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Promotion effect of various charcoals on the proliferation of composting microorganisms

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We prepared charcoals from bamboo, scrap wooden formwork and corn-cobs. Each of these charcoal powders was mixed with rice bran as a nutrient in a weight ratio of 1 : 1.15. (Rice bran had previously been used as a model biomass waste for composting.) The moisture content of the mixture was adjusted to 65 %. Aerobic complex microorganisms were added to the biomass waste mixture to seed the composting. Samples were maintained in ambient air (RH 53 %, 23 ℃) and stirred vigorously with a spatula once a day for aeration. Scanning electron microscopy revealed microorganisms on the surface of the charcoal and in the mouths of the charcoal pores. Measurement of adenosine triphosphate concentrations of the samples revealed that the microorganisms had proliferated in the systems containing charcoal.

It was suggested that this proliferation was attributed to supplying abundant oxygen for microorganisms because the charcoal has a lot of pores. Another reason why the charcoal facilitated microorganisms proliferation might be that the microorganisms on the surface of the charcoal were able to be supported in the pores of a sub-micron scale in the charcoal as a matrix might be.

KEYWORDS : Charcoal, Compost, Microorganism, Proliferation, ATP

1. Introduction

Charcoals, ashes and composts from biomass wastes have been used for a long time in Japan as soil improvers and fertilizers on farms[1]. Sugiura and Ogawa reported that the addition of charcoal to farm soils had a proliferative effect on symbiotic microorganisms such as root nodule bacteria and mycorrhizae[2),3)]. It is well known that symbiotic microorganisms play important roles in growing plants. 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[4),5)], 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. One of the authors (S. Y.) 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[6]. 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. In our previous paper[7], we 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. In this case the rice bran was used as a model biomass wastes for garbage and livestock waste. In another study, rice bran as a nutrient for *Pleurotus ostreatus* was added to charcoal made from used paper, and accumulation of mycelia in the charcoal was reported[5].

Here, we added charcoals made from bamboo, wood and corn-cobs to complex microorganisms used for composting. We studied the proliferation of the microorganisms by measuring the incubation time dependence of the concentrations of adenosine triphosphate (ATP) from the microorganisms, and we observed the morphology of the microorganisms in the mixture by scanning electron microscopy (SEM). To the best of our knowledge this is the first

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study to use SEM observation of composting microorganisms on and in charcoals.

2. Experimental

2.1 Sample preparation and measurement

Charcoals were prepared from moso bamboo (*Phyllostachys pubescens* Mazel ex Houzeau de Lehaie), waste wood from concrete formwork and corn-cobs. These raw materials were carbonized at 600 to 700 ℃ in a batch-type furnace (Venture Viser Inc., Type-IV). **Table 1** shows the characteristics of the various charcoals. The pH of the charcoals ranged from 8.9 to 9.5 ; it was estimated by measuring the pH value of an aqueous solution containing ions leached out from the charcoal immersed in the solution, in conformity with Japanese Industrial Standard 1474-1991 ("Test Methods for Activated Carbon"). Distilled water (100 ml) was added to 3 g of charcoal pulverized with a mortar. The mixture was boiled for 5 min then cooled to room temperature, and then distilled water was added to adjust the total volume to 100 ml. The pH value was measured with a glass electrode.

The bulk densities of the bamboo and corn-cob charcoals were smaller than that of the wooden formwork charcoal. 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 corn-cob charcoal (230 m^2/g) was smaller than those of the bamboo and wood waste charcoals, because bamboo and wood have finer structures, including finer vascular tracheids or finer pores.

The relative pore volume of the charcoal was measured by a mercury porosimeter (Shimadzu Corp., Autopore III 9420).

Fig.1 is a flow chart of sample preparation. 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[5]. The moisture content of the mixture was adjusted to 65 % by adding distilled water. The mixture was then treated at 120 ℃ for 60 min in a high-pressure sterilizer. Aerobic complex microorganisms (10 g) were added to seed the mixture. The samples were maintained in an incubation chamber with a relative humidity (RH) of 53 % at 23 ℃ and stirred vigorously with a spatula once a day for aeration.

In order to compare with the charcoal addition experiment, glass beads (1 mm diameter) were mixed with the rice bran. The amount of glass beads was adjusted to give the same weight (wt) % or volume (vol) % as those of the charcoal.

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.

Table 1 Characteristics of the three types of charcoal used.

Characteristics \ Charcoal	Bamboo	Waste formwork wood	Corn-cob
pH	9.2	8.9	9.5
Bulk density [g/cm³]	0.17	0.25	0.18
Specific surface area [m²/g]	420	580	230

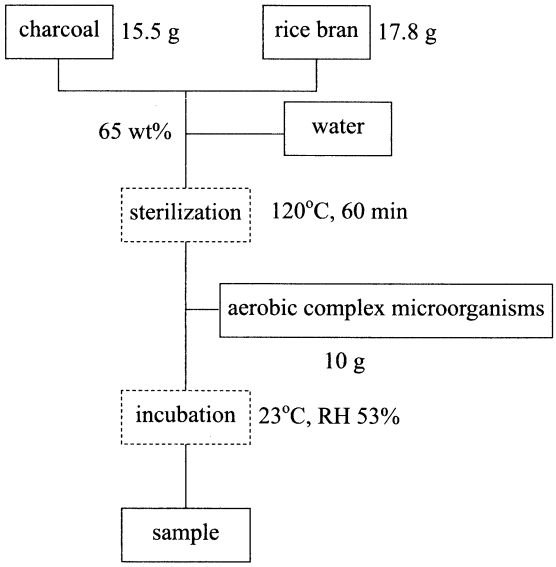

Fig.1 Flow chart of sample preparation.

The concentration of microorganisms was estimated by measuring the ATP concentration in the sample. Because ATP exists in mitochondria in the cytoplasm, the concentration of ATP can be used as an indication of microorganism activity. ATP changes to adenosine monophosphate (AMP) by addition of D-luciferin in the presence of luciferase and Mg^{2+}, leading to the light emission at a wave length of 560 nm[8]. By this reaction, the amount of ATP is determined using a luminometer (Meidensha Corp., UPD-4000). Experimentally, 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 leached from the charcoal immersed in the solution ; [9] 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.

3. Results and discussion

3.1 Charcoal systems

The relative pore volume distributions of the bamboo charcoal,

wood waste charcoal and corn-cob charcoal are shown in **Figs.2** (a), (b) and (c), respectively. The peak pore size distributions of the charcoals from the bamboo and wood waste were centered on 0.1 to 1 μm ; that of the corn-cob charcoal (10 to 1000 μm) was thus much larger.

SEM photographs of the microorganisms found on the surface of the mixture of bamboo charcoal and rice bran after 696 h of incubation are shown in **Fig.3**. Granules, such as the spores of *Actinomadura*, which had many bumps, were detected (**Fig.3** (a)). The microorganisms shown in **Fig.3** (b) were similar to *Actinomycetes*, which has a ramified structure similar to that observed in *Cellulomonas* and *Agromyces*. **Figs.4** (a), (b) and (c) are SEM photographs of microorganisms on the surfaces of the charcoals from bamboo, wood waste and corn-cobs, respectively, after 336 h of incubation. Rod shaped and short-rod shaped microorganisms can be observed on the surfaces of the charcoals and in the mouths of the pores. There can be observed many microorganisms in the vicinity of pore of bamboo charcoal (**Fig.4** (a)). In **Figs.4** (b) and (c), arrows show typical small microorganisms in wood and corn-cob charcoals, respectively. 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 was not considered sufficient to characterize fully the microbial communities, DNA fragments isolated from the composting microbial communities need to be studied as a next step[10].

The incubation time dependence of ATP concentration and pH of the samples are shown in **Figs.5** (a) and (b), respectively. In the systems that used charcoal as a medium, the ATP concentration

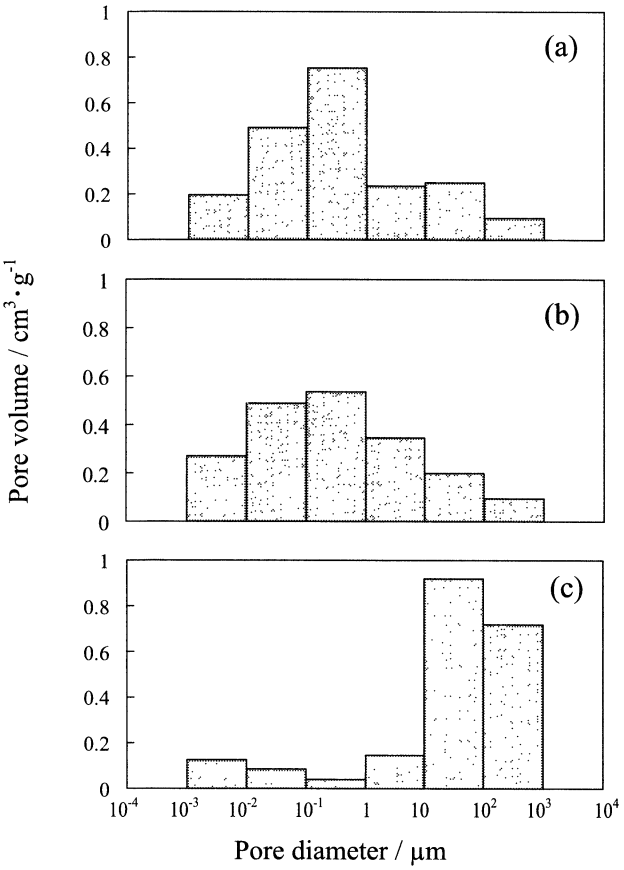

Fig.2 Pore distribution of various charcoals ; (a) bamboo charcoal, (b) scrap wooden formwork charcoal and (c) corn-cob charcoal.

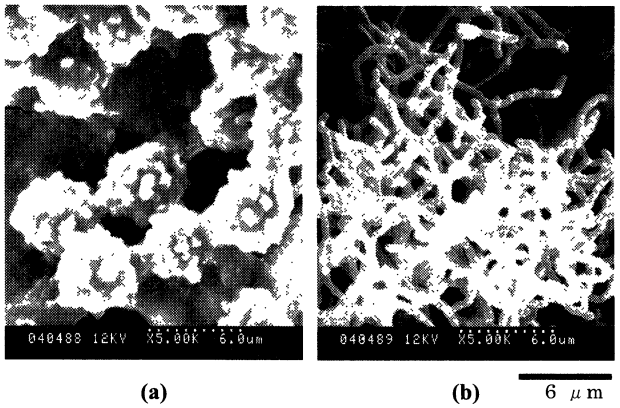

Fig.3 SEM photographs of the different types of microorganism on the mixture surface of bamboo charcoal and rice bran after 696 hours of the incubation.

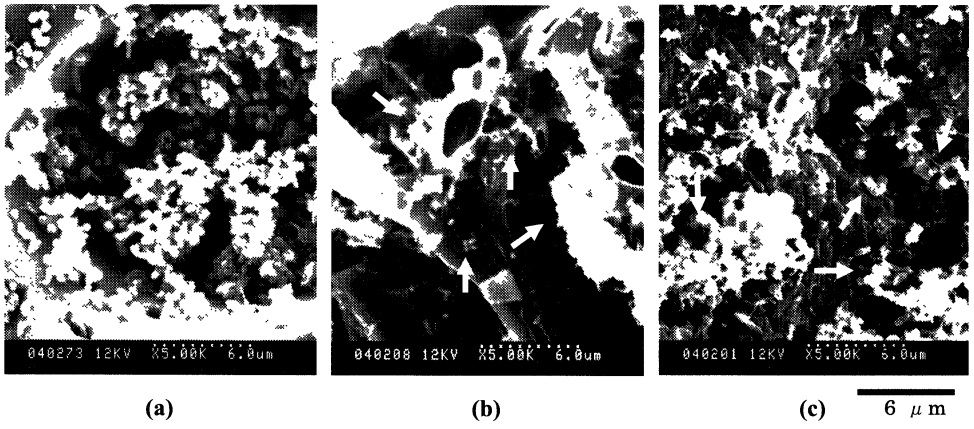

Fig.4 SEM photographs of microorganisms on various charcoals ; (a) bamboo charcoal, (b) scrap wooden formwork charcoal and (c) corn-cob charcoal after 336 hours of the incubation.

increased continuously up to 100 to 200 h and then decreased. pH increased linearly with increasing incubation time. Proliferation of the composting microorganisms was scarcely influenced by differences in pore diameter distribution and specific surface area among the charcoals from bamboo, waste wood, and corn-cobs. This is because the microorganisms proliferated only on the surface of the charcoal and in the vicinity of the pore entrances, where they could come into contact with the rice bran ; they could not proliferate inside the pores where there were no nutrients. Additionally, because the composting microorganisms ranged in size from submicron scale to several microns, it seems impossible that they could have intruded into the micropores (less than 2 nm in diameter) or mesopores (2 to 50 nm) in the charcoal. The composting microorganisms that we used can proliferate in the presence of oxygen (i.e. they were aerobic). Because the charcoal contained many pores, its addition to the system provided adequate oxygen for microorganism proliferation.

The ATP concentration and pH in the system without charcoal increased up to 50 to 100 h, but both then decreased. After 100 h the ATP level was almost zero, corresponding to the low pH of about 4. These results in the system without charcoal indicate that it reached an acidic state, accompanied by the production of lower molecular weight fatty acids such as acetic acid, isobutyric acid, *n*-butyric acid, isovaleric acid, and *n*-valeric acid[11]. This phenomenon is also supported by our other observation that the color of the system changed from dark to reddish brown and the smell became sour. These results suggest that the content of the system without charcoal was under anaerobic conditions despite vigorous stirring once a day.

3.2 Glass bead system

Glass beads were compared with the charcoal as a medium for the proliferation of microorganisms. The weight (wt) % or volume (vol) % of the glass beads were adjusted to the same as that of the charcoal. **Figs.6** (a) and (b) show different magnifications of SEM photographs of microorganisms on the surfaces of the glass beads after 696 h of incubation. Extraneous matter on the surface of the glass bead in **Fig.6** (a) was magnified to the same extent as shown in **Figs.3** and **4** and is shown in **Fig.6** (c). The object ranging from about 3 to 10 μm long are pieces of rice bran, and the scare, small structures around 1 μm appear to be microorganisms as shown by arrows.

The incubation time dependence of ATP concentration and pH of the

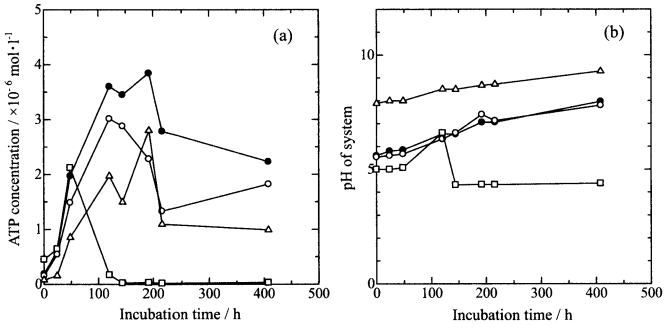

Fig.5 Incubation time dependence of (a) ATP concentration and (b) pH of the system. ● ; bamboo charcoal, ○ ; scrap wooden formwork charcoal, △ ; corn-cob charcoal and □ ; without charcoal.

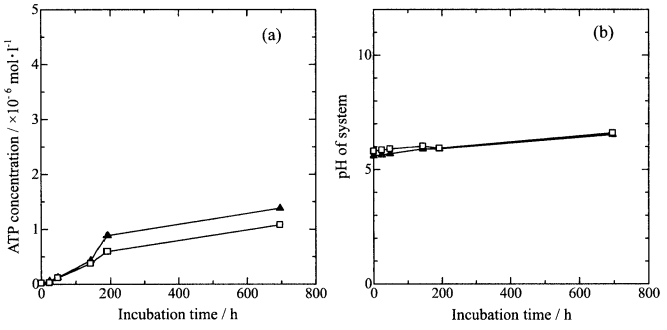

Fig.7 Incubation time dependence of (a) ATP concentration and (b) pH of the system with glass beads. ▲ ; same wt % with bamboo charcoal and □ ; same vol % with bamboo charcoal.

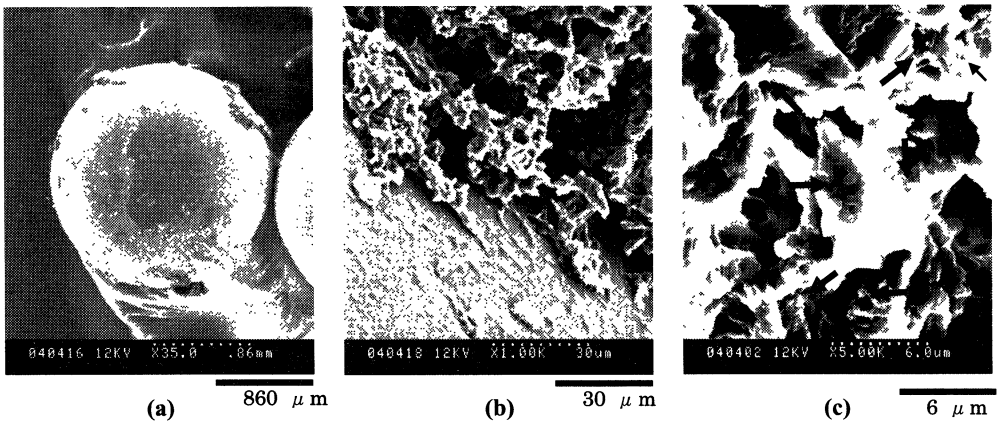

Fig.6 SEM photographs of microorganisms on the surface of glass beads after 696 hours of the incubation under different magnification.

glass bead samples are shown in **Figs.7** (a) and (b), respectively. ATP concentration and pH increased gradually with incubation time. Unlike in the systems with charcoal (**Fig.5** (a)), there was no ATP concentration peak in the range of 100 to 200 h. In the system with the glass beads, although aerobic conditions were maintained by vigorous stirring once a day, the extent of microorganisms proliferation was small.

Although the affinity between the charcoal and the microorganisms was not well defined, we found that aerobic microorganisms proliferated on the surface of the charcoal and in the vicinity of the pore openings. One of the reasons for this may have been the presence of the pores in the charcoal which is of a size suitable as a matrix for the microorganisms. And another reason is that the pores may supply adequate oxygen for the aerobic microorganisms. We can expect, therefore, that in practice the time required to make compost from various biomass wastes containing large numbers of microorganisms would be shortened by mixing charcoal into the waste.

4. Conclusions

Charcoal made from bamboo, waste wood, or corn-cobs was mixed with rice bran as a nutrient in a ratio of 1 : 1.15. Aerobic complex microorganisms for composting biomass waste were added to seed the mixture. We observed by SEM that the microorganisms proliferated on the surface of the charcoal and near the openings of the pores. An increase in ATP concentration in the system was also observed, indicating that the microorganisms had proliferated. For reasons of this proliferation, it was suggested that the large number of pores in the charcoal supplied adequate oxygen for the aerobic microorganisms. Another reason may be the presence of the pores in the charcoal which is of a size suitable as a matrix for the microorganisms.

Acknowledgments

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