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Comparison of organic matter degradation and microbial community during thermophilic composting of two different types of anaerobic sludge

Kiyohiko Nakasaki [a,*], Le Thi Hong Tran [a], Yoshito Idemoto [a], Michiharu Abe [a], Analiza Palenzuela Rollon [b]

[a] Department of Materials Science and Chemical Engineering, Shizuoka University, 3-5-1, Johoku, Naka-ku, Hamamatsu 432-8561, Japan
[b] Department of Chemical Engineering, University of the Philippines, Diliman, 1101 Quezon City, Philippines

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ABSTRACT

Changes in organic matter degradation and microbial communities during thermophilic composting were compared using two different types of anaerobic sludge, one from mesophilic methane fermentation, containing a high concentration of proteins (S-sludge), and the other from thermophilic methane fermentation, containing high concentrations of lipids and fibers (K-sludge). The difference in the organic matter degradation rate corresponded to the difference in the organic matter constituents; the CO_2 evolution rate was greater in the composting of S-sludge than of K-sludge; moreover, the NH_3 evolution resulting from the protein degradation was especially higher in the composting of S-sludge. Then the differences in the microbial communities that contributed to each composting were determined by the PCR-DGGE method. *Ureibacillus* sp., which is known as a degrader with high organic matter degradation activity, was observed during the composting of S-sludge, whereas *Thermobifida fusca*, which is a well known thermophilic actinomycete that produces enzymes for lignocellulose degradation, were observed during the composting of K-sludge.

1. Introduction

Anaerobic digestion has been used to treat various organic wastes including industrial wastewater, sewage sludge, agricultural wastes and the organic fraction of municipal solid waste (MSW). The residual sludge after anaerobic digestion, i.e., anaerobic sludge, can be treated further via composting in order to mineralize the remaining easily degradable portion of organics and to remove possible pathogens that may survive the anaerobic digestion process. After composting, the anaerobic sludge can be used on farmland as a high-quality hygienic fertilizer (Poggi-Varaldo et al., 1999).

In order to manage the composting process efficiently, the effects of various operational conditions have been investigated (e.g., Finstein and Morris, 1975; Golueke, 1977; de Bertoldi et al., 1983). Composting efficiency is strongly affected by oxygen supply, since the composting efficiency is directly linked to the composition and succession of the microbial community during the composting process. Sundberg and Jönsson (2008) demonstrated that increased aeration rate caused the higher microbial activity and increased pH at the biowaste composting and succeeded in shortening the time need to produce a suitable compost product. The most remarkable characteristic of the present study is that a drastic change of the microorganisms' environment, from anaerobic to aerobic conditions, occurred at the start of composting. Therefore, microbial succession from anaerobic to aerobic microorganisms was expected to occur as composting progresses. It is difficult, however, to ascertain this microbial succession by the traditional culture-dependent method of plating, since both aerobic and anaerobic incubations must be carried out simultaneously, which requires a great deal of time and labor.

PCR-DGGE, a culture-independent molecular biological method, has been used to analyze the genetic diversity of the complex microbial communities in natural environments. In principal, both aerobic and anaerobic microorganisms can be determined at the same time by the PCR-DGGE method. By using the PCR-DGGE method, many researchers have tried to analyze the microbial diversity of composting, and various valuable findings have been made (e.g., Ishii et al., 2000; Larsen and McCartney, 2000; Ishi and Takii, 2003; Takaku et al., 2006). However, to our knowledge there have been no studies of microbial succession during aerobic composting of anaerobic sludge. The purpose of the present study was to compare aerobic composting with two different types of anaerobic sludge in order to elucidate the changes in organic matter degradation and microbial community structure that occur with the progress of thermophilic composting as measured by the PCR-DGGE method.

2. Methods

2.1. Composting raw materials

Two types of residual sludge derived from two different anaerobic digestion processes were used for composting. One type was derived from the methane fermentation process used in S-city

* Corresponding author. Tel.: +81 53 478 1172; fax: +81 53 476 0095.
E-mail address: tcknaka@ipc.shizuoka.ac.jp (K. Nakasaki).

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under mesophilic conditions, where food wastes from the catering industry, i.e., cooking residues and leftovers from lunch boxes, were treated (S-sludge). The operation temperature and the solid retention time were 38 °C and 14 days, respectively. The other type was collected from a thermophilic anaerobic digester in K-city, where a mixture of 70% organic fraction of MSW and 30% garbage from restaurants was used (K-sludge). The anaerobic system was operated with high solids content at 55 °C. The solid retention time was adjusted to approximately 30 days. Some characteristics of the two different types of anaerobic sludge are shown in Table 1.

Two experimental runs, runs I and II, were carried out using S-sludge and K-sludge, respectively as the raw material for composting. The anaerobic sludge was mixed with sawdust as a bulking agent and an inoculum with the trade name Alles G (Matsumoto Laboratory of Microorganisms Co. Ltd., Matsumoto, Japan) in a ratio of 5:14:1 to create a raw composting mixture. At the start of all of the experiments, the pH level was adjusted to around 8.0 by the addition of slaked lime, and the moisture content was adjusted to 60% by the addition of distilled water.

2.2. Composting operation

The experimental system used in the present study was the same as that described in our previous paper (Nakasaki et al., 2004). A mini-reactor consisting of a cylinder (45 mm in diameter, 100 mm in depth) made of Pyrex glass was used. The air was first introduced into a flask containing NaOH solution to eliminate CO_2, then passed through a bubbler prior to reaching the reactor to saturate the air with moisture. The aeration rate was maintained at 5 mL/min throughout the experiment. It was ascertained that the aeration rate was sufficient for maintaining aerobic conditions in the preliminary experiment. The raw composting mixture was placed into the reactor at a wet weight of 12 g for runs I and II. Five reactors in total were used for the composting in each experimental runs I and II in order to measure the change in pH, moisture content, microbial cell density, and microbial diversity. The reactors themselves were placed in an incubator (Model LTI-1000; EYELA Co. Ltd., Tokyo, Japan) to regulate the composting temperature. The temperature was raised from room temperature to a set point of 60 °C at a constant rate of 2 °C/h, and the temperature of 60 °C was then maintained until the eighth day, when the composting operation was stopped. The exhaust gas from the composting reactor was introduced into a 5-l plastic bag made of polyvinyl fluoride (Tedlar Bag™; Omi Odoair Service Co. Ltd., Omihachiman, Japan) for 12 h, and the plastic bag was changed twice daily at 12-h intervals. The volume of exhaust gas captured in the plastic bag was measured, and the concentrations of CO_2 and NH_3 were analyzed with Kitagawa gas detector tubes (Komyo Rikagaku Kogyo K.K., Tokyo, Japan). CO_2 and NH_3 analysis employing the Kitagawa gas detector tube has been used for the rapidity and simplicity of the measurement (Nakasaki et al., 2001), although a method using gas chromatography (GC) is more accurate than that using a gas detector tube to measure the gas concentration. We confirmed, however, that the difference in the analytical results was not large between the two methods in our preliminary experiment: 4.53 ± 0.19% for the GC method and 4.5 ± 0.3% for the detector tube method as the results of the evaluation test using approximately 4.5% CO_2 gas standard. The CO_2 and NH_3 evolution rates during each 12-h period were determined by measuring the CO_2 and NH_3 concentrations and the exhaust gas volume. The cumulative CO_2 and NH_3 that had evolved up to a certain composting time were then calculated. The composting material inside the reactor was mixed by a sterilized spatula every 24 h. The compost sample was withdrawn from the mini-reactor and subjected to physicochemical and microbial analyzes.

Table 1
Some characteristics of two different types of anaerobic sludge

	Run I	Run II
	(S-sludge)	(K-sludge)
Moisture content (%)	84.1	76
Initial pH (%)	6.88	8.2
Ash content (%)	27.8	36.5
Organic content (%)	72.2	63.5
NFE (%)	18	13.4
Proteins (%)	43.3	15.4
Lipids (%)	9.2	19.9
Fibers (%)	1.7	14.8
C-content (%)	41.6	37.9
N-content (%)	6.21	2.27
C/N ratio (–)	6.7	16.7

Organic constituents, NFE (nitrogen free extract), Proteins, Lipids, and Fibers were measured by the standard method for food analysis.

2.3. Measurement of physicochemical parameters

The pH value was measured with a pH meter (Model F-8; Horiba Co. Ltd., Tokyo, Japan) in a suspension of compost mixed with distilled water (1:9 w/w) by weight. The electrical conductivity of a 1:10 (w/w) compost:distilled water suspension was determined with a conductivity meter (Model DS-14; Horiba Co. Ltd., Tokyo, Japan). The moisture content was obtained by drying the sample at 105 °C for three days in a drying oven (DS600; Yamato Scientific Co. Ltd., Japan). Total carbon and nitrogen of the solid compost sample and water extract of the sample (solid to distilled water ratio of 1:10 w/w) were determined using a Vario MAX CN analyzer (Elementar Analysesysteme GmbH, Hanau, Germany).

2.4. Microbial analyzes

For the microbial analyzes, we measured the cell density of microorganisms by a dilution plating method on Trypticase-soy agar and determined the microbial succession by the PCR-DGGE method. The composition of the Trypticase-soy agar medium was as follows: trypticase peptone, 17 g; phytone peptone, 3 g; K_2HPO_4, 2.5 g; NaCl, 5 g; glucose, 2.5 g; agar, 20 g; distilled water, 1000 mL; pH 7.3. The incubation temperature was the same as the temperature of composting, 60 °C, with an incubation period of three days. The cell density of the microorganisms was expressed in terms of colony-forming units per unit of dry weight of the composting material (CFU/g-DW).

In the PCR-DGGE analysis, DNA was extracted from the compost samples and PCR was conducted using the DNA as a template. The PCR products were then subjected to DGGE analysis. Each step of the experimental procedure was as follows.

2.5. DNA extraction from compost sample

The DNA was extracted from the compost samples taken on days 0, 2, 4, 6 and 8 by the ISOIL method for the Beads Beating kit (Nippon Gene Co. Ltd., Toyama, Japan). A 0.1 g wet weight composting sample was put into the 2 mL plastic tube and suspended by adding 950 μL of the Lysis Solution BB together with 50 μL of the Lysis Solution 20S. The suspension was then shaken vigorously (5500 rev/min) on a beadbeater (Micro Smash; TOMY MEDICO., Ltd, Tokyo, Japan) for 45 s and centrifuged at 12,000 g for 1 min. The supernatant was collected and mixed with the Purification Solution. The mixture was deproteinized with chloroform and centrifuged at 12,000 g for 15 min. The top supernatant including DNA was precipitated by adding the Precipitation Solution and then centrifuged at 16,000 g for 15 min at 4 °C, and washed with the Wash Solution. The pellet of DNA was purified by the addition of