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# Morphological Change of Microbial Community Structure during Composting Rice Bran with Charcoal

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Bamboo charcoal powder was mixed with rice bran as a nutrient for the composting microorganisms. The moisture content of the mixture was adjusted to 65%. Aerobic complex microorganisms were added to the mixture of the charcoal and the rice bran to seed the composting. Samples were maintained in ambient air (RH 53%, 23 °C) and stirred vigorously with a spatula once a day for aeration. Measurement of adenosine triphosphate (ATP) concentrations of the samples revealed that the microorganisms proliferation was accelerated with the increase of the charcoal amount accompanied with three peaks of the ATP concentration. Microbial community was observed by scanning electron microscopy on the surface of the charcoal. It was suggested that the charcoal functioned as a matrix for the microorganisms. The microorganisms were morphologically diverse corresponding to the change of the ATP concentration.

**Keywords:** Charcoal, Biomass waste, Composting, Microorganisms, Microbial community

## 1. INTRODUCTION

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 of garbage generated by homes, restaurants, and food industries and of livestock waste.

Wood and bamboo have pores that range from several to several tens of microns in diameter and originate from tracheids. Charcoal prepared from carbonized wood and bamboo has pores of almost the same size. It was found that the proliferation of composting microorganisms was enhanced on and in bamboo and scrap wooden formwork and corncobs charcoals as a medium to which rice bran had been added as a nutrient [1][2]. In this case the rice bran was used as a model biomass wastes for garbage and livestock waste.

The successful composting of a mixture of charcoal and garbage from 55 houses was previously verified over a 2-month period in April and May of 2005 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 [3][4].

In this study, we added charcoals made from bamboo to rice bran with aerobic complex microorganisms (ACM) used for composting. We studied the effect of charcoal amount on the proliferation of the microorganisms by measuring the incubation time dependence of the concentrations of adenosine triphosphate (ATP) from the microorganisms, and morphological change of microbial community structure on the surface of the charcoal during composting rice bran by scanning electron microscopy (SEM) technology.

## 2. EXPERIMENTAL

### 2.1 Sample Preparation

Charcoals were prepared from 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 $N_2$ adsorption isotherms for charcoals measured with an adsorption apparatus (BELSORP 18). The specific surface area of the charcoal was 420 $m^2/g$. The relative pore volume of the charcoal measured by a mercury porosimeter (Shimadzu Corp., Autopore III 9420) was distributed from several nm to 1 mm. The peak was centered in 0.1 – 1 μm.

Flow chart of sample preparation is shown in Fig. 1. Charcoal pulverized and sifted into particles 1 to 3 mm in diameter was used as a medium. The charcoal of 1.0 g, 5.9 g or 15.5 g was mixed to rice bran (17.8 g) as nutrient in a 300-ml flask. 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 0.5 g was added to seed the mixture. The samples were maintained in an incubation chamber with a relative humidity (RH) of 53% at 23 °C and stirred vigorously with a spatula once a day for aeration.

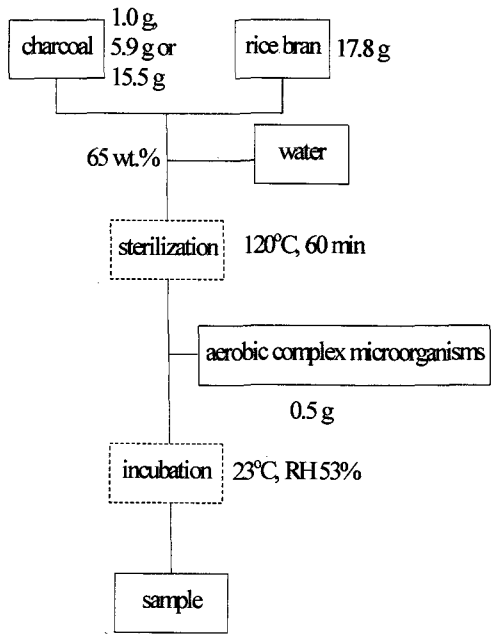


Fig. 1. Flow chart of sample preparation.

### 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) [5]. 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 2,500 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 [6]; 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.

## 3. RESULTS AND DISCUSSION

### 3.1 Proliferation of Microorganisms

The incubation time dependence of ATP concentration of the samples is shown in Fig. 2. In the system mixed with charcoal, rice bran and ACM, the ATP concentration increases accompanied with three concentration peaks with increase of incubation time. In the systems, the mixture of charcoal and rice bran without ACM and the single component, only ACM, only charcoal, and only rice bran, no increase of the ATP concentration is observed.

For studying influence of the charcoal amount on the microorganisms proliferation in the mixture of charcoal, rice bran and ACM, the logarithmical incubation time dependence of the ATP concentration in the mixture with different amount of the charcoal, 1.0 g, 5.9 g and 15.5 g, is shown in Fig. 3. Increase rate and extent of the ATP concentration are dependent on the charcoal amount in the system. The result that ATP

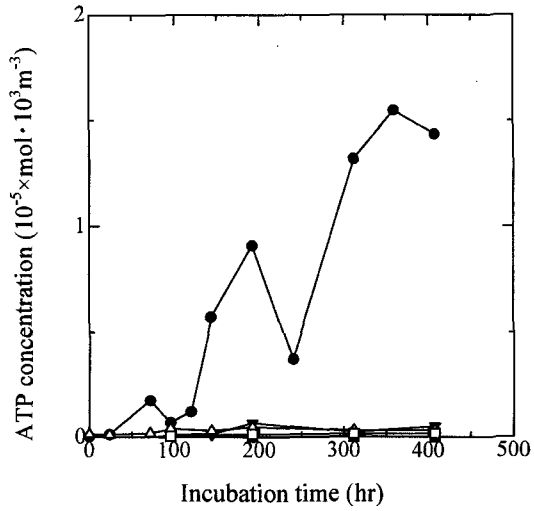


Fig. 2. Incubation time dependent of ATP concentration of the systems. •; ACM, charcoal and rice bran, ▼; Charcoal and rice bran, △; ACM, ■; Charcoal, and □; Rice bran.

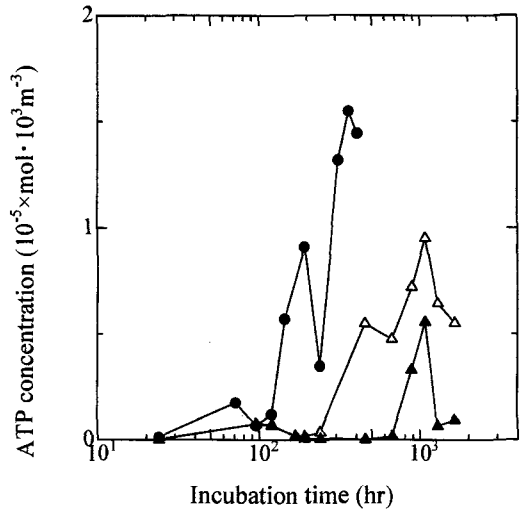


Fig. 3. Incubation time dependent of ATP concentration of the systems. Charcoal amount : ▲; 1.0 g , △; 5.9 g and •; 15.5 g.

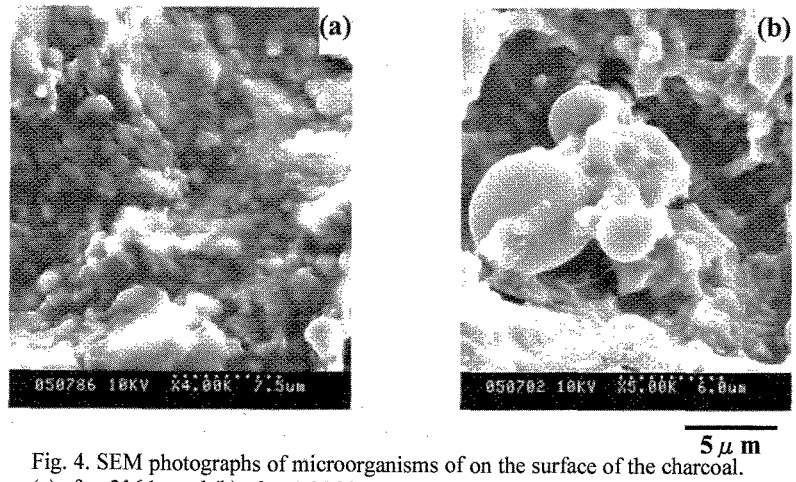


Fig. 4. SEM photographs of microorganisms of on the surface of the charcoal. (a) after 216 hr and (b) after 1,056 hr. The charcoal amount; 1.0 g.

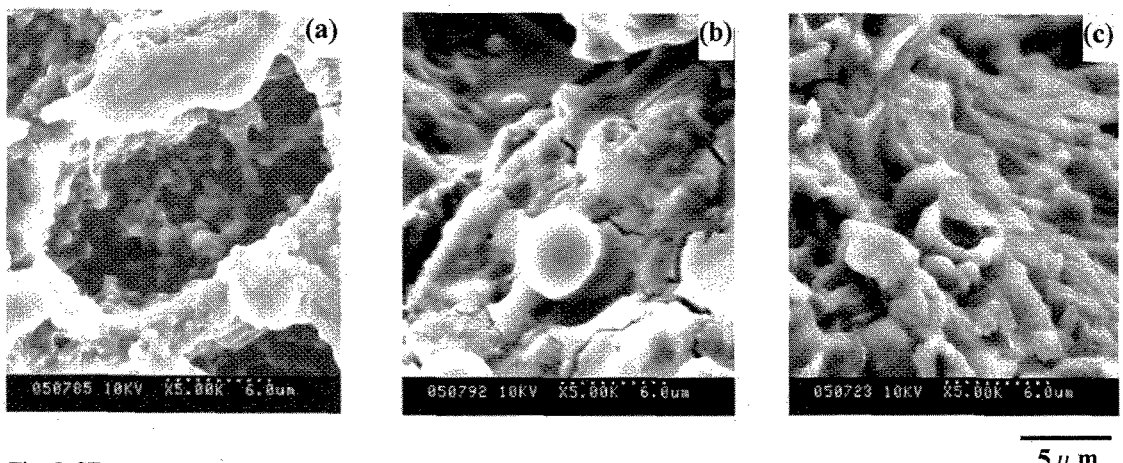


Fig. 5. SEM photographs of microorganisms on the surface of the charcoal. (a) after 216 hr, (b) after 432 hr, and (c) after 1,056 hr. The charcoal amount; 5.9 g.

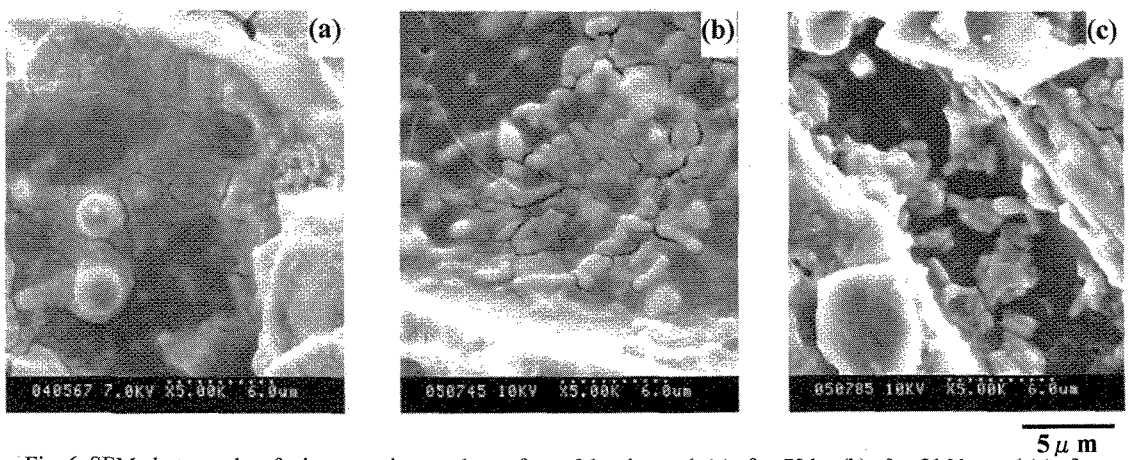


Fig. 6. SEM photographs of microorganism on the surface of the charcoal. (a) after 72 hr, (b) after 216 hr, and (c) after 408 hr. The charcoal content; 15.5 g.

concentration increases in the mixture system with charcoal, rice bran and ACM, means that the charcoal can proliferate microorganisms in the system. There are found several peaks of the ATP concentration; two peaks at about 100 hr and 1,000 hr in the system with 1.0 g of charcoal, and three peaks at 100 hr, 500 hr and 1,000 hr

with 5.9 g of the charcoal, and three peaks at 70 hr, 200 hr and 400 hr with 15.5 g of the charcoal. The peak shifts in the incubation time dependent on the amount of the charcoal. It is found that as the amount of the charcoal increases, the microorganisms proliferation is accelerated remarkably. From those results, there should be at least three kinds of microbial community in the system whose proliferation rate is different. It is suggested that this comes from difference of the proliferation rate of adaptive microorganisms as a specific response to the presence of nutrient such as glucide, protein and lipid contained in rice bran as a main component. The proliferation rate may reflect the biodegradation property of the nutrients.

### 3.2 SEM Observation of Microorganisms

SEM photographs were taken in a pore of the charcoal in the mixture, which were sampled near the peak of the ATP concentration. Figs. 4, 5 and 6 show the SEM photographs of the samples with the charcoal amount of 1.0 g, 5.9 g and 15.5 g, respectively. Many kinds of microbial community can be observed; cocci, rod and short rod bacteria. We confirmed that charcoal functioned as a matrix for these microorganisms and that the composting microorganisms on the charcoal were morphologically diverse. However, because SEM observation is not considered sufficient to characterize fully the microbial communities, DNA fragments isolated from the composting microbial communities need to be studied as next step [7].

## 4. CONCLUSIONS

Charcoal powder 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 ACM increased, the ACM concentration increased, and three peaks in the ATP concentration were observed. The charcoal functioned as a matrix for the microorganisms. ACM proliferation rate is dependent on addition amount of the charcoal. The microorganisms were morphologically diverse corresponding to the change of the ATP concentration.

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