J. Rakuno Gakuen Univ., 20(1): 81～90 (1995)

Changes of Microbial Population on Liquid Composting of Dairy Cattle Slurry

Eiryu OKAMOTO[1], Eiichi MIYAGAWA[1], Yukio MATSUI[1] and Juzo MATSUDA[2]

(June, 1995)

Abstract

On the liquid composting treatment of dairy cattle slurry, the changes of microbial population in the slurry of undergoing aerational treatment were investigated for 28 days at a well-managed dairy farm. By the continuous aeration in the beginning for 3 days the number of aerobic mesophilic bacteria and Enterobacteriaceae increased significantly, and anaerobic mesophilic bacteria decreased slightly. Temperature and pH of the slurry rose up from 27.3°C to 38.2°C and from 7.39 to 8.03, respectively. In the intermittent aeration started from 4th day to 7th day, the number of anaerobic mesophilic bacteria increased, volatile fatty acids such as acetic and propionic acids were detected, and the pH values fell from 8.03 to 7.90. Thereafter the number of filamentous fungal spores, *Pseudomonas*, and aerobic mesophilic bacteria decreased, while mesophilic actinomycetes increased. Superiority of mesophilic actinomycetes was recognized comparing with viable counts of aerobic mesophilic bacteria on the 28th day. During the experiment, the most superior microorganisms were obligate anaerobic mesophilic bacteria rather than facultative anaerobes.

Introduction

Livestock excrements in Japan have become one of important subjects in environmental pollution such as offensive odor, water pollution and hygiene[14]. Formerly, the excrements had been restored to be utilized as manure. There are numerous reports concerning solid compost changes of ingredients and microflora[6),7),17]. Furthermore, the investigations have made progress in judge tests in maturity of wastes and hastening decomposition by the addition of subsidiary materials[8),12),13),15].

Nowadays, it is becoming more difficult to resolve the problems in enlaeged treatment loads with high levels of moisture content by multiple breeding and with excrements at free-stool bedding[3]. Generally, liquid waste treatment was classified as either aerobic or anaerobic. Concerning the generation of offensive odor and the treatment period required for treatment, it is clear that aerobic

1) 酪農学園大学酪農学科（農業微生物学） 岡本英竜，宮川栄一，松井幸夫
Department of Dairy Science (Agricultural Microbiology), Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan.

2) 北海道大学農学部農業工学科 松田従三
Department of Agricultural Engineering, Hokkoido University, Sapporo. Hokkaido 060, Japan.

treatment is more efficient than anaerobic.

Studies on solid composting have been well researched. There are also many engineering reports on liquid composting[2),4),5),11)]. However, only a few investigations of microbiological research on liquid composting have been reported[1),19),20)].

This study aims to obtain more fundamental knowledge on time-interval changes of microbial population on liquid composting.

Materials and Methods

Sampling procedure: Cattle slurry used in this experiment was the fluids resulted from separation of wastes into solid and liquid in the dairy farm in Teshio, Hokkaido. The liquid composting was carried out by an ejector aerator in continuous aeration for an initial 3 days and subsequent intermittent aeration for 1 hour every 4 hours per day for 25 days. The samples were collected from the aeration tank of a Slurry System using a water-sampler, immediately in the beginning of aeration and also were collected after 3, 7, 14, 21 and 28 days, respectively (Fig. 1). These samples were kept at 4°C and transported to our laboratory.

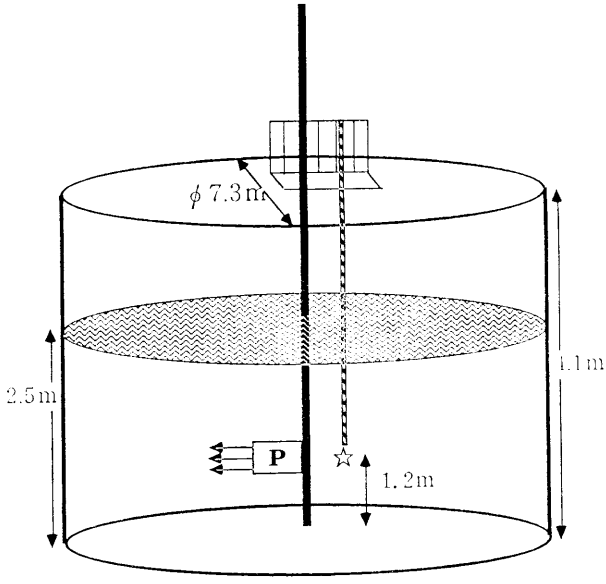

Fig. 1. Aeration tank

Microbial analysis: For microbilogical assays, after mixing of the slurry specimens, a series of ten-fold dilutions to 10^{-8} for each specimen was prepared using the sterilized saline solution for aerobes and anaerobic diluents for anaerobes, respectively. Microflora in the slurry was analyzed by the media and culture as shown in Table 1. The cultures were performed by the plate method for aerobes and by the roll-tube method[23)] or anaerobic steel-wool method for anaerobes. Each sample dilution was inoculated in three repicate plates or tubes. Each culture was counted as colony-forming units (CFU). In case of pronounced colonies could not be distinguished, their numbers were estimated by the most-probable-number

Table I. Incubation of microorganisms

Microorganism	Medium	Inucbation	
Aerobic bacteria	Nutrient agar (added 0.5% glucose)	psychrophilic	10°C–48 hrs.
		mesophilic	30°C–48 hrs.
		thermophilic	55°C–48 hrs.
Anaerobic bacteria	YL agar	psychrophilic	10°C–5 days
		mesophilic	30°C–5 days
		thermophilic	55°C–48 hrs.
Enterobacteriaceae	DHL agar		37°C–24 hrs.
Thermostable bacteria	Nutrient agar (added 0.5% glucose)		30°C–48 hrs.
Pseudomonas	Pesudomonas C-F-C agar		25°C–48 hrs.
Actinomycetes	HV agar	mesophilic	30°C–7 days
		thermophilic	55°C–7 days
Molds	PG agar		25°C–5 days
Yeasts	PG agar		25°C–3 days

method. The identification of microbes was performed by colonial and cellular morphologies, Gram-reaction, spore formation and growth condition.

Slurry Analysis: Temperature, pH and dissolved oxygen concentration were measured by electrodes. Volatile fatty acids (VFA) and non-volatile fatty acids (non-VFA) were analyzed by gas chromatograph equipped with a flame ionization detector with N_2 as the carrier gas, by injecting 1 $\mu\ell$ of deproteinized samples with 24% meta-phosphoric acid in 5N-H_2SO_4[21]. Biological oxygen demand (BOD_5) was assayed according to Japanese Industrial Standard (JIS) K 0102[16]. Chemical oxygen demand (CODcr) was estimated by colorimetric method (HUCK COMPANY, USA). The total solids (TS) and ignition losses (IL) were analyzed by dry method at 105°C for slurry and by ignition method at 600°C for dry matter, respectively. TS was expressed as the dry weight per mℓ of slurry, and IL was expressed as the ratio per gram dry weight of TS.

Results

Microflora analysis: The changes of the aerobic bactria were shown in Fig. 2. The number of psychrophilic bacteria (10^6 CFU/mℓ) that survived in the beginning of aeration could not be detected on the 3rd day. On the other hand the mesophilic bacteria were increase from 1.2×10^7 CFU/mℓ to 7.9×10^7 CFU/mℓ in the first 3 days, thereafter, decreased gradually to the 28th day. No evident changes was observed in thermophilic bacteria.

Changes in the number of anaerobic bacteria were shown in Fig. 3. The psychrophilic bacteria could not be detected during the experiment. The number of mesophilic bacteria was high, approximately 10^7 CFU/mℓ, with transient increase in 7th day. No remarkable changes was observed in thermophilic bacteria.

Figures 4 and 5 showed the changes of other microorganisms. Enterobac-

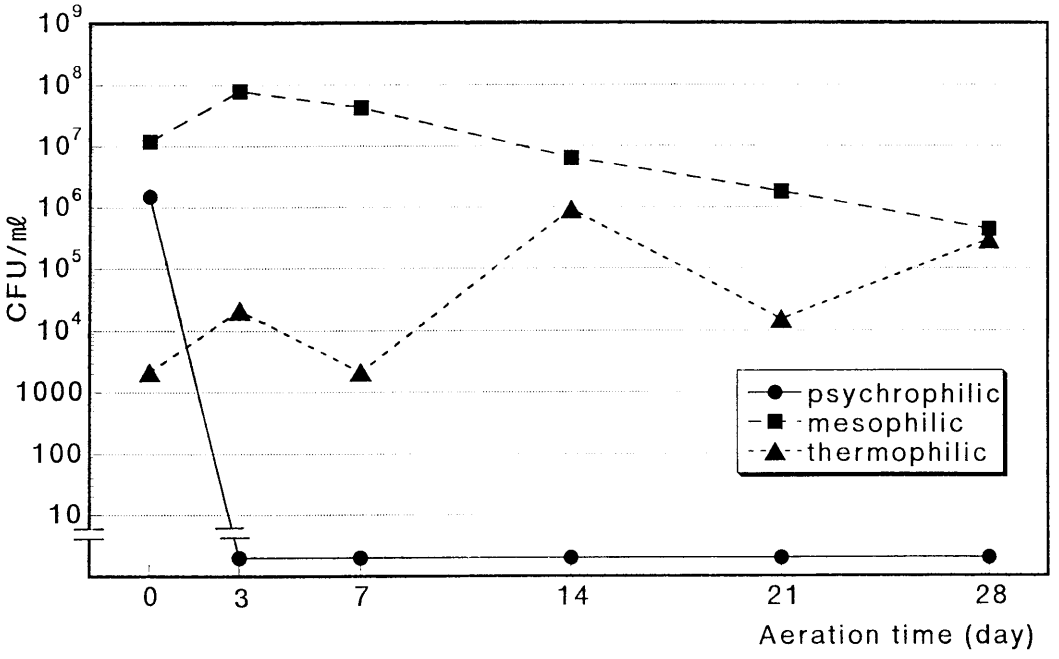

Fig. 2. Change in viable counts of aerobic bacteria during aerational treatment of slurry.

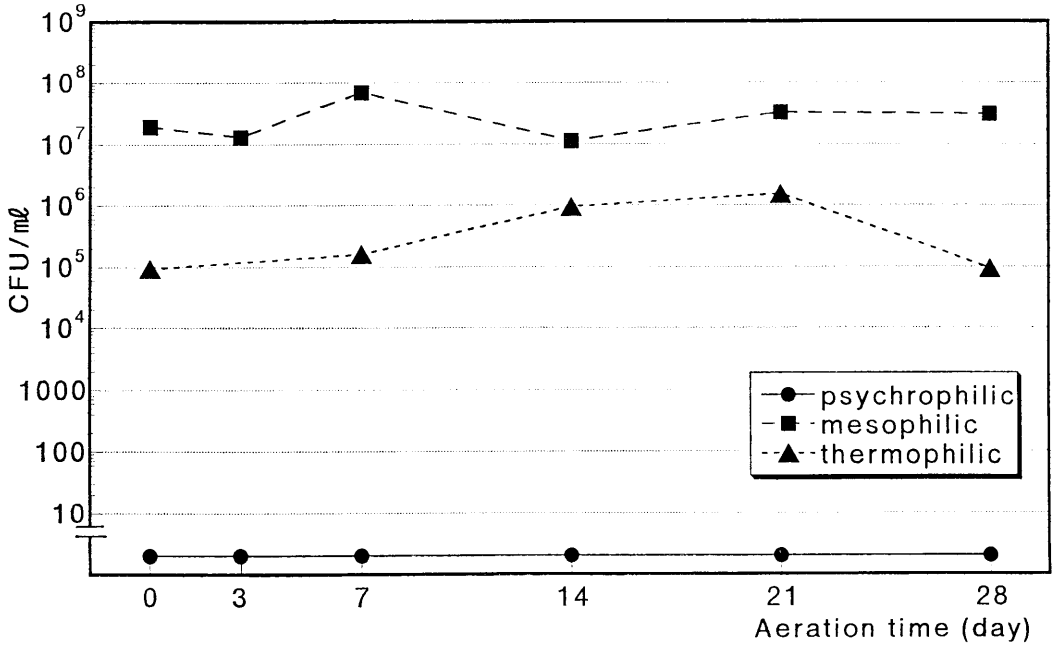

Fig. 3. Change in viable counts of anaerobic bacteria during aerational treatment of slurry.

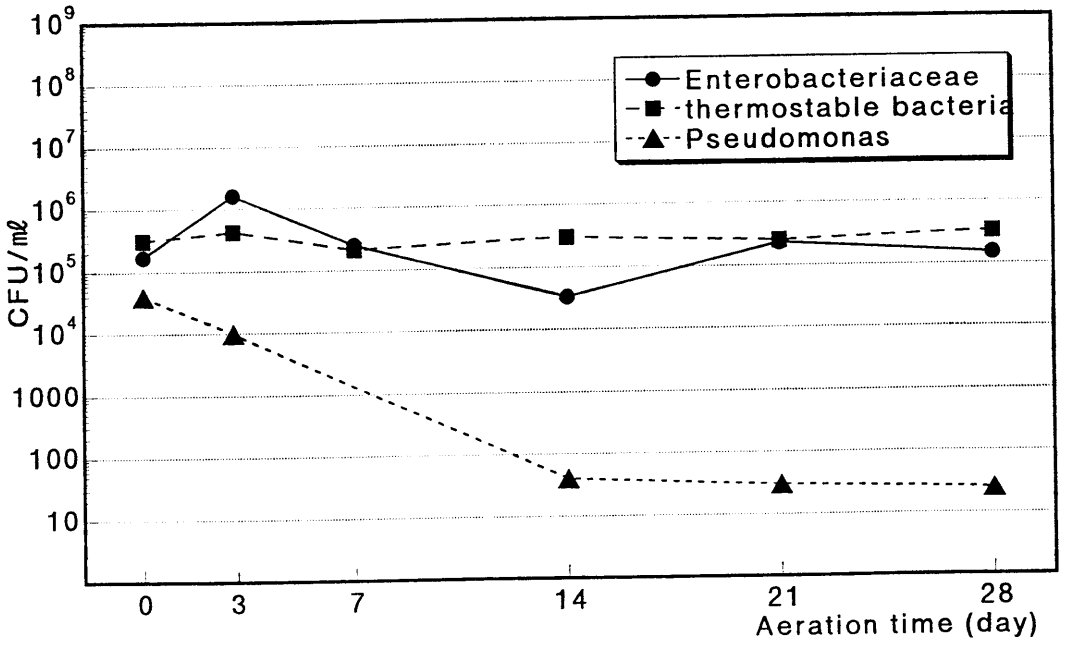

Fig. 4. Change in viable counts of certain bacteria during aerational treatment of slurry.

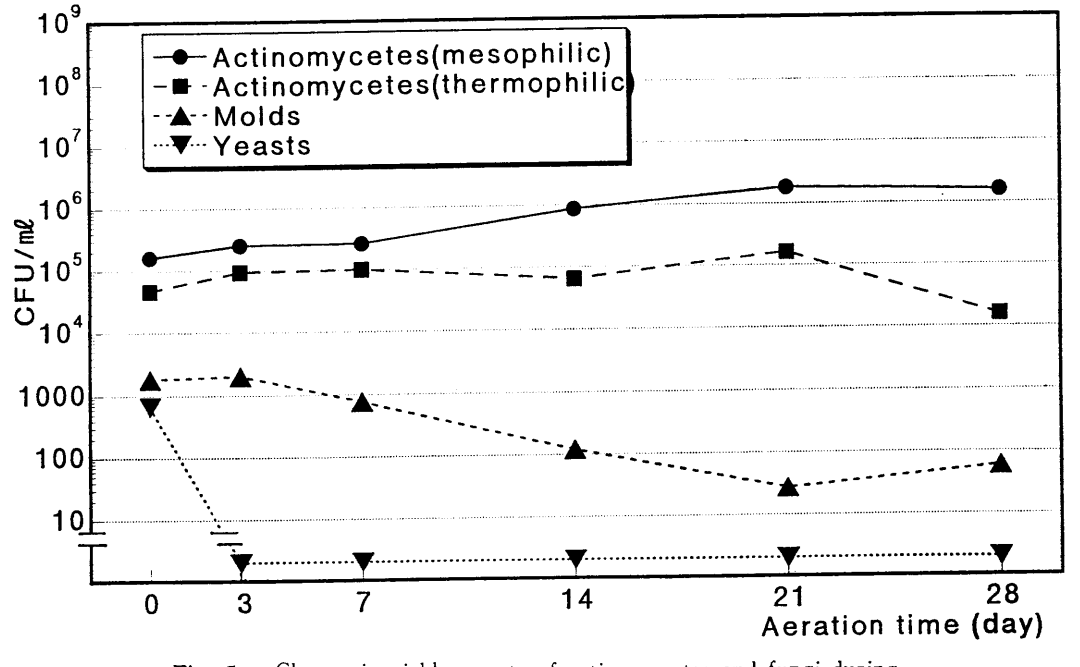

Fig. 5. Change in viable counts of actinomycetes and fungi during aerational treatment of slurry.

teriaceae was recognized to increase about ten-fold on the 3rd day and decrease to 3.3×10^4 CFU/mℓ on the 14th day, finally, it became approximately 10^5 CFU/mℓ. No evident changes was observed in thermostable bacteria. The number of *Pseudomonas* that had survived about 10^4 CFU/mℓ in the beginning decreased to 10^1 CFU/mℓ by the 14th day gradually, otherwise no other changes from 14th day to 28th day. In addition, a define in the mesophilic actinomycetes was recognized. Moreover, these bacteria became the predominant population exceeding the other aerobic mesophilic bacteria on the 28th day. No change was evident in the thermophilic actinomycetes. The spore numbers of filamentous fungi tended to decrease along with aeration time. The yeasts that had survived at about 10^3 CFU/mℓ in the beginning of aeration and could not be detected by the 3rd day.

The most predominant microorganisms examined in this experiment were the anaerobic mesophilic bacteria. Table 2 shows the ratio of the number of the facultative anaerobes to anaerobic strains obtained by the roll-tubes. In spite of aerobic treatment, it was recognized that obligate anaerobes were more numerous.

Table 2. Proportion of facultative anaerobe to the total anaerobic bacteria isolated from slurry

	slurry samples [aeration time (day)]					
	0	3	7	14	21	28
Number of anaerobic strains[a]	40	45	35	37	16	48
Number of obligate anaerobes	19	42	34	35	15	46
Number of facultative anaerobes	21	3	1	2	1	2
proportion of facultative anaerobes to anaerobic strains (%)	52.5	6.7	2.9	5.4	2.2	4.2

a: Clolonies appeared on VL agar at 10^{-5} dilution.

Slurry properties: Changes of temperature, pH and dissolved oxygen concentration of slurry during aeration treatment were shown in Fig. 6. The temperature was 27.3°C in the beginning, was raised to 38.2°C by the 3rd day, and thereafter was about 40°C at maximum. The pH was 7.39 in the beginning and changed to 8.03 on the 3rd day, 7.90 on the 7th day, 8.15 on the 28th day. The dissolved oxygen concentration could not be detected by the 14th day, after that start to appeared in concentration of 0.06 mg/ℓ on the 21st day, and 3.29 mg/ℓ on the 28th day.

The amounts of VFA and non-VFA in the slurry were shown in Table 3. Acetic, propionic, *n*-butyric and *iso*-butyric acids were detected in the beginning of aeration period, aeter that no fatty acids could be detected. However, on the 7th day, acetic and propionic acids were detected in a small amount, thereafter, they could not be detedted again.

Remarkable degradation in BOD_5 was observed in the first 3 days, then gradually declined. The CODcr also degraded (Fig. 7). Remarkable decrease in TS and IL were recognized during the continuous aeration, thereafter, degraded gradually (Fig. 8).

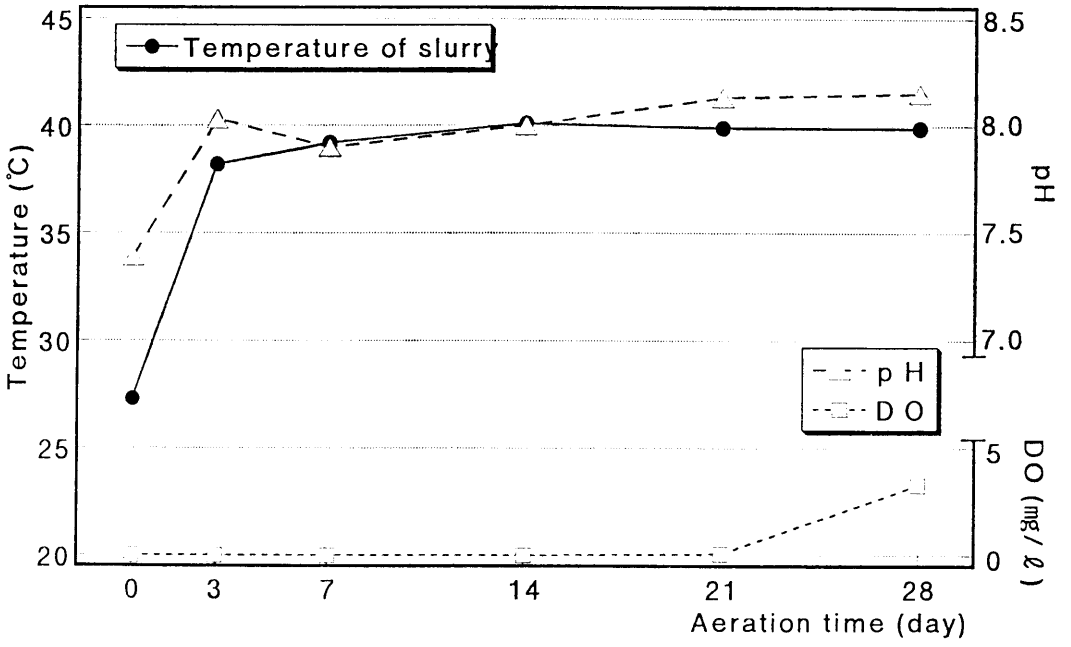

Fig. 6. Change in temperature, pH and DO of slurry during aerational treatment.

Table 3. Amounts of VFA and non-VFA in slurry

Samples Aeration time (day)	VFA (mM/dℓ)				non-VFA (mM/dℓ)		
	Ace.	Pro.	*n*-Buty.	*iso*-Buty.	Fum.	Suc.	Lac.
0	1.937	0.518	0.528	0.072	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND
7	0.307	0.091	ND	ND	ND	ND	ND
14	ND	ND	ND	ND	ND	ND	ND
21	ND	ND	ND	ND	ND	ND	ND
28	ND	ND	ND	ND	ND	ND	ND

ND: not detected.

Discussion

The aerational treatment in this study was performed by economical operation in the dairy farm with continuous aeration for 3 days in the beginning and followed by intermittent one. During the 3 days of continuous aeration, it was recognized that BOD_5, TS and IL decreased conspicuously, temperature and pH rose and VFA was not detected. In this time, the number of aerobic mesophilic bacteria and Enterobacteriaceae increased with remarkable degradation of organic matters. It is presumed that these bacteria are important for liquid composting in the initial period. On the other hand, it was observed that anaerobic mesophilic bacteria increased, VFA were detected and pH fell in the intermittent aeration from 4th to the 7th day. From the results, it was suggested that the whole

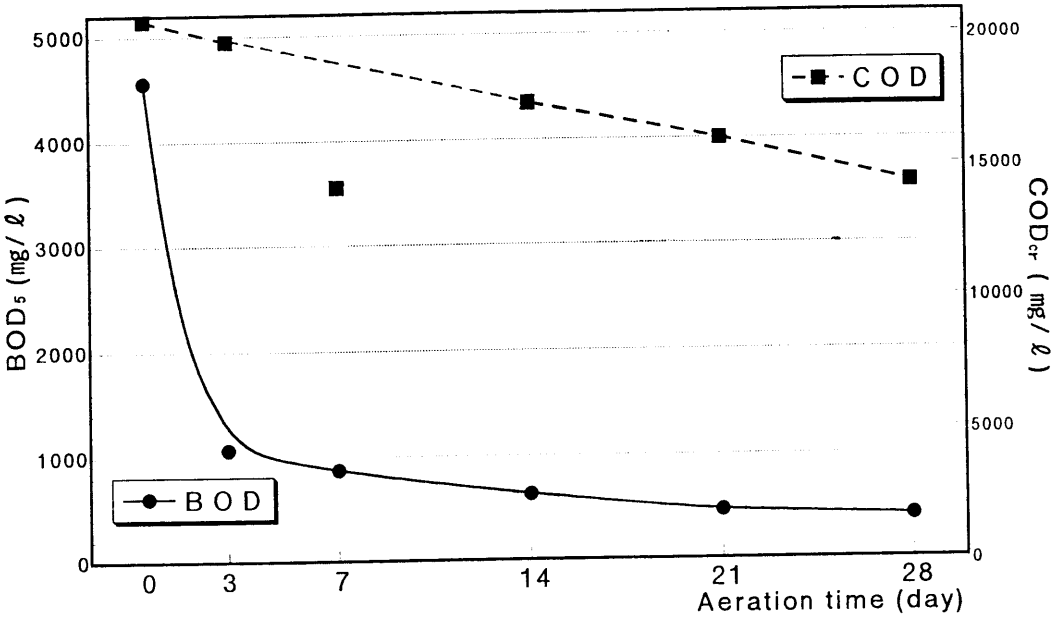

Fig. 7. Change in BOD_5 and COD_{cr} of slurry during aerational treatment.

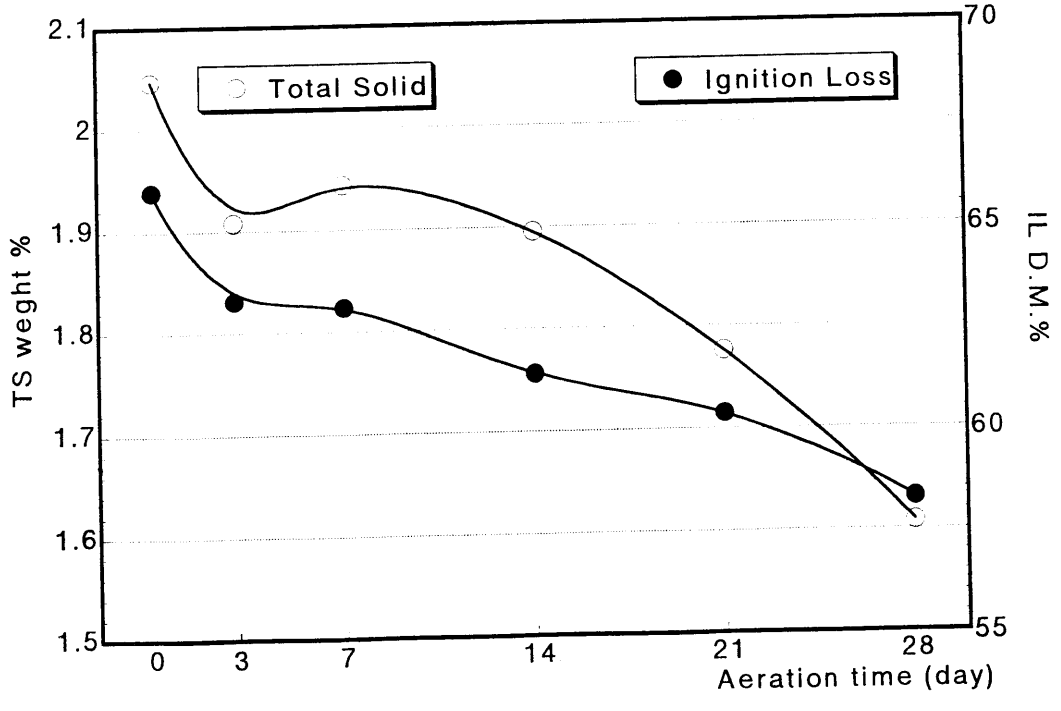

Fig. 8. Change in TS and IL of slurry during aerational treatment.

slurry tended to become anaerobically. However, no VFA was subsequently detected, pH rose, and BOD_5, CODcr, TS and IL were decreased, in spite of similar operation.

Some researchers reported that the growth inhibition of coliforms by rising the temperature of composting[6),9)]. Inversely, there are also several reports mentioned that coliforms decrease temporarily but can not excluded completely[3),18)]. The result in the present study is consistent with latter. Further research is needed to clarify whether or not existence of coliforms is necessary on composting process.

On solids compost, it is well known that the number of bacteria initially increases, thereafter, following actinomycetes, molds finally increased[10),22)]. In the present study, mesophilic actinomycetes exhibited a tendency to increase after the increase of aerobic mesophilic bacteria. This phenomenon is partially in accordance with reports of solids compost hitherto[6)]. It is necessary to determine whether or not the number of filamentous fungal spores increase in number, thereafter.

During this experimental period, the most superior microorganism was anaerobic mesophilic bacteria, in particular obligate anaerobic bacteria, regardless of aerational treatment. Therefore, further work is also needed to investigate on aeration condition.

Acknowledgment

This work was supported in part by Doboku Kanri, Co., Sapporo.

References

1) Acea, M. J. and T. Carballas, 1983. Characteristics of the microbial population of a cattle slurry. An. Edafol. Agrobiol., **42**: 149-159. (in Spanish).

2) Boutin, P., M. Torre, R. Serceau and P. J. Rideau, 1988. Atmospheric bacterial contamination from landspreading of animal wastes: Evaluation of the respiratory risk for people nearby. J. Agric. Engng. Res., **39**: 149-160.

3) Carroll, E. J. and D. E. Jasper, 1978. Distribution of Enterobacteriaceae in recycled manure bedding on California dairies. J. Dairy Sci, **61**: 1498-1508.

4) Crauer, L. S. and B. Hoffman., 1974. Technical aspects of liquid composting. J. Milk Food Technol., **37**: 293-301.

5) Cumby, T. R., 1987. A review of slurry aeration, 1. Factors affecting oxygen transfer. 2. Mixing and foam control. 3. Performance of aerators. J. Agric. Engng. Res., **36**: 141-215.

6) Finstein, M. S. and M. L. Morris., 1976. Microbiology of municipal solid waste composting. Adv. Appl. Microbiol., **19**: 113-151.

7) Forshell, L. P., 1994. Composting of cattle and pig manure. J. Vet. Med. B. **40**: 634-640.

8) Godden, B., M. J. Penninckx and C. Castill, 1986. On the use of biological and chemical indexes for determining agricultural compost maturity: extension to the field scale. Agric. Wastes, **15**: 169-178.

9) Golueke, C. G., 1972. A study of the process and its principles. In Composting., pp. 65-68, Rodale Press, PA.

10) Goueke, C. G., 1976. In Biological reclamation of solid waste. pp. 8-17, Rodale Press, PA.

11) Grant, F. A., 1975. Liquid composting of dairy manure. Managing livestock wastes, pp. 497-506.

12) Haga, K. and Y. Harada, 1983. Maturity of cattle waste compost: Characterization by biochemical oxygen demand. Proc. 5th World Conferece on Animal Production. 2, pp. 831–832.

13) Harada, Y. and K. Hara, 1983. Maturity of cattlewaste compost: Decomposition of organic matter during the composting. Proc. 5th World Conference on Animal Production. 2, pp. 833–834.

14) Harada, Y., 1985. Composting of animal wastes. Proceedings of the Symposium of the Composting of Organic Wastes (in Japanese). pp. 9–15.

15) Harada, Y., 1990. Composting and application of animal wastes. FFTC Extension Bulletin., **311**: 20–31.

16) JIS Handbook, 1981. Testing Methods for Industrial Waste Water., JIS K 0102., Nippon Kikaku Kyokai.

17) Kaneko, H., Y. J. Bae and K. Fujita, 1993. Profile of microbial numbers and bacterial growth activity in composting process. J. Jpn. Soc. Waste Management Experts, **4**: 35–41.

18) Mote, C. R., B. L. Emerton, J. S. Allison, H. H. Dowlen and S. P. Oliver, 1988. Survival of coliform bacteria in static compost piles of dairy waste solids intended for freestall bedding. J. Dairy Sci., **71**: 1676–1681.

19) Nodar, R., M. J. Acea and T. Carballas, 1992. Poultry slurry microbial population: composition and evolution during storage. Bioresource Technolo., **40**: 29–34.

20) Saiki, T., T. Beppu, T. Izawa, K. Morimoto, Y. Misaizu, and S. Higaki, 1978. Changes in microbial flora, including thermophiles, during composting of animal manure. In Biochemistry of Thermophily. (S. M. Friedman, ed.), pp. 103–115, Acad. Press, New York.

21) Suto, T., 1973. In Ushi no Rinshokensaho, (R. Nakamura, T. Yonemura and T. Suto eds.) pp. 6, Nousangyoson Bunkakyokkai, Tokyo.

22) Poincelot, R. P., 1975. The biochemistry and methodology of comosting. Conn. Agric. Exp. Sta. (New Haven) Bulletin, **754**: 1–18.

23) Ueki, A., E. Miyagawa, H. Minato, R. Azuma and T. Suto, 1978. Enumalatioa and isolation on anaerobic bacteria in sewage digester fluids. J. Gen. Appl. Microbiol., **24**: 317 332.