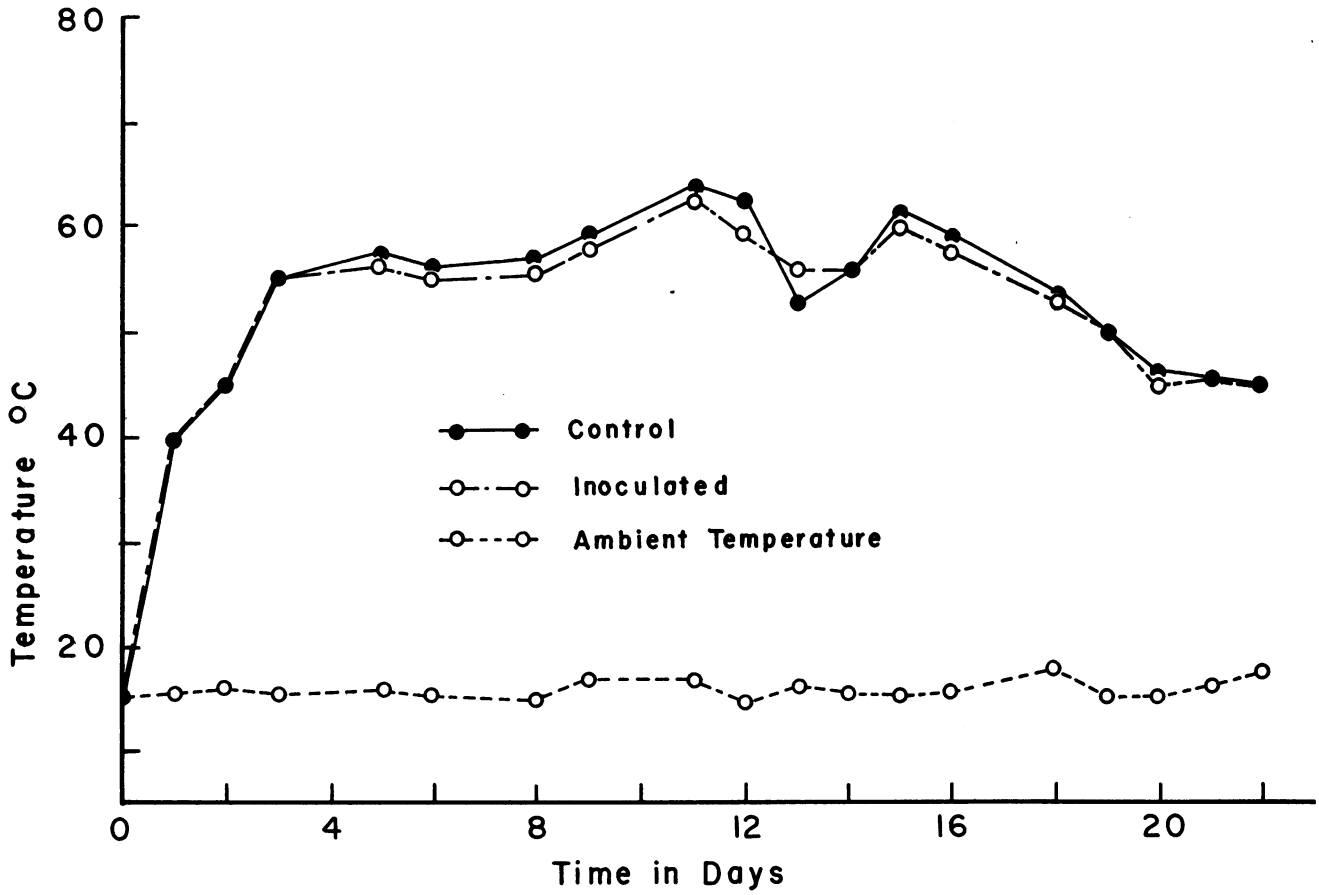

FIG. 3. Temperature curves of two composts, one of which was inoculated with a commercial preparation of bacteria.

and odor by the end of the nineteenth day. Table 3 shows that the chemical composition of both piles was very similar.

The identical rise in temperature through the first 3 days, even to the slight plateau on the second day when the transition from mesophilic to thermophilic populations took place (fig. 3), demonstrated that the indigenous population was responsible for the performance of both inoculated and uninoculated material.

TABLE 3. *Analyses showing the similarity of uninoculated refuse and refuse inoculated with a commercial preparation of bacteria*

	INITIAL	THIRD DAY		FINAL	
		Uninoculated	Inoculated	Uninoculated	Inoculated
	%	%	%	%	%
Ash*	10.5	13.8	16.1	29.2	28.8
Moisture†	90.2	80.7	79.4	74.7	75.1
Carbon*	37.8	38.6	41.7	31.9	31.4
Nitrogen*	2.2	2.3	2.3	2.0	1.9
Phosphorus*	0.2	0.3	0.3	0.3	0.3
Potassium*	1.0	0.9	0.8	1.1	1.1
C/N	17.5	17.0	18.0	15.9	16.5

* Values expressed as per cent dry weight.
† Values expressed as per cent wet weight.

If the inoculum had been of any value, the population it provided would have eliminated the transition plateau in the inoculated sample.

Soil as an inoculum. Literature is replete with references to the isolation of thermophilic bacteria from soil (Black and Tanner, 1928; Feirer, 1927; van Tieghem, 1881; Tsiklinsky, 1899; Waksman, 1938). As previously described, plate counts had demonstrated that garden soil has an actinomycete population almost equivalent to that of horse manure. It seems plausible, therefore, to assume that if additional bacteria were beneficial to a compost, soil should be a very excellent source. Accordingly, soil was used in a final experiment of the series. This study was conducted on a field scale. Approximately 273 kg of top soil was added to 2134 kg of municipal refuse directly after shredding. For the purpose of comparison a pile of uninoculated shredded refuse of about the same size and composition was set up. Both piles were turned at 3-day intervals and both produced satisfactory compost in 16 days. The normal rise and decline of temperature in both piles, as shown in figure 4, indicate that the addition of soil had no significant effect on the composting process. This conclusion is borne out by the similarity in decrease in per cent carbon, and increase in per

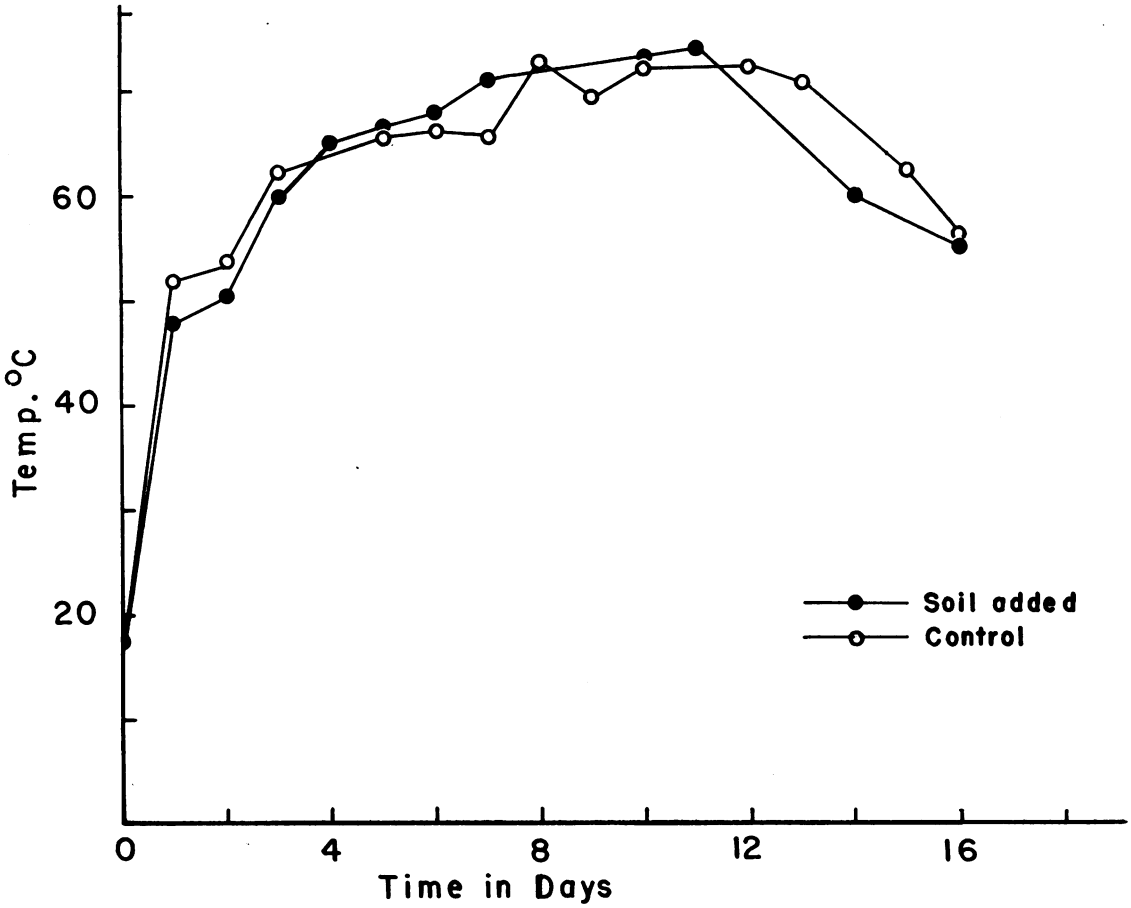

FIG. 4. The effect of soil on the temperature of composting municipal refuse.

TABLE 4. *Effect of the addition of soil on the composting process*

	INITIAL		FINAL	
	Refuse and soil	Control	Refuse and soil	Control
	%	%	%	%
Ash*	48.0	24.2	63.85	39.4
Moisture†	47.6	49.5	40.4	45.8
Carbon*	27.3	35.0	20.7	24.4
Nitrogen*	1.0	1.3	1.0	1.2
Phosphorus*	0.3	0.2	0.2	0.2
Potassium*	0.2	0.3	0.9	0.2
C/N	32		20	

* Values expressed as per cent dry weight.
† Values expressed as per cent wet weight.

cent ash, and final chemical analysis as is shown in table 4. Fungal and actinomycete growth was observed to be similar in both piles.

DISCUSSION

Although all of the inoculums tested were rich in bacteria, none of them accelerated the composting process or improved the final product. This fact leads to the conclusion that inoculums are of no demonstrable value in composting and that garbage and other organic refuse require no additional bacteria for satisfactory composting because of an inevitable exposure to all of the organisms involved in its decomposition. Other studies on composting made by the University of California have confirmed these conclusions and the experience of successful composting operations in many parts of the world, notably in Holland (Weststrate, 1951), Germany (Straub, 1950), New Zealand (Adams, *et al.*, 1951), El Salvador (U. of Calif. Tech. Bull. 9, 1953), South Africa (Van Vuren, 1949) is that bacteria are rarely a limiting factor in composting.

Failure of inoculums to benefit the composting process stems from the adequacy of the microbial population already existing on the material and from the nature of the process itself. Both mesophilic and thermophilic bacteria are always present in abundance on exposed refuse, and whenever the environment is appropriate they multiply with great rapidity and composting proceeds without any delay. In all of the University of California studies on inoculation, the composting process proceeded with equal rapidity on inoculated and uninoculated material, thus demonstrating that added bacteria did nothing to hasten the onset of decomposition.

Inoculation would be of value to the composting process only if the bacterial population in any emerging environment was unable to develop rapidly enough to take full advantage of the capacity of the environment to support a bacterial population. In such a case a time lag would result, which could be overcome by supplementing the initial population indigenous to the refuse. No such time lag occurred in the studies, thus demonstrating that the rate of growth of native bacteria was limited by the capacity of the environment. Composting is a dynamic process, representing the combined activity of a wide succession of mixed bacterial and fungal populations associated with a similar succession of environments, one overlapping the other and each emerging gradually as a result of continual changes in temperature and substrate. The substrate changes are due to a progressive breakdown by bacteria of complex foodstuffs to increasingly simple compounds. Temperature increases steadily in proportion to biological activity, so that initial mesophilic conditions are soon superseded by thermophilic conditions. Because the process is dynamic and any individual group of organisms can survive in a rather wide environmental range, one population begins to emerge while another is flourishing, and yet another is disappearing. Inasmuch as any group of bacteria is capable of multiplying at a pace equal to that of its developing environment, the addition of similar organisms as an inoculum would be superfluous.

ACKNOWLEDGMENTS

Appreciation is expressed to Professor Harold B. Gotaas, Director of the Sanitary Engineering Research Laboratory, who directed the studies on composting, and to other members of the Laboratory staff for valuable contributions to the investigation.

SUMMARY

A study was made of the effect of various inoculums on the composting of garbage and mixed municipal refuse. Garden soil, horse manure, partially decomposed organic material, and a commercial preparation of special bacterial cultures were tested in a series of experiments which were a part of an extensive study of the fundamentals of composting. No measurable effects resulted on the course of temperature, increase in per cent ash, or decrease in per cent carbon.

It is proposed that the nature of the composting process, and the presence of an adequate population of bacteria already existing on the material, make superfluous the addition of other bacteria.

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Low Temperature Storage for Maintaining Stable Infectious Bacterial Pools

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The maintenance of unchanging virulence is of concern to all those who deal with bacterial infection of experimental animals. Chemotherapy studies, in particular, require the production of uniform and reproducible mortality rates. Since cellular changes may be expected to occur spontaneously at any point in the artificial life history of a pathogenic culture, it becomes necessary for such cultures to be examined periodically for changes in virulence. If loss of virulence has occurred, the investigator must increase the size of the inoculum or raise the level of virulence by means of animal passage. This report describes a procedure for the use of low temperature storage whereby it is possible 1) to avoid the use of cultures which must be repeatedly transferred on laboratory media, 2) to maintain infectious material without loss of virulence, and 3) to prepare bacterial cultures which, over long periods of time, give reproducible infections. The method has been employed routinely in this laboratory during the past 4 years and has given satisfactory results with such species as *Salmonella typhosa, Salmonella choleraesuis, Shigella sonnei, Klebsiella pneumoniae, Vibrio comma, Streptococcus pyogenes* and *Bacillus anthracis.*

METHODS

Materials. Ringer-Locke solution is prepared by dissolving the following chemically pure salts in 1 liter of distilled water: NaCl, 9.0 g; KCl, 0.42 g; $CaCl_2$, 0.48 g; NaHCO, 0.20 g. Adjust the pH of the solution to 7.2–7.4 with 0.5 N NaOH. The solution is sterilized by Seitz filtration, tested for sterility in thioglycollate broth (Difco) and stored in a refrigerator. Ten per cent hog gastric mucin suspension is prepared by suspending 50 g of granular mucin (Wilson Labs., Type 1701-W[1]) in 477.5 ml of cold physiological saline in a 750-ml Erlenmeyer flask. The suspension is slowly agitated until the mucin is thoroughly wetted and then mixed with an electrical stirrer until a homogeneous suspension is obtained. Autoclave for 10 minutes at 15 lb pressure. Allow the suspension to cool to room temperature and adjust the pH to 7.2–7.3 using sterile 0.5 N NaOH (approximately 22.5 ml of 0.5 N NaOH). Test for sterility in thioglycollate broth and store at 4 C.

Preparation of bacterial pools. In view of the marked alterations which bacterial cells undergo on artificial

[1] Wilson Laboratories, Chicago 9, Illinois.