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Effects of Temperature and Aeration on Microflora during Liquid Composting of Dairy Cattle Slurry

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Summary

This experiment was conducted to determine the effects of temperature and aeration on the degradation of organic matter in the liquid composting of dairy cattle slurry. In comparison with aerational treatment at 40℃, static treatment at the same temperature showed no degradation of BOD_5, although it did show an accumulation of volatile fatty acids. The existence of coliforms was not to be found, even during static treatment. Under aeration, treatment at 40℃ was found to significantly decrease BOD_5 in slurry ($p<0.05$). Coliforms were regularly detected in aerational treatment at 20℃ over a period of 5 days. However, this was less than that detected for such treatment at above 30℃.

Introduction

Recently, environmental pollution has become a social problem, and it is said that the very existence of the livestock industry in its current form is in doubt as offensive odor and water-pollution are by-products of its operation. In practical terms, it has become increasingly difficult to resolve such problems because of the increased excrement produced by the intensive management and high level of moisture content characteristic of free-stall bedding found on dairy-farms throughout Japan. The desirability of composting and the importance of the role of microorganisms in the treatment of livestock excrement is well known. Indeed, a great number of engineering studies on liquid composting have been reported[2-4]. However, as yet, only a few microbiological investigations are available[1),8),9)]. This study aims at increasing the body of fundamental knowledge on liquid composting, especially as regards the effects of temperature on the degradation of BOD_5 and time-interval related changes in the microbial population.

Materials and Methods

Samples and treatment conditions

Fresh excrement from steers fed on roughage was collected, and the fresh feces, urine and distilled-water mixed to a ratio of 2:1:2 (vol). These fluids, filtrated using a poly-ethylene

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net (pore-size: 3mm), were used as the samples in our experiment. To obtain liquid composting, the fluids (1000mℓ) were put in a Sakaguchi-flask (2000mℓ), and surface aeration was carried out using a shaking-incubator (120 r.p.m.). To avoid excessive build up of carbon dioxide during incubation, small amounts of air were aseptically supplied. Static and aerational treatment were performed at 40℃. Furthermore, to elucidate the effect of temperature on aerational treatment, it was performed at temperatures of 20℃, 30℃, 40℃ and 60℃, respectively. Each treatment was performed over a period of 5 days, and the samples collected prior to, and on days 1, 3 and 5 of the treatment period. Incidentally, each treatment in this experiment was carried out using fluids adjusted on different days.

Slurry properties analysis

Dissolved oxygen concentration (DO), pH and oxidation-reduction potential (ORP) were measured using electrodes (TOA Electronics Ltd.). Amounts of ammoniac nitrogen (NH_4-N) were measured using the semimicro Kjeldahl distillation method. Volatile fatty acids (VFA) were analyzed with a gas chromatograph equipped with a flame ionization detector using N_2 as the carrier gas. One $\mu\ell$ of deproteinized sample containing 24% meta-phosphoric acid in 5N-H_2SO_4 was injected into the gas chromatogragh[10]. Biological oxygen demand (BOD_5) was assayed according to the Japanese Industrial Standard (JIS) K 0102[6].

Microbial analysis

For microbiological assay, after mixing the samples, a series of ten-fold dilutions to 10^{-8} for each specimen was prepared using a sterilized saline solution for aerobes. From the appropriate dilutions, 0.1mℓ aliquots were spread onto agar plates: trypticase soy (TS) agar for the total number of bacteria, deoxycholate hydrogen sulfide lactose (DHL) agar for the *Enterobacteriaceae*, humic-acid vitamin (HV) agar for the actinomycetes, potato dextrose (PD) agar with chloramphenicol for the molds and yeasts. Then each dilution of sample was heated at 80℃ for 15 min, and 0.1mℓ of each dilution spread onto nutrient agar with added 0.5% glucose for thermo-stable bacteria. The plates for the total number of bacteria were incubated at both 30℃ and the temperature of each aerational treatment; on the other hand, the other plates were incubated at 37℃ for *Enterobacteriaceae*, at 28℃ for fungi, at 30℃ and for both actinomycetes and thermo-stable bacteria, respectively. All were performed in three replicate plates and counted. Numbers of microorganisms were expressed as the median of colonyforming units (CFU). The identification of microbes was performed by colonial and cellular morphologies, Gram-reaction, spore formation and characteristic growth, etc[5].

Results and Discussion

Comparison with static incubation

Experiments on still standing were performed at 40℃ for comparison with aerational treatment. Changes in the slurry properties are shown in Tables 1,2,3,4. With still standing, pH rose as time progressed, but did not attain 9.0. DO indicated zero. ORP remained in a considerably reductive condition. A remarkable accumulation of NH_4-N concentration was, however, recognized. Changes in VFA are shown in Table 5. With aerational treatment, propionic, *n*-butyric and *iso*-varelic acids were not detected throughout the 5 days treatment period. However, with still standing, an accumulation of VFAs, especially acetic and *iso*-varelic acids, was recognized. No decrease in BOD_5 was recognized with still standing (Fig.

Table 1 pH of slurry at 40℃

	days			
	0	1	3	5
Aeration	7.47	8.51	9.07	9.18
Still Standing	7.75	8.43	8.67	8.68

Table 2 DO of slurry at 40℃

	(mg/ℓ)			
	0	1	3	5
Aeration	0.00	0.04	1.27	3.22
Still standing	0.00	0.00	0.00	0.00

Table 3 ORP of slurry at 40℃

	(mV)			
	0	1	3	5
Aeration	-41	-46	82	258
Still standing	-24	255	-236	-257

Table 4 NH_4-N of slurry at 40℃

	(mg/ℓ)			
	0	1	3	5
Aeration	423	775	1151	891
Still standing	258	2516	2036	2059

Table 5 Amounts of VFA in slurry at 40℃

Sample	Days	VFA (mM/dℓ) Ace.	Pro.	*n*-Buty.	*iso*-Val.
Aeration	0	1.693	0.420	0.115	0.019
	1	0.530	nd	nd	0.010
	3	0.369	nd	nd	nd
	5	0.317	nd	nd	nd
Still standing	0	1.462	0.271	0.096	0.002
	1	3.720	0.230	0.134	0.106
	3	3.744	0.305	0.122	0.173
	5	3.182	0.264	0.089	0.166

nd: not detected

1). Changes in microbes are shown in Fig.2. With still standing, the total number of bacteria increased. Compared to aerational treatment, coliforms were still below the detection threshold on day 1, and the pink colonies were still below the detection threshold after 5 days of still standing. No evident changes were observed in the actinomycetes. The numbers of fungi showed similar tendencies to each other.

From these results, it was found that volatile fatty acids accumulated causing offensive odor, but that no decrease in BOD_5 was recognized with non-aerational treatment. Aeration was found to be effective in the liquid composting of cattle slurry.

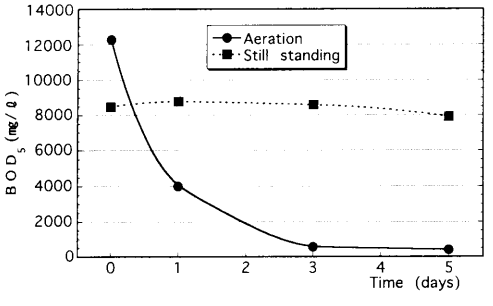

Fig. 1 Changes in BOD_5 at 40℃

Effect of temperature on liquid composting

Changes in DO concentration, ORP, pH and NH_4-N are shown in Fig.3. In all the treatments, the DO concentration rose to 3 ~4mg/ℓ, and the ORP changed from a reductive condition to 250~300mV. Each NH_4-N concentration, 300~400mg/ℓ at the beginning, increased rapidly. However, it seemed that the falling-off over 1 to 3 days during the 60℃-treatment depended on the

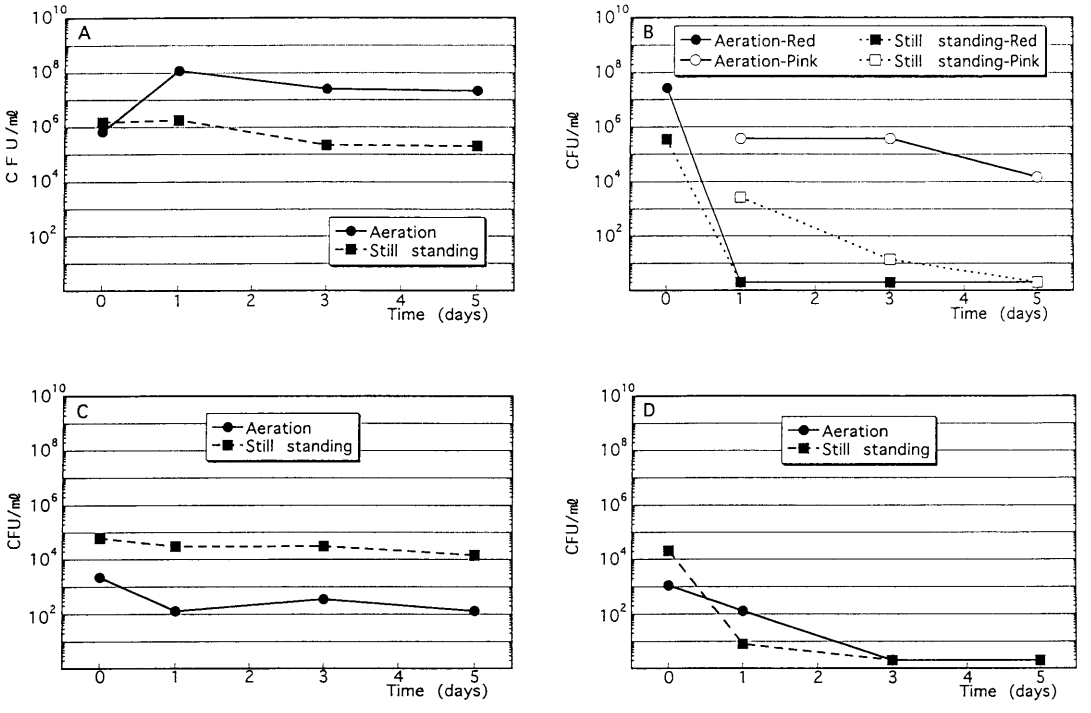

Fig. 2 Changes in microorganisms in slurry at 40℃
A. total number of bacteria B. bacteria on DHL medium C. actinomycetes D. fungi

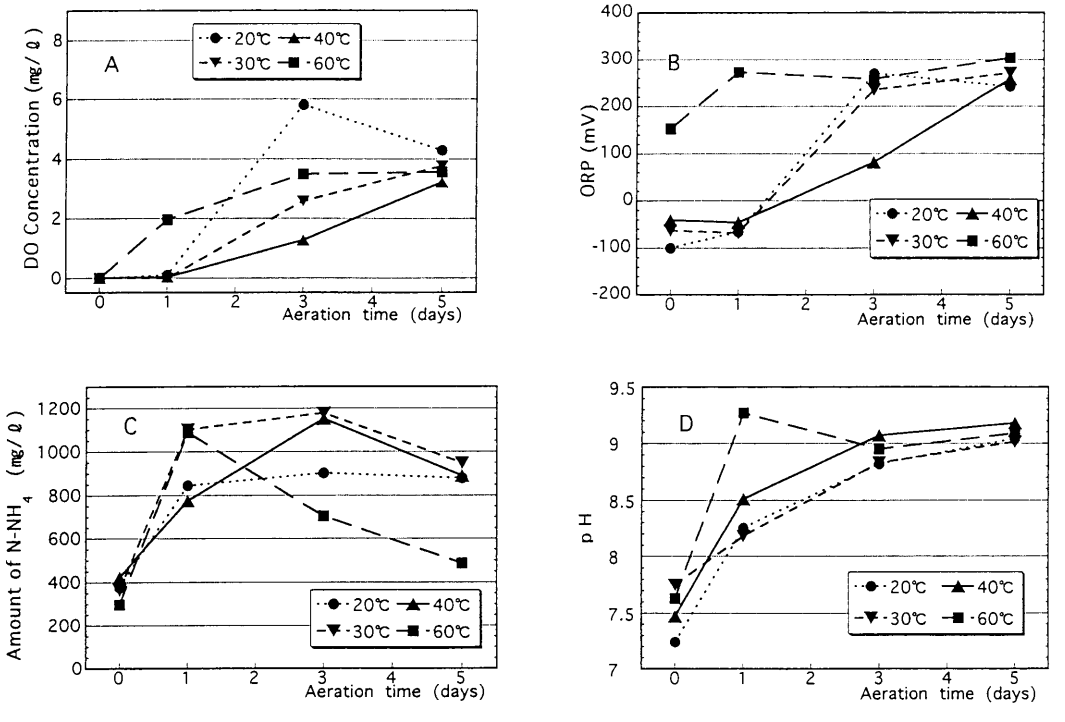

Fig. 3 Changes in properties of slurry
A. DO B. ORP C. NH4-N D. pH

effects of vaporization caused by thermal temperature, in addition to the effect of rising pH. pH over 9.0 was found in all the treatments. Changes in BOD_5 are shown in Fig.4. A decrease in BOD_5 was recognized in all the treatments. However, because it was not clear as to how the difference in BOD_5 changed with the effect of treatment temperature, the data were treated with an inclination conversing curvilinear regression (students-t test by Microsoft Excel). As a result, the 40℃-treatment was found to decrease BOD_5 in slurry most significantly ($p<0.05$).

As to changes in the number of bacteria, the number of bacteria on DHL medium, actinomycetes and fungi are shown in Fig.5. In each treatment, except the 60℃-treatment, the total bacteria increased from 10^6 CFU/mℓ to 10^8 CFU/mℓ with aeration (Fig.5 A). However, the total number of bacteria gradually decreased with the 40℃-treatment. The total number of bacteria incubated at 60℃ with the 60℃-treament increased. No evident changes were observed in the thermo-stable bacteria. Changes in the total number of bacteria with the 60℃-treatment incubated at 30℃ were nearly in accordance with the changes in the thermo-stable bacteria from the beginning of treatment. Changes in viable count classified by characteristic colony on DHL medium are shown in Fig.5 B. Mote *et al*[7] reported how to detect *Escherichia coli* using MacConkey agar. The red colonies, identified as fecal coli (i.e. *Escherichia coli*) by the coliforms-test, existed at 10^5/mℓ in the initial samples. The fecal coli

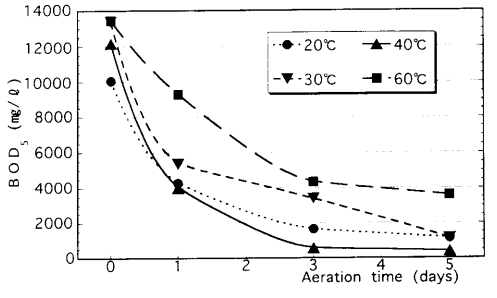

Fig. 4 Changes in BOD_5

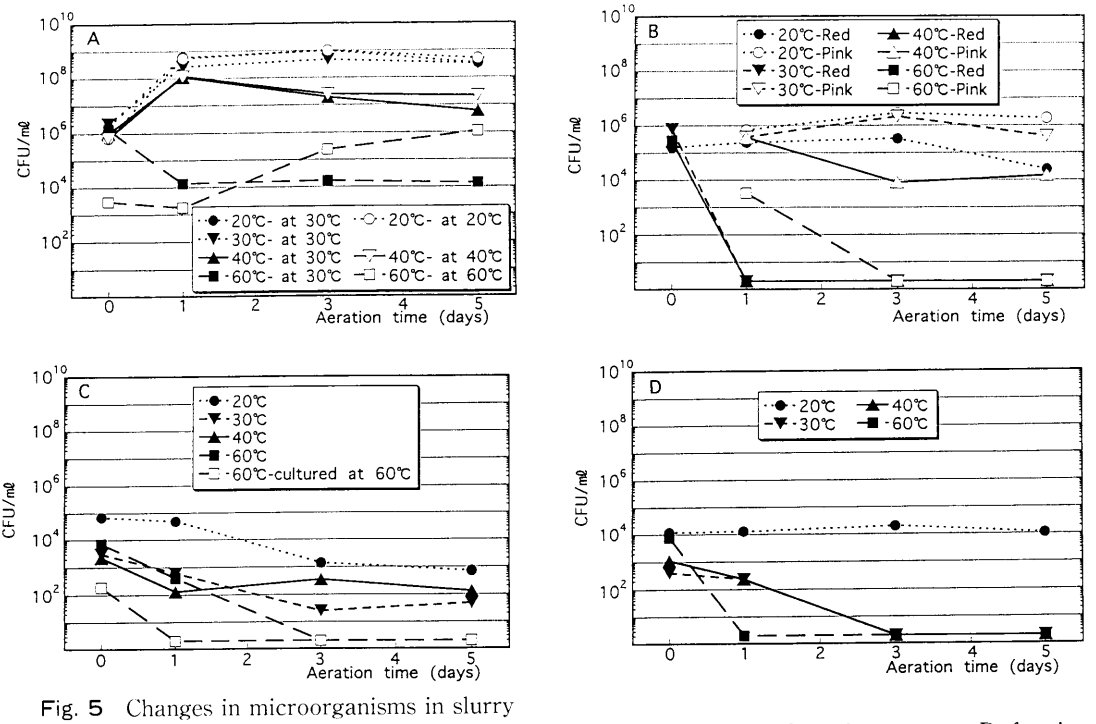

Fig. 5 Changes in microorganisms in slurry
A. total number of bacteria B. bacteria on DHL medium C. actinomycetes D. fungi

fell below the detection threshold during aeration, except at 20℃-treatment. The pink colonies were detected on day 1 after treatment, and were still in existence with the 20℃, 30℃ and 40℃-treatment over 5 days. From these results, it was clear that the pink colonies on the DHL agar were not *Enterobacteriaceae*. The pink colonies were identified as *Moraxella osloensis* or *Oligella urethralis* (Table 6). The actinomycetes tended to decrease, but did not fall below the detection threshold, except with the 60℃-treatment at the end of the aeration period. Okamoto *et al*[9] reported that actinomycetes increased through aerational treatment over the long term in dairy farming. However, in this experiment, no increase in actinomycetes was observed over a 5 day period. It is suggested, therefore, that thermo actinomycetes did not exist in the fresh excrement. The fungi fell below the detection threshold at the end of the treatment period, except with the 20℃-treatment.

It may not be appopriate to define fecal coli as an indication of hygiene. However, if fecal coli are indeed an indication of hygiene in the environment, then liquid compost that does not reach 30℃ would appear to be unsafe.

Acknowledgments

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Table 6 physiological and biochemical characteristics of bacteria showing the pink colony on DHL medium isolated from slurry

Characteristics	Number of Strain	
	20	10
Gram reaction	—	—
Cell shape	rod	rod
Motility	—	—
Growth anaerobically	—	—
Of test	NC	NC
Oxidase	+	+
Catalase	+	+
Urease	—	—
Utilization of:		
Glucose	—	—
Arabinose	—	—
Cellobiose	—	—
Lactose	—	—
Raffinose	—	—
Citrate	—	—
NO_3 reduced to NO_2	—	—
NO_2 reduced to NH_4	+	—
ONPG	—	—
Production of Indole	—	—
Hydrolysis of Tween80	—	—
Growth on MacConkey	+	+
	Oligella urethralis	*Moraxella osloensis*

NC: no changed

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要　　約

乳牛糞尿の液状コンポスト化について，有機物減少に及ぼす曝気処理温度の影響を検討した。40℃での曝気と静置処理を比較したところ，静置したものは，BOD_5 の減少が認められず，揮発性低級脂肪酸の蓄積が顕著であった。曝気および静置処理ともに糞便系大腸菌の存在は処理開始以降は認められなかった。曝気処理温度を代えて各々の変化を比較したところ，40℃での曝気処理が有意に BOD_5 を減少させた（$p<0.05$）。糞便系大腸菌は，20℃処理では曝気5日間存在していたが，30℃以上の曝気では処理開始以降検出限界以下であった。