Proliferation of aerobic complex microorganisms during composting of rice bran with charcoal

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SUMMARY: In Japan biomass charcoal has been used for a long time as soil improver in a firm. Bamboo charcoal was added to rice bran as nutrient with composting aerobic complex microorganisms. It was observed that the microorganisms proliferated on surface and in the pores of the charcoal in the mixture. Microorganisms proliferated by mixing charcoal with rice bran having three peaks of concentration and microorganisms. Two major bands of 16S rDNAs from ACM were amplified by PCR, and analyzed by DGGE. Dominance of the bands changed with peaks of growth (ATP) and this phenomenon was different depending on the initial amount of ACM. It was suggested that ACM proliferation rate is dependent on addition amount of ACM.

charcoal, composting, microorganisms

1 Introduction

Recently, two technologies have been receiving attention in the field of biomass waste recycling. One is the carbonization of biomass wastes such as waste construction materials, waste paper, and wood and bamboo forest thinnings, and another is the composting and use of garbage generated by homes, restaurants, and food industries and of livestock waste. It was reported that the addition of charcoal to farm soils had a proliferative effect on symbiotic microorganisms such as root nodule bacteria and mycorrhizae (Sugiura, 1984, Ogawa, 1984, 1987). It is well known that symbiotic microorganisms play important roles in growing plants. It was expected, therefore, that addition of charcoal to composting garbage enhances microorganisms proliferation, leading the composting time to be shortened. Yoshizawa (2005) and Yoshizawa et al. (2005) previously verified the successful composting of a mixture of charcoal and garbage from 55 houses over a 2-month period in the city of Suwa, Japan. Aged compost was obtained after 1 or 2 months, and composting microorganisms were observed on and in the charcoal in the compost.

Wood and bamboo have pores that range from several to several tens of microns in diameter and originate from tracheids, and charcoal prepared from carbonized wood and bamboo has pores of almost the same size. Tanaka et al. (2005) found that the proliferation of composting microorganisms was enhanced on and in bamboo charcoal as a medium to which rice bran had been added as a nutrient.

Here, we added charcoals made from bamboo to rice bran with aerobic complex microorganisms (ACM) used for composting. We studied the proliferation of the microorganisms by measuring the cultivation time-dependence of the concentrations of adenosine triphosphate (ATP) from the microorganisms, and we analyzed 16S rDNAs of microorganisms in the compost by denaturing gradient gel electrophoresis (DGGE) after amplification by polymerase chain reaction (PCR) method.
2 Experimental

2.1 Sample Preparation

Charcoals were prepared from thinned Moso bamboo (*Phyllostachys pubescens* Mazel ex Houzeau de Lehaie). The raw material was carbonized at 650°C in a batch-type furnace (Venture Viser Inc., Type-IV). The pH of the charcoals was 9.2. The specific surface area of the charcoal was estimated by the Brunauer-Emmett-Teller equation applied to N₂ adsorption isotherms for charcoals measured with an adsorption apparatus (BELSORP 18). The specific surface area of the charcoal was 420 m²/g. The relative pore volume of the charcoal measured by a mercury porosimeter (Shimadzu Corp., Autopore III 9420) is shown in Figure 1. The peak is centered in 0.1 – 1 μm.

![Flow Chart of Sample Preparation](image)

Figure 2 Flow Chart of Sample Preparation
Figure 3  Cultivation Time-Dependence of ATP Concentration in the System

Figure 4  Cultivation Time-Dependence of pH in the System

Flow chart of sample preparation is shown in Figure 2. Charcoal pulverized and sifted into particles 1 to 3 mm in diameter was used as a medium. Rice bran (17.8 g) as nutrient was added to 15.5 g charcoal powder in a 300-ml flask, giving a weight ratio of charcoal to rice bran of 1:1.15 (Imanishi and Sakawa, 2003) The moisture content of the mixture was adjusted to 65% by adding distilled water. The mixture was then treated at 120 °C for 60 min in a high-pressure sterilizer. ACM of 0 g, 0.01 g, 0.1 g 0.5 g 1 g and 5 g were added to seed the mixture, respectively. The samples were maintained in an cultivation chamber with a relative humidity (RH) of 53% at 23 °C and stirred vigorously with a spatula once a day for aeration.

2.2 Measurement

A part of the mixture was periodically sampled for measurement. Microorganisms that proliferated on the surface of the charcoal were observed by SEM. After freeze-drying of the sample in liquid nitrogen in a vacuum, the sample was fixed by osmic acid evaporation. The surface was then coated with a thin film of sputtered Pt-Pd alloy.

The concentration of microorganisms was estimated by measuring the ATP concentration in the sample (Meidensha Corp., Luminometer UPD-4000) (Inamori, 1988). Because ATP exists in mitochondria in the cytoplasm, the concentration of ATP can be used as an indication of microorganism activity. When ATP to which d-luciferin has been added changes to adenosine monophosphate in the presence of luciferase and Mg$^{2+}$, light at a wavelength of 560 nm is emitted. Distilled water (20 ml) was added to 2 g of the sample and stirred
with a tube mixer at 2500 rpm for 1 min. Then 250 μl of this suspension was withdrawn with a micropipette and an ATP measuring kit (Meidensha Corp., Lucifer AS) added. The pH of the sample was determined by measuring the pH values of an aqueous solution containing ions exuded from the charcoal immersed in the solution (Japan Soil Association, 2004); distilled water (20 ml) was added to 2 g of the sample and the mixture was stirred with a tube mixer at 2500 rpm for 1 min. pH was measured with glass electrode. DNAs was extracted from 2 g of sample, and 16S rDNAs was amplified with PCR method using primers with GC cramps, and the PCR products were analysed by DGGE method with denaturing agent concentration of 0–80% at 200 V for 7 h.

3 Results and Discussion

The cultivation time-dependence of ATP concentration of the samples is shown in Figure 3. In the systems that used charcoal as a medium, the ATP concentration increases showing three peaks around 70 h, 190 h and from 300 to 350 h, respectively. This suggests that there are some kinds of microbial community whose proliferation rate is different. The cultivation time-dependence of pH of the samples is shown in Figure 4. The pH decreases to 5.5 around 80 h and increases to 6.5 with increasing cultivation time. The first ATP concentration peak corresponding to decrease of pH comes from microbial community which produces lower molecular weight fatty acids such as acetic acid, isobutyric acid, \( n \)-butyric acid, isovaleric acid, and \( n \)-valeric acid (Eya, 2003).
SEM photographs of the microorganisms found on the surface of the mixture of bamboo charcoal and rice bran sampled with ACM 0.5 g at peak 1, 2 and 3 after 70 h, 190 h and 350 h are shown in Figures 5(a), (b) and (c), respectively. Rods and short rods can be observed on the surfaces of the charcoals and in the mouths of the pores. We confirmed that charcoal functioned as a matrix for these microorganisms.

Figure 6 shows photograph of DNA-bands of the ACM and the composts sampled at the peak 1, 2 and 3 containing ACM 5 g and 0.5 g. In electrophoresis patterns of DNAs from the compost added with ACM 5 g and 0.5 g, the main band is separated into two sub-bands. In the DNAs sampled at the peak 1 of the compost with ACM 5 g, the lower sub-band is observed, and two sub-bands emerge at the peak 2. The upper sub-band is diminished at the peak 3. In the compost with ACM 0.5 g, two sub-bands are observed at the peak 2 and 3. This suggests that ACM proliferation rate is dependent on addition amount of ACM.

4 Conclusions
Charcoal made from bamboo was mixed with rice bran as a nutrient. Aerobic complex microorganisms (ACM) for composting biomass waste were added to seed the mixture. As incubation-time of the mixed system went by, the ACM concentration increased. Three peaks in the ATP concentration vs. the incubation-time were observed. Two major bands of 16S rDNAs from ACM were amplified by PCR, and analyzed by DGGE. Dominance of the bands changed with peaks of growth (ATP) and this phenomenon was different depending on the initial amount of ACM. It was suggested that ACM proliferation rate is dependent on addition amount of ACM.

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