ウマ糞を用いた堆肥化とその促進要因

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論文要約

家畜が産生する悪臭は公衆衛生上の 1 つの課題である。本研究では，ウマ糞堆肥化における副資材と堆肥化温度の影響について，化学組成と微生物の多様性の観点から検証した。有機酸分析の結果，堆肥化前では 4 種類の有機酸（乳酸，酢酸，プロピオン酸，n-酪酸）が検出されたが，堆肥化後，とくに 30℃で堆肥化を行った後では，それらの有機酸は殆ど検出されなかった。炭素/窒素比はもみ殻を用いて 30℃での堆肥化で減少した。細菌叢を T-RFLP 法で解析した結果から，堆肥化前後で大きく変化していることが判明し，とくに 30℃で堆肥化すると *Lactobacillus* 属の割合が増加し，もみ殻では *Bifidobacterium* 属も検出された。以上より，堆肥化の副資材にはもみ殻を用い，温度は 30℃で腐熟させるのが適切と考えられた。これらの知見は堆肥の腐熟にとって重要であり，公衆衛生の課題を解決するために有用と思われる。

キーワード：ウマ糞、悪臭、有機酸、菌叢、堆肥の腐熟

Compost Manufacturing by Use of Horse Droppings and its Effective Factors

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ABSTRACT

Odor originating from livestock is one of the issues related to public health. In this study, we examined the chemical compositions and microbial variations of composting with horse droppings. In the result of the organic acid analysis, four kinds of organic acids (lactic acid, acetic acid, propionic acid, and n-butyric acid) were detected before composting ; on the other hand, they were rarely detected, especially after composting at 30º C. The ratios of carbon and nitrogen decreased on composting with rice husks at 30º C. In the results of T-RFLP analysis, it was demonstrated that bacterial flora changed dramatically by comparison before and after composting, in particular, the ratio of *Lactobacillus* genus increased after composting at 30º C and *Bifidobacterium* genus was detected from the rice husks group. Based on these results, the appropriate condition to prepare was considered to be 30º C using rice husks. It was considered that these findings were important to mature the compost and might be useful to resolve public hygiene problems.

Keywords: *horse dung, odor, organic acids, microflora, compost maturity*

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1. Introduction

It has been well known that carbohydrates are converted to methane and carbon dioxide by methane fermentation and these are released into the air. It is urgent for global warming to reduce them because they are mainly greenhouse gases[1]. On nitrogen metabolism, it is also found that ammonia generated from organic nitrogen is converted to nitric acid by the nitrification of nitrifying bacteria and ammonia is not only a kind of offensive odor but also a derivate of N_2O from nitric acid by denitrification[2]. N_2O has a similar effect on global warming and occupies approximately 6.2% of greenhouse gases[1]. Control of compost is a global problem and must be addressed over the long term.

In addition, odor originating from livestock farming is a kind of serious social problem and it is necessary to develop innovative technologies for reduction. In Japan, the Offensive Odor Prevention Law has been enforced since 1971, and the Act on the Appropriate Treatment and Promotion of Utilization of Livestock Manure has been established since 1999. At present, it accounts for approximately ten percent of all of the complaints of odor[3]. If it may be limited to complaints from livestock, offensive odor is approximately 60 percent of the complaints[4]. Odor is changed according to the excrement condition, *i.e.*, fresh excrement causes strong odor due to including lower fatty acid as a main ingredient[4]. In the composting process, it is well known that the high temperature phase is very important for odor production. At the same time, ammonia and sulfur compounds generate at high levels initially during the high temperature phase[4]. It is found that the fatty acid level was lower when ammonia and sulfur compounds became higher[4]. The ratio of carbon and nitrogen is very important for the compost and the reduction of the carbon rate is one of the determination indexes of the compost decay degree[5]. Furthermore, odor generation repeats increases and decreases by cutbacks[5-6]. The development of effective compost manufacturing requires odor control measures as soon as possible.

There are many reports on the compost of bovine, swine, and porcine[7-14]. Especially, the relation with cellulase activity, cellulose degradation, and the ß-glucosidase activity of microbes in the compost made from cattle manure and rice straw, was reported on the point of the global carbon cycle in recent years[15]. On the other hand, commercial compost from horse droppings is rare and there are almost no reports with scientific verification on the characteristics of its compost. In this study, we tried to make compost using horse droppings and verified the effect of mature relating with secondary materials, environmental conditions, and bacterial flora. We suggested creating high quality compost from the perspective of multiple viewpoints.

2. Materials and Methods

2.1 Sample

Horse droppings excreted by four houses native to Hokkaido were provided from the POLO horse riding club (Hachinohe, Aomori prefecture, Japan).

2.2 Making the compost

Horse droppings (wet weight 76%) and rice husks (dried to 0% of water contents after autoclaving (121ºC, 15 min) or rice charcoal were mixed and prepared to 55% water contents. The prepared droppings were divided into two groups and made into compost for 60 days in the outdoors or in the incubator at 30º C. During the preparation of the compost the outside temperature, relative humidity, oxygen density and inside temperature of samples were measured every day and were turned over three times a week.

2.3 Measurement of viable bacterial cell numbers

Samples were collected 1 g each from the composts every seven days and suspended in 9 ml of sterilized 0.85% saline. After serial diluting to 10^3 - 10^6, 100 µl of each dilution was smeared on a standard plate count agar (Eiken Chemical Co., Ltd., Tokyo, Japan) and cultured aerobically at 37º C for 24 hours. After incubation, the colony forming units per 1 g of the compost were measured.

2.4 Measurement of carbon/nitrogen contents and statistical treatment

The droppings before and after composting were dried at 50º C for 24 hours and crushed with mil. Materials which had been passed through a sieve were used for the measurement of the carbon and nitrogen contents. The total carbon and nitrogen in the organic compounds were measured by the Turin and Kjeldahl methods, respectively. The ratios of carbon and nitrogen (C/N) were calculated from each five measurements. Statistical analyses were conducted by the Kruskal-Wallis test and multiple comparisons by

the Steel-Dwass test. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria)[16]. This measurement depended on the Aomori Industry Technology Center (Noheji, Aomori pref., Japan).

2.5 Analysis of organic acids

The organic acids in the composts were analyzed by HPLC. In this analysis, nine kinds of organic acids (succinic acid, lactic acid, acetic acid, propionic acid, formic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, and n-valeric acid) were monitored. The lower detection limit of the first four kinds was set at 0.05 mg/g, whereas for the later five kinds it was set at 0.1 mg/g. The detection conditions were indicated as follows. This measurement depended on the TechnoSuruga Laboratory Co., Ltd. (Shimizu, Shizuoka pref., Japan).

System: Shimadzu organic acid analysis system
Column: Shim-pack SCR-102(H) 300 mm x 8 mm, Connected two columns in series
Eluent: 5 mmol/L p-toluenesulfonic acid
Reaction solution: 5 mmol/L p-toluenesulfonic acid, 100 μmol/L EDTA, 20 mmol/L Bis-Tris
Flow rate: 0.8 ml/min
Temperature of column oven: 45° C
Detection: Electrical conductivity detector CDD- 10A

2.6 Analysis of bacterial flora in the compost

Horse droppings before and after composting were collected, approximately 1 g aseptically, and offered to the T-RFLP for analyzing bacterial flora. This measurement also depended on the TechnoSuruga Laboratory Co., Ltd. DNA extraction, PCR condition, restriction enzyme treatment, fragment analysis, and cluster analysis are each described below.

DNA extraction: ISOL for Beads Beating kit (Nippon Gene, Japan)
PCR primer: 516F(6-FAM)-1510R (All the microbial species belonging to eubacteria 16S rDNA)
PCR condition: Nagashima *et al.*[17, 18]
Restriction enzyme: Fast Digest *Bsl* I (Thermo Fisher Scientific, USA)
Restriction enzyme treatment: 37° C, 10 min
Fragment analysis: ABI PRISM 3130 xl Genetic Analyzer System (Applied Biosystems, USA)
Cluster analysis software: Gene Maths (Applied Maths, Belgium)
Clustering method: UPGMA
Distance function: Pearson's correlation coefficient

3. Results and Discussion

3.1 Transition of environmental conditions during the manufacturing of the compost

In order to examine the effects of external environmental factors, the inside and outside temperatures of compost, relative humidity, and oxygen concentration were measured (Fig. 1-3). In the outdoors, the outside temperature passed within 4-22°C, the relative humidity was within 55-90%, and

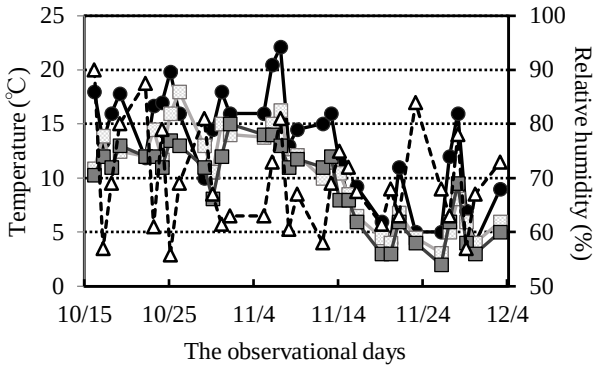

Fig. 1 The process of environmental conditions during the manufacturing of compost outdoors. Outside temperature (●) and relative humidity (△) were monitored during a compost preparing period (from 17th Oct 2017 to 5th Dec 2017). The inside temperatures of the compost were also checked in the different kinds of secondary materials (rice husks (□) and rice charcoal (■)).

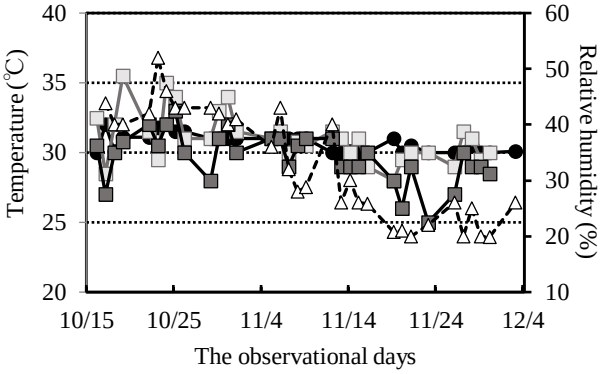

Fig. 2 The process of environmental conditions during the manufacturing of compost in an incubator at 30° C. Outside temperature (●), relative humidity (△), and inside temperature with the secondary materials (rice husks (□) and rice charcoal (■)) were monitored in the same period as Figure 1.

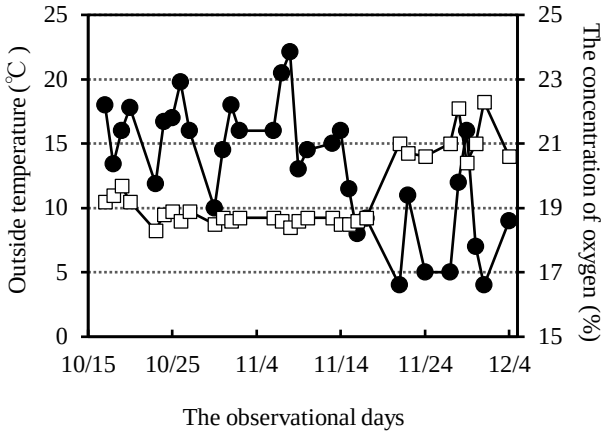

Fig. 3 The processes of outside temperature and oxygen concentration during the manufacturing of compost outdoors. The outside temperature (●) and the concentration of oxygen in the air (□) were indicated in the process during the compost manufacturing.

the oxygen concentration was within 18.3- 22.3%. On the other hand, using the incubator at 30º C, the outside temperature was almost constant and the relative humidity was lower compared to the outdoors compost. It has been well known that the high temperature phase of fermentation is very important for making compost and affects its quality[3-4]. In this study, the high temperature phase wasn't recognized during manufacturing; therefore, microflora in the compost might not have been affected by the high temperatures. More viable cells were found in the 30ºC compost than in the outdoors compost through a given period (Table 1). The difference of in secondary materials (rice husks and rice charcoal) was also compared. Both the outdoors and incubator (30º C) groups, inside temperatures of the rice husks were higher than the rice charcoal. Viable bacterial numbers in the rice husks had a tendency to be larger than in the rice charcoal (Table 1). It was considered that the state of carbon (organic or inorganic) affected the manure.

Based on these results, it was suggested that manufacturing with rice husks at 30º C was a better condition.

3.2 Effect of the C/N ratio by composting

The carbon and nitrogen contents before and after composting were measured and the ratio of carbon and nitrogen (C/N) was calculated on these data (Table 2). The results showed a tendency to decrease at 30º C, it was remarkable on the group with rice husks. On the other hand, the C/N ratio increased in the outdoors groups and this was particularly notable in the group of rice charcoal. It has been well known that the reduction of the C/N ratio indicates the compost decay degree[4] and the C/N ratio of domestic animals are usually about 15, and 25 or less is classified as rank A according to the standard of Ministry of Agriculture, Forestry and Fishery in Japan[4]. Based on these

Table 1 Viable bacterial cell numbers in the composts (CFU/ml)

Date	Control	Incubator (30 ºC)		Outdoors	
		Rice husks	Rice charcoal	Rice husks	Rice charcoal
10/17	$1.10 \pm 0.27 \times 10^5$	-	-	-	-
10/25		*	*	$1.63 \pm 0.13 \times 10^7$	$2.18 \pm 0.15 \times 10^7$
10/31		$3.42 \pm 0.37 \times 10^8$	$2.91 \pm 0.53 \times 10^8$	$4.8 \pm 0.8 \times 10^7$	$1.54 \pm 0.56 \times 10^7$
11/9		$5.2 \pm 1.4 \times 10^8$	$2.2 \pm 0.6 \times 10^8$	$1.95 \pm 0.25 \times 10^7$	$1.19 \pm 0.21 \times 10^7$
11/15		$1.40 \pm 0.31 \times 10^8$	$1.32 \pm 0.20 \times 10^8$	$2.65 \pm 0.38 \times 10^7$	$3.99 \pm 0.36 \times 10^7$
11/22		$2.85 \pm 0.74 \times 10^8$	$1.18 \pm 0.41 \times 10^8$	$3.9 \pm 6.4 \times 10^7$	N.D.
11/29		$3.3 \pm 1.4 \times 10^7$	$1.08 \pm 0.21 \times 10^8$	$9.3 \pm 5.2 \times 10^6$	$9.4 \pm 2.7 \times 10^6$

Measured via three plates. CFU: colony forming unit, -: Not applicable,
*: Not countable by a lot of colonies, N.D.: Not detected at 10^5 diluted solution

Table 2 Influence on the C/N ratio of secondary materials and environmental condition (%)

Secondary materials	Before composting	After composting	
		30º C	Outdoors
Rice husks	26.0 ± 2.0^{a}	22.0 ± 0.6^{b}	26.5 ± 0.5^{a}
Rice charcoal	19.5 ± 1.4^{A}	18.7 ± 0.5^{A}	23.0 ± 0.8^{B}

Table 3 The ratio of bacterial genus before and after composting via T-RFLP analysis (%)

Estimated bacteria	Before composting	After composting			
		Incubator (30 ºC)		Outdoors	
		Rice husk	Rice charcoal	Rice husk	Rice charcoal
Bifidobacterium	0.0	0.8	0.0	0.0	0.0
Lactobacillales	1.7	50.2	48.5	8.0	7.9
Bacteroides	34.3	39.7	33.4	58.8	54.2
Prevotella	0.0	0.0	0.0	0.0	0.0
Clostridium cluster IV	39.5	0.0	0.0	2.4	0.0
Clostridium subcluster XIVa	19.8	3.0	5.3	9.4	8.6
Clostridium cluster IV	1.3	3.6	9.2	0.8	0.9
Clostridium cluster IV	0.0	0.0	0.0	18.4	22.1
Clostridium cluster IV	1.1	0.0	0.0	0.0	0.0

findings, the result that manufacturing with rice husks at 30º C was the best condition in this study.

3.3 Change of microflora before and after composting and the relation with organic acid

For evaluating bacterial flora, comparing before and after composting, T-RFLP analysis was performed (Table 3). The *Clostridium* cluster was classified phylogenetically according to the base sequence of 16S rDNA and included unfiled taxa and genus other than *Clostridium*[19]. This result showed that the composition ratio of bacteria flora before composting consisted of 39.5% *Clostridium* cluster IV, 19.8% *Clostridium* subcluster XIVa, 34.3% *Bacteroides* and 1.7% Lactobacillales. On the other hand, after composting, *Clostridium* cluster IV wasn't detected expect in the rice husks outdoors group, instead, *Clostridium* cluster XI and *Bacteroides* became higher in the outdoors groups, and *Clostridium* cluster IX became higher in the 30º C groups. Especially, the ratio of Lactobacillales remarkably increased after composting at 30º C and *Bifidobacterium* was also detected in the rice charcoal. Based on this result, it was found that microflora led to change via the composting and temperature and directly affected the compost quality.

Then, organic acid compositions were analyzed (Fig. 4). As a result, four kinds (lactic acid, acetic acid, propionic acid, and n-butyric acid) of organic acids were detected before composting, but three organic acids, excluding acetic acid were not detected after composting. This indicated that odor from horse droppings decreased or disappeared by manufacturing the compost. It was found that *Clostridium* IV and XIVa were able to produce butyric acid from lactic acid or acetic acid[19]. It was guessed that the inability to detect n-butyric acid was caused by the decrease of these clusters after composting. In this study, the ratio of Lactobacillales after composting was increased, especially at 30º C, however, lactic acid was not detected at all after composting. It was found that lactic acid was transformed into propionic acid or butyric acid by the genus *Bacteroides* and *Clostridium*[20]. It was considered that lactic acid was used completely by other bacteria. Furthermore, small amounts of acetic acid were detected after the outdoors composting. This might be related with the increase in the genus of *Bacteroides* and *Clostridium* cluster XI.

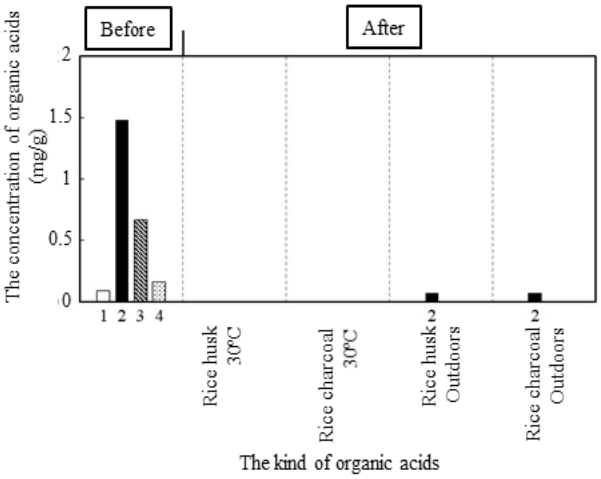

Fig. 4 Organic acid analysis before and after the composting. The organic acids in the composts were qualitatively and quantitatively analyzed by HPLC. In this figure, 1, 2, 3 and 4 are indicated as lactic acid, acetic acid, propionic acid, and n-butyric acid, respectively.

In this study, methane fermentation must also be considered to manufacture compost. It has been well known that acetic acid and propionic acid are main intermediate metabolites on methane fermentation, these are reduced into methane and/or carbon dioxide by methanation archaebacterium possessing substrate specificity[21]. In regards to the remaining acetic acid in the outdoors compost, it was not only due to the influence of intestinal bacteria but also the inactivation of the methanation archaebacteria, and as such needs to be investigated in detail.

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