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Temperature control strategy to enhance the activity of yeast inoculated into compost raw material for accelerated composting

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ABSTRACT

The effects of inoculating the mesophilic yeast *Pichia kudriavzevii* RB1, which is able to degrade organic acids, on organic matter degradation in composting were elucidated. When model food waste with high carbohydrate content (C/N = 22.3) was used, fluctuation in the inoculated yeast cell density was observed, as well as fluctuation in the composting temperature until day 5 when the temperature rose to 60 °C, which is lethal for the yeast. After the decrease in yeast, acetic acid accumulated to levels as high as 20 mg/g-ds in the composting material and vigorous organic matter degradation was inhibited. However, by maintaining the temperature at 40 °C for 2 days during the heating phase in the early stage of composting, both the organic acids originally contained in the raw material and acetic acid produced during the heating phase were degraded by the yeast. The concentration of acetic acid was kept at a relatively low level (10.1 mg/g-ds at the highest), thereby promoting the degradation of organic matter by other microorganisms and accelerating the composting process. These results indicate that temperature control enhances the effects of microbial inoculation into composts.

1. Introduction

Food waste originating from households, restaurants, and food processing factories is free from contamination with heavy metals and other toxic substances; thus, it is suitable for reclamation as compost for agricultural use. Therefore, composting is considered a promising method for food waste treatment, and much recent research has focused on composting of food waste (Al-jabi et al., 2008; Chang and Chen, 2010; Cheung et al., 2010; Li et al., 2013; Ermolaev et al., 2015; Pandey et al., 2016).

One of the significant characteristics of food waste composting is a decrease in pH in early stages of composting (Nakasaki et al., 1996; Cheung et al., 2010). Food waste contains a large amount of easily degradable carbonaceous materials such as carbohydrates, and the degradation of these materials occurs earlier than that of other organic constituents. The pH can decrease during storage and collection of waste, before composting begins, because of the production of acidic intermediates termed organic acids. Among the organic acids, it is important to note that the volatile fatty acids severely inhibit the activity of compost microorganisms (Cheung et al., 2010). Low pH associated with organic acid production inhibits vigorous organic matter degradation during composting (Nakasaki et al., 1996; Cheung et al., 2010). This problem has been solved by the addition of chemical compounds (Gray et al., 1971; Yu and Huang, 2009; Wong et al., 2009) and the inoculation of microorganisms and recycled compost materials into the raw compost (Nakasaki et al., 1996; Choi and Park, 1998; Nakasaki et al., 2013; Sundberg et al., 2013).

Nakasaki et al. (1996) observed the effects of inoculation with a thermophilic bacterium, *Bacillus licheniformis* HA1, which prevents the pH decrease in early stages of composting by producing NH_3 from the protein in the compost raw materials, although no effect was observed when the initial composting conditions were acidic. By inoculating an acid-degrading microorganism, organic acids contained in the raw material were degraded; thus, inoculation of such a microorganism is expected to be effective in accelerating the compost even when the raw material contains high concentration of organic acids. In fact, Choi and Park (1998) demonstrated the positive effects of inoculation with a thermophilic yeast, *Kluyveromyces marxianus* Y60, under controlled temperature conditions of 50 °C throughout the food waste composting process. Nakasaki et al. (2013) used a mesophilic strain of the yeast *Pichia kudriavzevii* RB1, which is highly active in degrading organic acids, as an inoculum. The RB1 strain rapidly degraded the organic acids present in compost raw material composed of model food waste with a C/N ratio of 18.1, leading to an increase in pH above neutral. Therefore, organic matter degradation was accelerated by enhanc-

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ing the activity of bacteria that favors neutral or weak alkaline conditions.

In an earlier composting method that involved inoculation with yeast, the yeast died owing to high temperatures in early stages of composting, and thus the yeast did not contribute in thermophilic stages of composting; however, its contribution was sufficient to enhance the activity of bacteria that vigorously degraded organic materials (Nakasaki et al., 2013). The organic acids did not accumulate again in the composting material after the yeast died, resulting in accelerated composting. It is known that the production of organic acid depends strongly on the types of raw compost materials. In fact, when higher amounts of easily degradable carbonaceous materials are present in the raw compost material, higher amounts of organic acid are produced. In such cases, the organic acids accumulate after the yeast dies; the pH drops, and thus the organic matter degradation is severely inhibited. It is considered that organic acids that are originally present in the raw material and those generated as composting progresses could be effectively degraded if the temperature is controlled to prevent the death of the inoculated yeast—the inoculation of yeast combined with temperature control to activate the yeast may be effective in preventing accumulation of organic acids and accelerating organic matter degradation during composting.

In a previous study, a composting method that combined microbial inoculation with temperature control was shown to suppress plant diseases (Nakasaki et al., 1998b). In order to allow the inoculated *Bacillus subtilis* to proliferate in the raw composting material and reach a high cell density, the temperature was held at 40 °C during the heating phase, and then the temperature was increased to 60 °C for accelerated and hygienic composting. That study aimed to produce a special compost containing a specific microorganism at high cell density in the final compost, rather than to solve the low pH problem during composting. In the present study, a new method was devised that uses a combination of microbial inoculation and temperature control to accelerate composting while solving the low pH problem. Maintaining a temperature favorable for the inoculated microorganism was expected to be effective in promoting the growth of that microorganism, thus mitigating the low pH. To the best of our knowledge, this is the first study to demonstrate the effects of inoculating a microorganism from pure culture in combination with temperature control to accelerate organic matter degradation during the composting process.

2. Materials and methods

2.1. Composting material and preparation of the inoculum

In Japan, food waste from each household contains a low amount of carbohydrates, whereas the food waste from restaurants, convenience stores, and food factories sometimes contain a high concentration of carbohydrates from sources such as cooked rice, bread, and pasta. In order to simulate food waste with high carbohydrate content, two types of raw materials were prepared by mixing commercial rabbit food (Rabbit Food Timothy, Easter Co. Ltd., Tatsuno, Japan) with cooked rice at a ratio of 7:3 and 5:5, respectively, on a dry weight basis. Dry weight of each raw material was set at 600 g. The C/N ratios of the rabbit food and the cooked rice, determined by elemental (CHN) analysis, were 18.1 and 48.5, respectively. The moisture content and pH value for the rabbit food were 8.22% and 5.92, and for the cooked rice, were 64.9% and 7.02, respectively.

Each of the rabbit food and cooked rice mixtures was then supplemented with sawdust and seeding material (Alles G; Matsumoto Laboratory of Microorganisms Co. Ltd., Matsumoto, Japan) at a ratio of 10:9:1 on dry weight basis to create a raw compost mixture. Thus, dry weight of sawdust in the raw compost mixture was 540 g. Sawdust was used as a bulking agent, since it absorbs the water that fills the free air space between the solid particles of raw compost mixture and improves aerobiosis. The bulk density of the raw compost mixture was approximately 0.484 g/cm^3.

The cell densities of mesophilic and thermophilic bacteria and mesophilic fungi in the seeding materials were determined in our laboratory by the dilution plating method, using trypticase soy agar medium supplemented with amphotericin B for bacteria and potato dextrose agar medium supplemented with chloramphenicol for fungi. The incubation temperatures for the mesophilic and thermophilic microorganisms were 30 °C and 60 °C, respectively, with an incubation period of 3 days. The cell densities expressed in terms of colony-forming units (CFU) per gram dry solid weight of the seeding material for mesophilic and thermophilic bacteria were 8.13×10^7 and 5.37×10^6 CFU/g-ds, respectively. The cell densities of the mesophilic fungi were less than 10^3 CFU/g-ds. Previous studies have shown that four types of organic acids are characteristic of food waste: acetic acid, propionic acid, butyric acid, and lactic acid, present at concentrations of 2.90, 3.02, 2.43, and 12.45 g/kg-ds, respectively (Sundberg et al., 2011). The raw compost mixture used in the present study contained acetic acid and lactic acid at concentrations of 0.12 and 0.08 g/kg-ds, respectively, but the other two organic acids, i.e., propionic acid and butyric acid, were not detected. Thus, the concentrations of these organic acids in the raw compost mixture were lower than those present in the food waste reported in the previous study. Therefore, reagents of acetic acid, propionic acid, butyric acid, and lactic acid were added in the raw compost mixture. The pH of the compost raw material was approximately 5.2 after addition of these organic acids.

Six experimental composting runs from Run A through E, as well as Runs B-1 and B-2, were carried out with varying types of compost raw material and experimental conditions, i.e., with and without inoculation of the yeast strain RB1 and with varying periods of temperature control at 40 °C during the heating phase (see Table 1). The RB1 strain of the yeast *Pichia kudriavzevii*, isolated in a previous study (Nakasaki et al., 2013), has the ability to degrade organic acids and it was used as an inoculum. The RB1 strain was stored at −80 °C and pre-cultured twice in potato dextrose liquid medium at 30 °C while shaking to prepare the RB1 culture at a concentration of 10^9 CFU/mL. The RB1 strain was centrifuged and washed with physiological saline three times and suspended in physiological saline at a concentration of 10^7 CFU/mL to prepare the inoculum. Ten milliliters of the inoculum were added to 1000 g-ds of compost raw material for an RB1 cell density of 10^5 CFU/g-ds. A previous study confirmed that there was no significant difference in the composting acceleration effect when more than 10^5 CFU/g-ds of RB1 was inoculated. At the beginning of all experimental runs, the moisture content was adjusted to 60% by the addition of distilled water.

2.2. Composting operation

Composting experiments were conducted in a bench-scale composting reactor (diameter: 300 mm, depth: 400 mm, volume: 28.3 L). This reactor was made of polyvinyl chloride, surrounded by an electrical ribbon heater, and embedded in polystyrene foam for heat insulation. At the beginning of the experiment, approximately 3000 wet-g of the raw compost mixture was transferred to the reactor, and thus the working volume of the composting material was approximately 7 L. The temperature of the composting material was set at 27 °C, with air introduced from the bottom of the reactor at a minimum flow rate of 45 L/h to maintain aerobic conditions. The temperature was allowed to self-heat as a result of