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Automation of Solid-state Bioreactor for Oyster Mushroom Composting

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This study focused on the production of high quality compost for the growth of aero-thermophilic fungi, which has a promoting effect on the growth rate and production of oyster mushrooms. The automated solid-state bioreactor system was designed on the basis of a Three-Phase-One system, which controls the serial steps of prewetting, pasteurization and fermentation processes. High numbers of thermophilic fungi and bacteria were recovered from the mushroom composts prepared by this solid-state bioreactor. The rates of composting process were depended on physical as well as chemical factors. Among these factors, the parameters of moisture content and temperature were found to be particularly important. In our automated system, constant levels of moisture content, temperature and ventilation via mixing were provided by a centralized control apparatus including PLC, water tank and water jacket systems. These features induced higher microbiological activity of aero-thermophiles.

KEYWORDS: Automation, Mushroom composting, Solid-state bioreactor

The production of oyster mushroom (*Pleurotus ostreatus*) has increased rapidly for the last decade and its production was ranked second after the white mushroom (*Agaricus bisporus*). The production and consumption of the mushroom have become extremely popular in Asia, especially in China and Korea (Chang and Miles, 1991; Lelley and Jansen, 1993). Meanwhile its production in Europe and North America remain unchanged practically over the last 15 years (Lelley and Jansen, 1993). This was primarily due to the low yield of the mushroom cultivated at a commercial scale in these regions. In order to cope with this problem and to maximize economic efficiency, the development of high quality compost is the most urgent problem to be solved in mushroom industry.

Composting is the process of preparing necessary nutrients through high temperature fermentation by thermophilic fungi. These important organisms grow optimally at temperature of greater than 40°C (Crisan, 1973). Thermophilic fungi grow extensively during the last phase of the composting process (Straatsma *et al.*, 1989; Wiegant, 1992), at which compost quality is usually determined. This process is especially important because it provides a selective pressure on the mushrooms to grow by reducing ammonia concentration, immobilizing nutrients, and positively affecting the extension rate of mycelium (Wiegant *et al.*, 1992).

The growth-promoting effect of a thermophilic fungus, *Scytalidium thermophilum*, on *Agaricus bisporus* has been well established. Some *Sepedonium* species were known as growth-promoting thermophilic fungi on oyster mushroom (*Pleurotus ostreatus*) while *S. thermophilum* showed no growth-promoting effect on oyster mushroom (Lee and Hyun, 2000). The optimum temperature for the growth of thermophilic fungi is around 45~50°C and the maximum temperature is less than 60°C for all thermophilic fungi although their spores may survive at much higher temperatures.

Composting is an aerobic process in which organic matters are partially mineralized and humidified. In order to produce good quality compost, the makers should consider the following three fundamental rules; 1) a relatively short process with low energy consumption, 2) a guarantee of the compost reliability, and 3) free from harmful pathogens. For these reasons, composting should be operated in a controlled system in order to produce end products of high quality.

Sinden and Hauser (1950, 1953) developed the first composting device attached with pre-wetting and mixing systems. On the basis of this composter, a three-phase-one system (Derks, 1973) and Phase I and Phase II of Spawn Run type composter (Edwards, 1977) were developed. Because the pre-wetting and Phase I stacks are usually controlled in part, temperature and oxygen levels within the compost fluctuate widely from 20°C to 80°C and from 0% to 21%, respectively (Randle and Flegg, 1978). These result in an inefficient composting (Smith, 1983), as well as atmospheric pollution from the production of ammonia in anaerobic regions within the stacks. Also odorous compounds (particularly sulfur compounds) and liquid run-off from the pre-wetted materials and Phase I stacks are sources of pollution (Derikx *et al.*, 1990).

For these reasons, several researchers have attempted to exert greater control over composting by manipulating the entire process in an enclosed system (Derks, 1973; Bech,

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1978; Smith, 1983; Laborde *et al.*, 1987; Perrin and Gaze, 1987; Gomez, 1998). However, many attempts to prepare the compost in an uniform manner were unsuccessful because of difficulty of operation, contamination of harmful microorganism, and a low yield of mushroom.

The purpose of this study is to design an upgrade bioreactor armed with a computerized controller which can control the fermentation environments and to evaluate the bioreactor for the commercial use. Therefore, in this study, we investigated environmental conditions such as temperature, carbon dioxide flux, and activity of thermophilic fungi during composting by the developed bioreactor.

Materials and Methods

Features of bioreactor. The principle underlying the construction of the solid-state bioreactor was to design a system that keeps moisture content constant, conserves heat, and maintains good aeration.

An experimental bioreactor was designed on the basis of a three-phase-one system and modified as a dynamic system that is closed and horizontal reactor with forced mixing screw (Fig. 1). A dynamic composting system was designed to control the exact temperature, amounts of oxygen, and moisture content of the compost. The main chamber of a bioreactor was constructed for composting 6,000 kg of compost as an end product. The size of bioreactor was 2.14 m (diameter), 2.17 m (height), 2.14 m (length) and 15.01 m^3 (volume). The main body was covered by a water jacket with a lined water tank for the exact control of temperature.

An automatic controller, PLC FPO-C32 (Matsushita Electric Co.) was equipped for main programmable logic controller and control panel with touch screen, MT 506L (Weinteck Labs.) was used for easy operation. IBM compatible PC with Pentium processor was used to input environmental factors and main O/S was Microsoft Windows 98. A 30HP geared motor with 1/60 motor reducer was used to mix the 6 tons of composting material. The dual ribbon mixer was contrived for easy mixing by bi-directional forcing. The ventilation system consisted of a 1HP air compressor (600 *l*/hour) and hepa filter on airlines. Air inlet line was mounted at the bottom cover of the bioreactor to provide a controlled flow of air through the compost. Overall ventilating air was composed of 80~90% recirculated air and 10~20% fresh air. Water circulation system with a water tank, water jacket, pump, sensors, solenoid valves, and PLC was installed for the maintenance of optimum temperature. The total volume of hot water in water jacket was calculated as 0.30092 m^3, the dimension and capacity of the water tank were 0.79× 0.70×0.90 m (W/L/H) and 0.498 m^3, respectively. Sensors and solenoid valves were attached to water and steam lines in the inside of the bioreactor (Fig. 2).

Organisms. *Sepedonium* sp. S-2 as thermophilic fungi was used for this study. Spore suspensions were obtained from growth on a potato dextrose agar (PDA, Difco) at 45°C. The commercial strain, *Pleurotus ostreatus* (KACC 500128), was obtained from the National Institute of Agricultural Science and Technology (Suwon, Korea), and used as mushroom mycelium in all experiments.

Media and growth conditions. *Sepedonium* sp. was maintained on PDA at 25°C. The fungi were cultured at 45°C in a medium containing 60 g of potato extract, 15 g

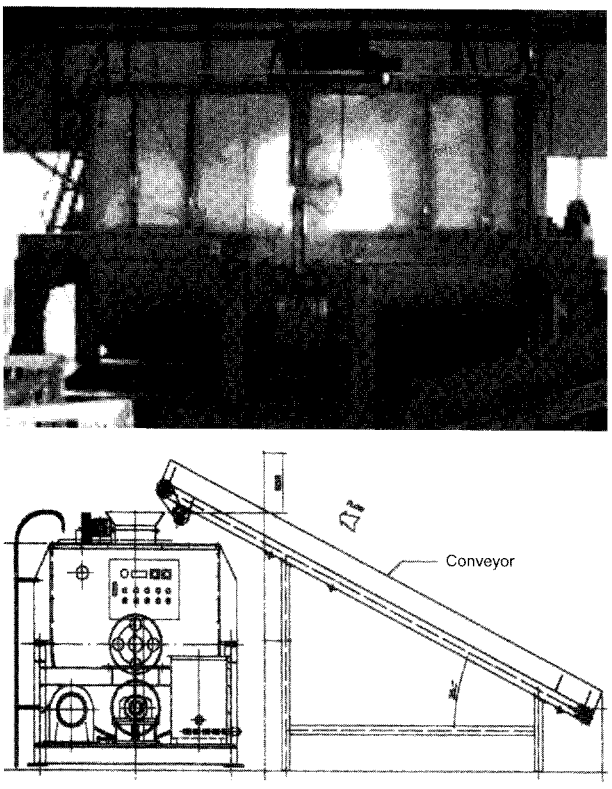

Fig. 1. Front and side view of solid-state bioreactor.

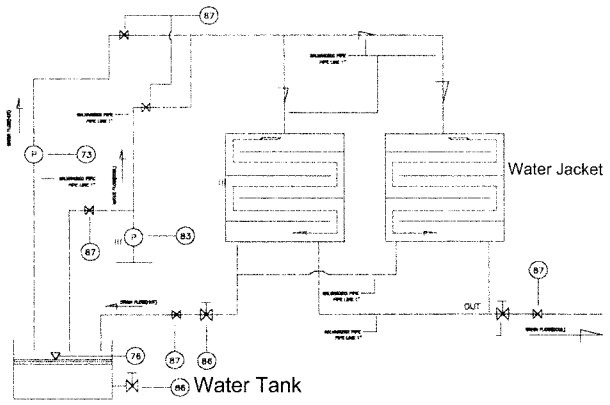

Fig. 2. Water circulation system for temperature control.

of commercial sugar, and 1 *l* of distilled water and harvested by filtration through sterile cheesecloth in a laminar flow hood.

P. ostreatus (KACC 500128) were grown on malt extract agar (MEA, Difco) plates at 25°C for 14 days. After 5mm diameter plug of an inoculum was removed by cork borer from the cultures of *P. ostreatus* (KACC 500128), the plug was placed on the center of agar plate of sterile composts prepared with different procedures and the growth rate was measured after the incubation at 25°C for 14 days.

Compost materials. The organic ingredients of the substrates were new season chopped rice straw, rice bran produced in Korea, and cotton waste imported from Pakistan. Bales of straw were chopped into pieces of 3 to 5 cm long in a bale chopper.

Composting procedure. The composting procedure consisted of three steps on the basis of the conventional Phase II composting; (1) wetting with water to achieve a moisture content of 70%, (2) steam pasteurization, and (3) high-temperature fermentation. Air was passed into the bioreactor at the rate of 0.5 ton per minute. The outdoor air temperature was 23~29°C during experiments. The moisture content of composting materials was adjusted to 70%. The air was shut off and the device was stopped at intervals to check temperature and carbon dioxide. Air samples for carbon dioxide determination were taken 1 minute after the air supply was shut off, and then measured by an infrared multi gas detector (SD 8313300, Drager, Germany). Temperature was measured with standard temperature probes.

In this study, three composting procedures were compared as follows; Procedure A: The compost was sterilized at 65°C for 8 hours, and then fermented at 45°C for 48 hours. Procedure B: After the compost was sterilized at 65°C for 8 hours, sterilized compost was inoculated with the thermophilic fungi, and then fermented at 45°C for 48 hours. Procedure C: The compost was fermented at 45°C for 48 hours, and then sterilized at 65°C for 8 hours.

Count of fungal population. 10-gram samples taken from the solid-state bioreactor were blended with 100 m*l* of distilled water, and then solids were removed with sterile cheesecloth. Either compost suspensions and dilutions, or washed compost particles were plated onto PDA plate containing ganamycin (100 mg/liter) to estimate fungal CFU per gram of fresh weight, or the percentage of particles showing recovery of thermophilic fungi, respectively. The amount of compost in the undiluted suspension was 0.1 g/m*l*. The resulting detection limit was 1 CFU/g on one colony per five plates. Plates were incubated at 45°C and were observed for up to 7 days.

Effect of composting condition on fungal population. Precise control of temperature is very essential to the maintenance of water content and fungal population.

The temperature control systems of two composters, one using a standard conventional temperature regime in mixer (CON) and the other using an automated controlled solid-state bioreactor (AUC) were compared during the composting of substrates made of cotton waste and rice straw.

Aeration time and mixing rate are the main variables affecting the composting temperature. In our bioreactor (aeration rate; 3 tons/hr), the relationship between temperatuer accuracy and fungal population was compared by the experiments under the combination of the aeration time (8, 15, and 30 min) and mixing rate (6, 12, and 18 rpm).

Results and Discussion

Comparison of composting procedure. The composting procedure differed fundamentally from the conventional process in that it maintained fermentation temperature with forced steam input. The automatically-controlled bioreactor system reduced the composting period to 2 days from 6 days in the conventional method. The data in Table 1 are typical experimental results. The number of thermophilic fungi increased during high-temperature fermentation. The carbon dioxide concentration increased rapidly, indicating an increase of fungal population. The data from the Procedure A and C show that thermophilic fungi grew logarithmically within 36 hours, even after pasteurization. This result indicated that the spores of thermophilic fungi were not killed all by pasteurization (Straatsma *et al.*, 1994a, 1994b). The procedure B, in which *Sepedonium* sp. was used as the

Table 1. Comparison of temperature, carbon dioxide, and number of thermophilic fungi in different composting procedures A, B, and C

Composting time (hour)	Temperature (°C)			Carbon dioxide (%)			Numbers of thermophilic fungi (Log_{10} CFU/g)		
	A	B	C	A	B	C	A	B	C
12	56	58	48	ND	ND	7.5	ND	ND	3.3
24	48	48	48	6.8	7.6	5.6	ND	3.2	4.8
36	46	49	49	6.2	12.2	10.3	2.1	5.3	5.6
48	47	48	47	8.8	9.8	8.6	3.3	6.6	6.4
60	48	49	62	7.6	8.8	ND	4.2	6.8	ND
72	47	51	65	6.8	7.2	ND	5.4	5.5	ND
84	45	48	53	7.2	8.6	4.8	5.6	5.8	2.4
96	48	47	49	7.6	6.6	3.4	5.6	5.2	3.6

ND means not detected.
The differences between composting procedures A, B, and C were elucidated in Materials and Methods.

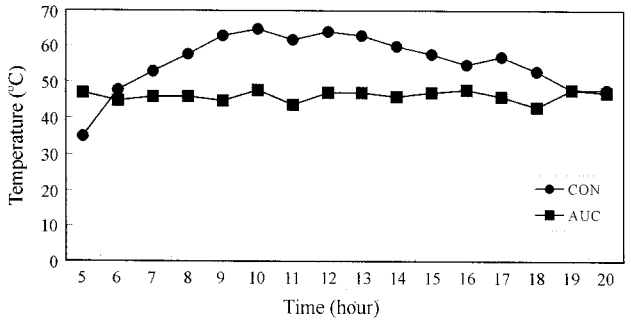

Fig. 3. Comparison of real temperature in compost controlled by conventional system (CON) and automatic bioreactor system (AUC). Temperature and mixing rate were fixed on 45°C and 8 rpm respectively and no aeration was maintained.

inoculant, resulted in the fastest composting process as suggested by Ross and Harris (1983).

Temperature accuracy of bioreactor. Figure 3 shows the accurate temperature readings in the newly developed solid-state bioreactor (AUC). As we hypothesized, the main computerized PLC controller was able to maintain the temperature within a 5% error limit. In order to control temperature accurately, we designed the system in such a way that a water jacket covers the main body of the bioreactor in a water tank. A controller was integrated into the both devices. The water tank and water jacket reduced the error rate to 5%, whereas the error rate of the bioreactor without this system was around 16%.

As in Fig. 4, the optimal temperature was maintained with 15 minutes of aeration time and 18 rpm mixing rate (B-3 group). The results indicated that over-aeration resulted in dry compost and inaccurate temperature control. Sufficient mixing dispersed heat and resulted in a uniform compost. In this study, aeration time was found to be the most important factor in controlling temperature accurately.

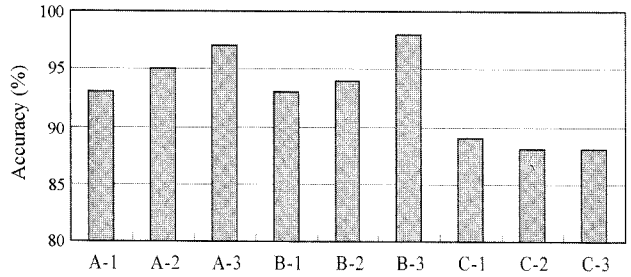

Fig. 4. Comparison of temperature accuracy by different aerating times and mixing rates on 45°C incubation. Experiments were carried out with different aerating times (minutes per hour); A (8 min), B (15 min), C (30 min), and mixing rates (rpm); 1 (6 rpm), 2 (12 rpm), 3 (18 rpm).

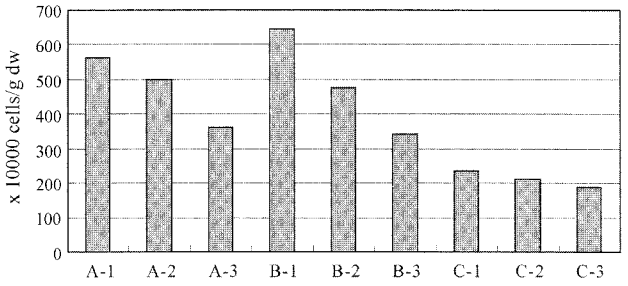

Fig. 5. Changes of population size of thermophilic fungi due to different aerating times and mixing times. Experimental conditions were the same as Fig. 4.

Effects of composting condition on fungal population. Figure 5 shows the change of fungal population size by using different composting methods after 60 hours of fermentation. Total fungal population was significantly increased in the first 20~30 hours by using well-cultivated thermophilic fungi as inoculate (1% volume over total input compost materials). The results indicate that the population dynamics of thermophilic fungi was strongly influenced by mixing time. This indicates that forced mixing resulted in mycelial cutting and inhibited fungal growth. From the results of Fig. 5, the authors concluded that B-1 group (6 rpm mixing and 15 minute aeration/hour) was the best condition for making compost in develped bioreactor.

Changes of growth rate of *Pleurotus ostreatus* (KACC 500128) by composting. The growth rate of *Pleurotus ostreatus* (KACC 500128) mycelium on autoclaved material without composting was 6~7 mm per day in petri dish (control group). Growth rate of mushroom mycelium was significantly increased by composting when compared with control and was almost as good as the group pre-incubated with *Sepedonium* sp. S-2. Maximum growth rate was achieved after 1 day incubation. Straatsma *et al.* (1989) also reported the effect of thermophilic fungi on mushroom growth.

The effect of thermophilic fungi on growth rate of mushroom mycelium was remarkable since the fungal

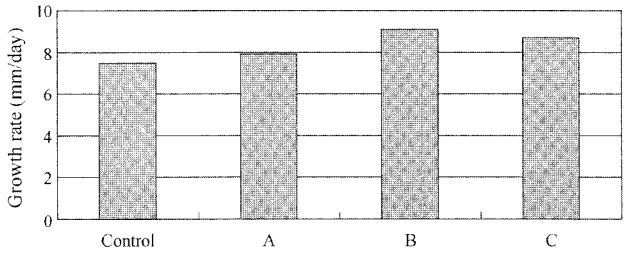

Fig. 6. Growth rates of *P. ostreatus* on compost in petri dishes. *P. ostreatus* was grown on sterilized materials without composting (Control) and composts prepared by procedure A (A), procedure B (B), and procedure C (C).

body was used as a food of higher quality with plentiful carbon and nitrogen sources. Wood and Fermor (1985) noted that the basic nutritional requirements for mushroom could be divided into four categories; carbon sources, nitrogen sources, minerals, and vitamins. For successful composting, compost makers must focus on the maintenance of near-optimal growth conditions for thermophilic fungi. These conditions were inadequately maintained by conventional methods (Stoller, 1987). Figure 6 shows the increased growth rates of oyster mushroom on compost fermented for only 48 hours in the newly developed solid-state bioreactor, which is successful.

Conclusion

The presence of thermophilic fungi is important for the successful colonization of mushroom mycelium in compost. The most important variable in making good quality compost is the maintenance of environmental conditions conductive for the growth of thermophilic fungi during composting process. The automatic control system of the solid-state bioreactor in this study can be applied to the industrial preparation of mushroom compost of high quality.

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