

Population Changes in Enteric Bacteria and Other Microorganisms During Aerobic Thermophilic Windrow Composting¹

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Composting of wastes from swine feeding operations was studied. The effects of the frequency of turning the wastes and addition of straw to improve the physical structure were studied to determine the most effective technique to rapidly increase the temperature and, consequently, destroy coliforms and *Salmonella*. Four different treatments were studied; the results showed that, with addition of 5% (wt/wt) straw and mechanical turning of the compost 20 times per week, the temperature reached 60 C within 3 days and enteric bacteria were destroyed within 14 days.

According to Wadleigh (12), animal farm waste production in the United States amounts to about two billion tons annually. Traditional methods for utilizing these wastes as fertilizers are not being employed widely, and the vast amounts that are accumulating present serious threats from both environmental and public health standpoints. Solutions to the problems of disposal of animal solid waste require complex environmental control. Methods must be utilized that do not cause contamination of water, pollution of the atmosphere, or desecration of the land. Burning of waste in open dumps or poorly designed incinerators is a major source of air pollution. Other disposal methods, such as sanitary landfill, aerobic and anaerobic lagoons, and spreading of liquid and solid on soil, may cause odors and chemical and microbial pollution of surface and ground waters.

Many of these problems can be avoided by composting; with proper control of this method, wastes are incorporated into the biogeochemical cycle without serious detriment to the ecosystem. Composting can be defined as the decomposition of organic wastes under semi-dry conditions by aerobic, thermophilic organisms. The products of composting are carbon dioxide, water, heat, and stable humus-like organic material.

Several investigators (2, 5, 10) have concluded that since relatively high temperatures are attained during the composting process any

pathogens present should be destroyed. Recently, Wiley and Westerberg (13) selected representatives from four groups of pathogens as indicators and related the temperature of a laboratory composter to survival of these organisms. Few studies of a similar nature have been reported on a large-scale field operation. This paper describes the survival of *Salmonella*, other enteric bacteria, and fecal streptococci during the composting of swine waste in windrows that were turned mechanically with a composting machine. Changes in the levels of actinomycetes, filamentous fungi, and cellulolytic microorganisms occurring during composting are also discussed.

MATERIALS AND METHODS

Preparation of compost. Swine waste was obtained from a hog feeding operation in southern New Jersey. The swine were fed hotel and restaurant garbage, which was cooked with live steam and dumped outdoors in concrete-floored pens. The swine waste—a combination of uneaten garbage, bottles, cans, plastic, paper, bones, other inedible material from the garbage, and swine feces—was scraped up daily with a front-loading tractor and removed. The material was trucked to the compost site and stockpiled over a 5-week period until a sufficient amount of waste accumulated for construction of four 40-ft (about 12.2 m) windrows, which were installed on a concrete floor. The swine waste was turned by a self-propelled composting machine (Roto-Shredder, Roto-Shredder Co., Division of Imco, Crestline, Ohio) which straddled the row and traveled its length, shredding, grinding, and pulverizing the material (Fig. 1). As the machine moved forward, the waste fell behind the machine, reforming the windrow. Through this mixing action (turning), oxygen (air) was incor-

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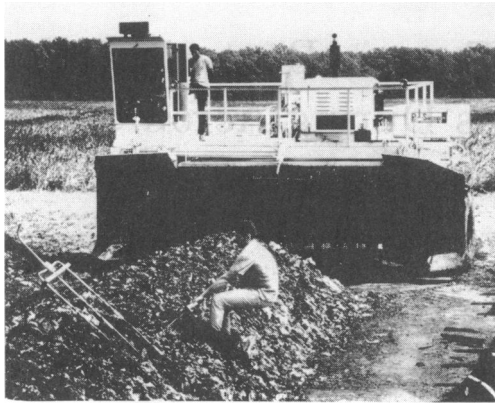


FIG. 1. Front view of the Roto-Shredder approaching the windrow.

porated into the mass. After turning, the windrow was less compact, and the particle size of materials such as paper, cardboard, vegetable matter, and bones was greatly reduced.

Windrow 1 contained approximately 40 tons (36 metric tons) of unsupplemented swine waste and was turned twice a week. Windrows 2, 3, and 4 were turned 20 times per week. Windrow 2 contained approximately 40 tons of swine waste; windrow 3 contained approximately 23 tons (21 metric tons) of waste and 18 tons (16 metric tons) of old compost that has been previously composted for 180 days with turning twice weekly. Windrow 4 contained 40 tons of stockpiled waste and 1.5 tons (1.36 metric tons) of straw.

Sampling procedure. Samples of compost to be assayed were collected from the windrow surface immediately after turning on the days indicated in Fig. 5 to 9. Samples weighing approximately 3 g were taken randomly along the windrow and combined in a sterile quart Mason jar. The total composite sample weighed about 1 kg. In the laboratory, the sample was mixed thoroughly, and duplicate portions (10 g) were taken for testing. These were suspended in 90 ml of sterile water, and 1-ml volumes of the suspension were diluted serially (wide-mouthed pipette). Samples (100 ml) of run-off water were collected from seepage at the foot of the pile; 1-ml volumes of these samples were diluted serially.

Determination of microorganism numbers. The standard plate count method (1) was used for estimating the numbers of microorganisms in the compost. Coliforms were counted on Levine eosin methylene blue agar (Difco) plates, as justified by Poelma (8) and used by the Food and Drug Administration. The identification methods used for *Salmonella* were, in general, those of Poelma (8). Plates for counting *Salmonella* were poured with brilliant green agar, *Salmonella-Shigella* agar, or bismuth sulfite agar (all Difco products), and incubated at 37 C for 2 days. Proportions of the colonies appearing on these plates were cultured and characterized by biochemical tests (fermentation of glucose, lactose, dulcitol, and mannitol; decarboxylation of lysine, ornithine, and asparagine; production of H₂S, indole, acid [methyl red],

acetoin [Voges-Proskauer]; and utilization of citrate, urea, malonate, and triple sugar-iron). About 75% were indicated to be *Salmonella* and 25% were *Proteus* types (urease positive). Serological tests were not performed. Counts in tables are based on presumed *Salmonella* from plate counts.

Fecal streptococci were counted on KF agar plates (1); *M*-enterococcus agar was also used and gave similar results.

For total bacterial counts, plates were poured with nutrient agar (Difco) and incubated at 37 C for 2 days. Only typical bacterial colonies were included in the counts; these were generally mucous or small, yellow, lenticular colonies. Total counts were also investigated on compost agar (9), but fewer colonies were observed. Fungi were enumerated on acid-agar or potato-glucose-novobiocin agar plates (9) incubated at 28 C for 5 days. Colonies counted as fungi were typically filamentous or large, round, mucous colonies with yeast-like appearance. Actinomycetes were enumerated on caseinate agar and Czapek-Dox agar (Difco). These plates were incubated for 7 days at 37 C, and only the small, powdery, wrinkled, or pasty colonies were counted as actinomycetes. When these colonies were isolated, their plates had the typical earthy odor associated with actinomycetes. Cellulolytic bacteria associated with compost were enumerated by plating the compost homogenate on a cellulose-mineral salts agar medium (6). The medium contained per liter: (NH₄)₂SO₄, 0.5 g; K₂HPO₄, 0.5 g; KH₂PO₄, 0.2 g; CaCl₂, 0.5 g; MgSO₄, 0.5 g; NaCl, 1.0 g; agar, 15 g; and 150 ml of a slurry of cotton-fiber cellulose, ball milled for 24 h in 4% HCl and washed with distilled water. The colonies which produced clearing zones on this medium were recorded as cellulolytic microorganisms. The plates were incubated aerobically for 7 days at 37 C.

Determination of windrow conditions. Temperatures of the composting windrows were determined by a thermocouple potentiometer and recorded on a battery-operated, 12-point Brown recorder (Minneapolis-Honeywell Regulator Co., Industrial Division, Philadelphia, Pa.). The thermocouples were mounted on a probe that could be inserted in the piles (one is visible at the lower left in Fig. 1). This probe had three parallel rods of 3/4-inch (about 1.89 cm) diameter galvanized pipe with an aluminum point on one end and a junction box on the other. Each rod had two openings—one each at 6 and 24 inches (about 15.24 and 60.96 cm, respectively) from the tip of the aluminum point. At each opening was a thermocouple (electrically insulated from the metal rod). The rods were 24 inches apart. They were inserted vertically into the top of the windrow and forced down until the tip touched the concrete base, so the readings were made 6 and 24 inches from the bottom of the piles. The three thermocouples at each of the two depths were connected in parallel, thus yielding an average reading. The thermocouples were connected to the recorder, which printed the average temperature. The printout was controlled by a timer set to record the temperature at 6-h intervals.

The pH of compost samples was measured on three replicate samples, each containing 10 g/500 ml of

distilled water. The suspensions were stirred with a magnetic mixer for 5 min, and the pH was determined with a Beckman Expandomatic pH meter, model 7600.

The oxidation-reduction potential (E_h) of the material in the windrows was determined with a redox probe constructed by Starkey and Wight (11). This 4-ft-long (about 121.9 cm) pointed probe was composed of inner and outer tubes; after the probe was inserted in the windrow, the inner tube could be rotated to make the openings in the two tubes coincide, exposing the electrodes (two platinum and one calomel half-cell) to the windrow material. Readings were made by connecting the calomel half-cell to the lower terminal on a Beckman model G pH meter and by connecting one of the two platinum electrodes to the 700 terminal. After the electrodes had stabilized and a reading was taken, the other platinum electrode was connected to the 700 terminal and a duplicate reading was taken.

The electrodes were cleaned before and after each reading by being washed successively with 5% acetic acid, 5% nacconol, water, 10% H_2O_2 , and distilled H_2O . The electrodes were standardized with solutions of known E_h value; the meter was standardized by adjustment to zero H^+ ion and checked by connecting the electrodes—in standard solution—to an Electronic Associates Inc. 6200 digital volt meter.

The moisture content was determined by weight loss of 25-g samples which were dried for 3 days at 103 C or until a constant weight was reached.

RESULTS

Windrow temperature, pH, and E_h .

Changes in temperature within the windrows during composting are shown in Fig. 2. Windrow 1 showed a lag period of 38 days before a rapid rise in temperature occurred. The temperature in the center of windrow 2 (swine waste turned 20 times per week) reached a thermophilic range of 55 to 65 C in 25 days. The temperature in windrow 3 (approximately 50% old compost) reached this range in 15 days, whereas windrow 4 (waste and straw mixture)

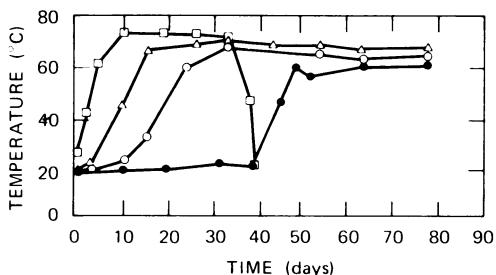


FIG. 2. Temperature variation in the windrows during composting. The temperature readings were taken at 24 inches from the bottom of the windrow. The symbols representing the windrows are: ●, 1; ○, 2; Δ, 3; and □, 4.

had reached 60 C within 3 days of composting. The highest temperature recorded at any position in any windrow was 72 C in windrow 4. After reaching thermophilic temperature, windrows 2 and 3 remained thermophilic until they were removed from the concrete floor on day 80. Windrow 4 cooled to ambient temperature (20 to 30 C) by day 38.

Changes in the pH of the waste during composting are shown in Fig. 3. Windrow 4 reached a pH of 8.0 in only four days—indicative of rapid decomposition in this windrow—whereas windrow 3 took 16 days to reach this pH; windrow 2 reached pH 7.5 in 25 days but did not reach pH 8.0; and windrow 1 took approximately 80 days to reach pH 8.0.

The initial E_h of the windrows was -450 mV (Fig. 4). In windrow 1, the E_h rose gradually to -200 to -250 mV over the first 18 days of composting and then remained constant for the next 20 days; active thermophilic composting, indicated by pH and temperature rise, began only after this period. The E_h rose to +50 to

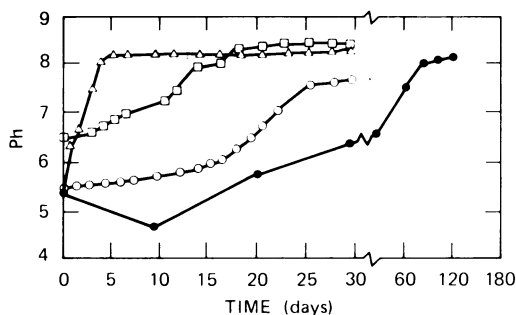


FIG. 3. Influence of composition on the progress of decomposition, as indicated by pH pattern. The symbols are: ●, windrow 1 (unsupplemented swine waste turned twice a week); ○, windrow 2 (unsupplemented waste turned 20 times a week); □, windrow 3 (waste plus 180-day-old compost); Δ, windrow 4 (waste plus 5% straw).

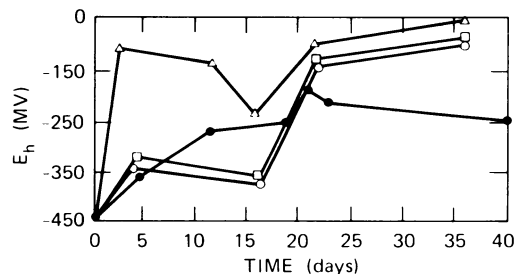


FIG. 4. Redox potentials in windrows of the composting process. All readings were taken before the daily turning at a 24-inch depth in windrows 1 (●), 2 (○), 3 (□), and 4 (Δ).

+100 mV immediately after the windrow was turned but decreased within 60 min to the level observed before the turning. In windrows 2, 3, and 4 during the active composting (pH >7, temperature >60 C), the E_n was -50 to -100 mV before the turning. Windrow 4 reached this level (from the initial -450 mV) within 3 days. These more aerobic conditions presumably result from greater porosity of the pile, especially in windrow 4.

Active composting (pH >7, temperature >50 C) began in windrow 1 only after the moisture content fell below 40%, 40 days after the composting process was begun; however, since windrows 2, 3, and 4 reached the active composting stage when moisture content was still 45 to 55%, dryness of the material was not solely responsible for active composting.

Effects of thermophilic composting on intestinal microorganisms. Enumeration of *Salmonella* in windrow 1 and its run-off water at various times indicated the number of organisms that could survive and possibly pollute water supplies. *Salmonella* numbers, after an initial drop, increased in the windrow (Table 1, 40 days). When the temperature rose above 48 C, the population of presumptive *Salmonella* decreased sharply and continued to decrease as temperature increased. Similarly, the numbers of *Salmonella*, coliforms, and streptococci in the run-off water increased initially (after a drop in coliforms), then decreased as the temperature in the center of the windrow passed 52 C (Table 2). The count of fecal coliforms, shown in Table 2, appeared to remain higher than fecal streptococci or *Salmonella*. The coliform count decreased rapidly as composting proceeded in windrows 2, 3, and 4 (Fig. 5). The coliform test was negative in windrow 4 at day 14 of composting when the temperature was 71 C. When windrow 4 was removed on day 40, presumptive *Salmonella* colonies could not be detected. The

TABLE 2. Presumptive *Salmonella*, fecal coliform, and fecal *Streptococcus* colonies in run-off water from windrow 1

Days of composting	Ambient temp (C)	Center windrow 1 temp (C)	<i>Salmonella</i> ^a ($\times 10^5$ cells/ml)	Fecal coliforms ($\times 10^5$ cells/ml)	Fecal <i>Streptococcus</i> ($\times 10^5$ cells/ml)
18	31	35	180	90	6
46	19	47	570	41	12
53	14	50	590	53	90
60	24	52	117	210	160
84	22	55		2	0.5
88	13	55		2	0.2
194	7	70	0.02	0.3	0.02

^a Isolated on bismuth sulfite agar.

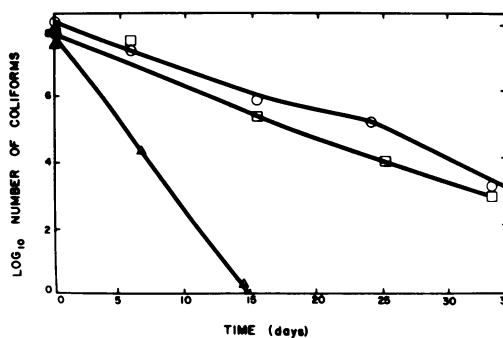


FIG. 5. Effect of composting action on the number of fecal coliforms in windrows 2 (○), 3 (□), and 4 (△). Each point is the average value from counting four replicate plates.

number of coliforms was reduced by 10^4 -fold in windrows 2 and 3.

In 40 days of composting, the number of mesophilic bacteria decreased between 10^4 - and 10^5 -fold in windrows 3 and 4 (Fig. 6). The bacterial population in windrow 2 increased during the first 15 days and then started to decline. The decline in mesophilic bacteria occurred at the time when the temperature began to rise rapidly in these windrows. The number of mesophilic fungi did not drop as much as the number of bacteria (Fig. 7). In windrow 4, fungi were reduced 10^3 -fold, but in windrows 2 and 3 the decrease was less than 100-fold. The population of mesophilic actinomycetes responded differently from either bacteria or fungi. This population increased by factors of over 10^3 in windrow 4, 10^3 in windrow 3, and less than 10^2 in windrow 2 (Fig. 8). The increase in windrow 3 followed a decrease in the first week from the high initial numbers present as a result of the addition of old compost. The number of cellulolytic organisms showed a re-

TABLE 1. Presumptive *Salmonella* colonies from windrow 1

Days of composting	Temp at center of windrow (C)	Bacteria ($\times 10^5$ cells/g) on		
		BS ^a	SS ^a	BG ^a
0	36	75	83	41
19	41	9	12	11
40	48	320	370	510
60	52	6	7	8
78	51	4	3	2
187	68	0.08	0.07	0.05

^a BS, Bismuth sulfite agar; SS, *Salmonella-Shigella* agar; BG, brilliant green agar.

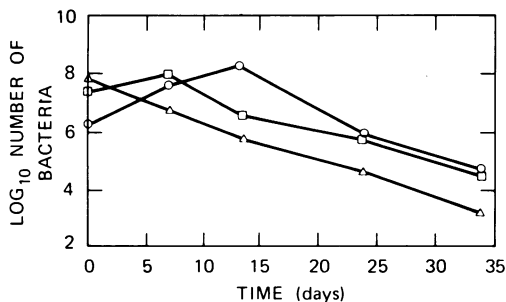


FIG. 6. Number of mesophilic bacteria during the composting process. The symbols represent windrows 2 (O), 3 (□), and 4 (Δ).

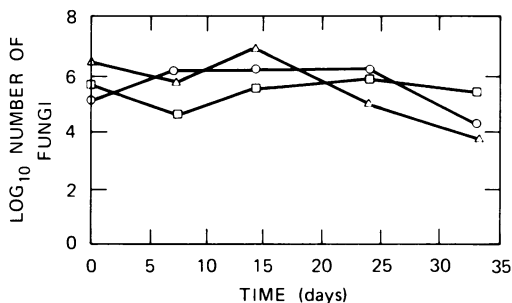


FIG. 7. Number of mesophilic fungi during the composting process. The symbols represent windrows 2 (O), 3 (□), and 4 (Δ).

sponse similar to that of the population of actinomycetes. Again, the population in windrow 4 developed more rapidly and attained a much higher number (Fig. 9).

DISCUSSION

Although considerable use is made of composting in the disposal of municipal wastes, garbage, sewage sludge, etc. (4, 5), little information is available on the numbers of enteric bacteria actually resulting from such operations. Typically, thermal death points of common pathogens present have been determined, and the assumption was made that attainment of these temperatures during the composting process would destroy the pathogens (2, 5). In one study (13), introduced indicator organisms (poliovirus, *Candida albicans*, *Ascaris lumbricoides*, *Salmonella newport*) were shown to disappear within 43 h from a pilot-scale composter maintained at 60 to 70 C. The possibility of survival of pathogens at the cool surface of a windrow operation, however, has not been disproven.

Our results indicate clearly a marked decrease in coliforms, salmonellae, and enterococci during the thermophilic stage of com-

posting. Before the thermophilic stage, there was presumably anaerobic decomposition of carbohydrates, proteins, and fats to form organic acids and other intermediate compounds that could be used by *Salmonella*, coliforms, and enterococci for growth under partially anaerobic conditions (7). Therefore, an increase in these organisms during the first stages of composting was expected. From a public health standpoint, it is desirable that this early increase be minimized and that the windrows maintain a temperature above 48 C long enough to destroy pathogens like *Salmonella*. These results indicate that, for maximal sanitary safety, the thermophilic stage of composting should be reached as soon as possible.

The noteworthy practical observation is that inclusion of straw (windrow 4) fostered more aerobic conditions and thereby facilitated very rapid attainment of thermophilic conditions and destruction of enteric bacteria. Frequent turning of the windrows was much less effective. The original rationale for addition of straw was increase of the carbon-nitrogen ratio, since excess nitrogen is liable to be eliminated as ammonia and other malodorous amines. However, the primary effect of addition of straw

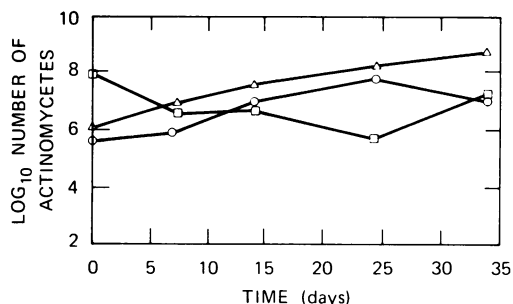


FIG. 8. Number of mesophilic actinomycetes during the composting process. The symbols represent windrows 2 (O), 3 (□), and 4 (Δ).

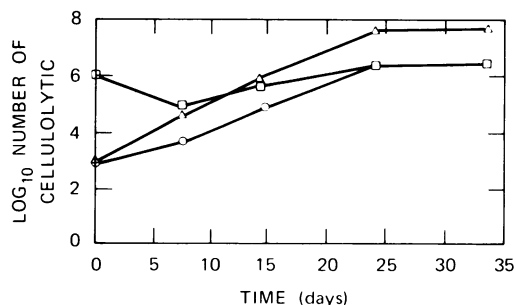


FIG. 9. Number of cellulolytic organisms during the composting process. The symbols represent windrows 2 (O), 3 (□), and 4 (Δ).

appeared to be improvement of the physical structure of the windrow, allowing more natural aeration (compare Fig. 4) and a rapid rise in temperature consequent upon intense aerobic microbial activity. This had the beneficial effect of rapid destruction of pathogens. Waste materials with similar structural properties, such as cornstalks, chipped wood, shredded municipal refuse, etc., should also have this effect. The possibility of use of vegetable wastes such as cornstalks, which are also becoming a disposal problem as open air burning is banned, to improve the composting of animal wastes is particularly attractive.

A succession of microbial populations was observed during the composting process. The bacteria increased in number before the temperature of the windrows rose and then declined, whereas cellulolytic organisms and actinomycetes in general increased in the thermophilic stage. Presumably, the mesophilic bacteria rapidly attack the more readily available organic constituents, resulting in a temperature increase. The increased temperature favors the cellulolytic organisms, and the mesophilic bacteria largely disappear. The actinomycetes appeared in the final stage to such an extent that the surfaces of the compost piles were white or gray. These organisms are known to play a role in the humification of organic matter, which results in a stabilized product (3).

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