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Characterization of Fruitbody Morphology on Various Environmental Conditions in *Pleurotus ostreatus*

Kab-Yeul Jang*, Chang-Sung Jhune, Jeong-Sik Park, Soo-Muk Cho[1], Hang-Yeon Weon, Jong-Chun Cheong, Sun-Gyu Choi and Jae-Mo Sung[2]

Div. of Applied Microbiology, National Institute of Agricultural Science and Technology, R.D.A., Suwon 441-707, Korea
[1]Div. of Agriproduct Science, National Institute of Agricultural Science and Technology, R.D.A., Suwon 441-707, Korea
[2]Department of Environmental Biology, Kwangwon National University, Kwangwon 360-763, Korea
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This study investigated the morphological differences of *P. ostreatus* grown in the artificial environmental conditions such as humidity, temperature, ventilation, and watering. Oyster mushroom, which was cultivated on artificial environmental condition, was shown to have different morphology of fruitbodies. The optimum CO_2 concentration for good morphology of *P.ostreatus* was 0.3%. But most fruitbody showed the morphologically low qualities in more than 0.5% of CO_2 concentration. In the humidity in excess of 80% at 13~16°C, the best morphology of *P. ostreatus* was investigated. The growth of fruitbodies of *P. ostreatus* in the ventilation system was good at the wind velocity ranging from 0.2~0.5 fpms and expouring type. In other conditions, *P. ostreatus* generally showed the morphology closing to malformation.

KEYWORDS: Morphological characteristics, *Pleurotus ostreatus*, Ventilation system

Asians have popularly consumed *Pleurotus ostreatus*, and the demand for it has increased each year. In Korea, *P. ostreatus* has been cultivated for many years since artificial cultivation methods were developed in 1980's (Cha *et al.*, 1989). Its productivity occurring almost 51% in Korea and 21.5% in the world trends to increase as 72,348 metric tons in the area of 7,085,615 m^2 in Korea (Ministry of Agriculture & Forestry, 2003). Despite this increase, the production quality is rather limited because national mushroom cultivation houses were not enough to control environmental conditions for mushroom cultivation. Environmental conditions, however, were very important factors to the mushroom's growth and ing. The environmental factors as temperature, humidity, gases, light and ventilation affect the shape and yield of mushroom (Lambert, 1933; Donald, 1963; Flegg, 1978; Raudaskoski, 1982; Sohi, 1989). Paul *et al.* (1983) reported that high gases produce long stems and small undeveloped caps in *Agaricus brunnescens* and *P. ostreatus*. Abnormal fruitbodies of *P. ostreatus* in experiment developed under treatment with ca. 6,000 ppm CO_2 (Kinugawa, 1986). But there were few written reports related to environmental factors affecting mushroom morphology within our country. This experiment determined in terms of shape, morphology, and yield, the effects of temperature, relative humidity, and CO_2 concentration. The results of this study would serve as a useful guide in the mass cultivation of this mushroom in Korea and other countries.

*Corresponding author <E-mail: pdveg@up.nic.in>

Materials and Methods

Strain. Protoplast fusion products of *Pleurotus ostreatus*, ASI 2180, was obtained from the National Institute of Agricultural Science and Technology, Rural Development Administration (RDA). The culture was incubated on Potato Dextrose Agar (PDA) at 25°C (Yoo *et al.*, 1993).

Substrates. The cotton wastes and paddy straws were used for substrates to investigate mycelial growth of *P. ostreatus* on different CO_2 concentrations. To investigate morphological differences of *P. ostreatus* on different CO_2 concentrations, the poplar sawdust with rice bran 20% was used, put into 1,000 m*l*-polyethylene bottles, and sterilized at 121°C for 90 minutes. Two or three spoonfuls of precultured mother spawn in the sawdust medium were inoculated to the sawdust culture medium in 1,000 m*l*-polyethylene bottles. The inoculated sawdust media were incubated at 25°C for 30 days and then induced pin-heading in the experimental conditions. Cotton wastes were used for substrates to investigate morphological differences of *P. ostreatus* on different temperature and relative humidity. Its moisture content was adjusted to about 67% by watering. Then each mixed substrates of 5 kg were put into a 1.78 m^2-box. The substrates were pasteurized at 60~65°C for six hours and were fermented from 50~55°C for three days in the fermenting room. The substrates were inoculated by precultured spawn of 500 g and incubated at 25°C. After about 20 days of incubation, grown-up substrates were moved to the mushroom cultivation houses which were controlled by various temperature and

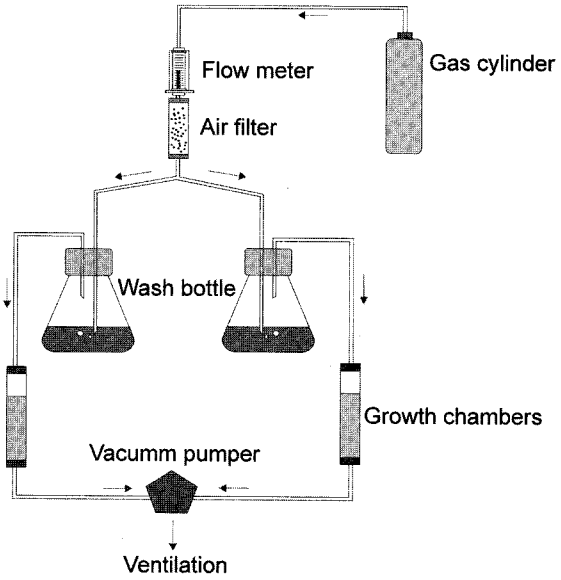


Fig. 1. CO_2 concentration controlling apparatus for the mycelial growth of *P. ostreatus*.

relative humidity.

Mycelial growth of *P. ostreatus* by CO_2 levels. The mycelial growth of *P. ostreatus* was measured using the CO_2 concentration controlling apparatus (Long, 1969). Namely, cotton wastes of which moisture content adjusted to 65% and weight of 100 g were put into test tube (ϕ3 cm × 20 cm) and sterilized at 121°C for 90 minutes. Inoculated cotton wastes were assembled at the apparatus (Fig. 1) and incubated at 25°C for 24 hours to evaluate the initial growth of mycelia. CO_2 concentrations were regulated in the ratio of 0.03, 0.1, 5, and 10% by the air mixture (Precision scientific, USA) and in the influx volume of 200 ml/min by the flow-meter in the 25°C.

Fruitdody yield and morphological characteristics of *P. ostreatus* in the CO_2 concentrations. The growth chamber (100 × 50 × 50 cm) made from acryl plate was used to investigate the morphological characteristics on different CO_2 concentrations (Fig. 2). In addition, the old spawn of the precultured sawdust media were scratched for regular primordial formation and covered with newspapers for maintenance of high humidity. Immediately after primordial formation, CO_2 gases in the concentration of 0.03, 0.1, 0.3, 0.5% flowed continuously in the growth chambers. Then temperature was adjusted to 13~16°C with cold water supplied by the copper pipe lying in the growth chamber. To maintain high humidity a damp absorbent cotton was placed.

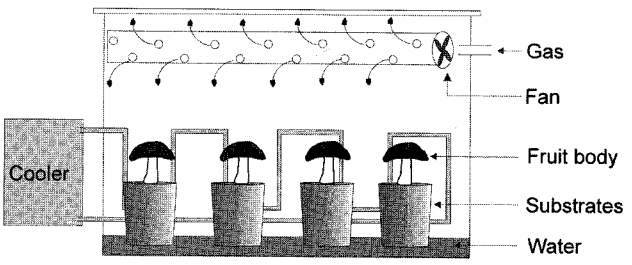


Fig. 2. CO_2 concentration controlling apparatus for the fruitbody development of *P. ostreatus*.

Morphological characteristics of *P. ostreatus* on different temperature and relative humidity. To investigate the morphological characteristics of *P. ostreatus* on different temperatures and relative humidity, temperature of mushroom cultivation house was adjusted to 13~16°C and 16°C or more, and relative humidity was adjusted to 60% or less, 60~80%, and 80% or more.

Characteristics of fruitbody morphology of *P. ostreatus* on the different ventilation system. To examine the morphological differences of *P. ostreatus* at different ventilation systems in the mushroom cultivation house, the mushroom was grown under controlled the inpouring ventilation system (IVS) and expouring one (EVS) adjusted to temperature of 13~16°C and humidity of 80% or more. The air of IVS flowed to the cultivation house through the unit-cooler and ones in EVS flowed out through the fan (Fig. 3). The conventional system (CVS) depending on natural ventilation served as the control.

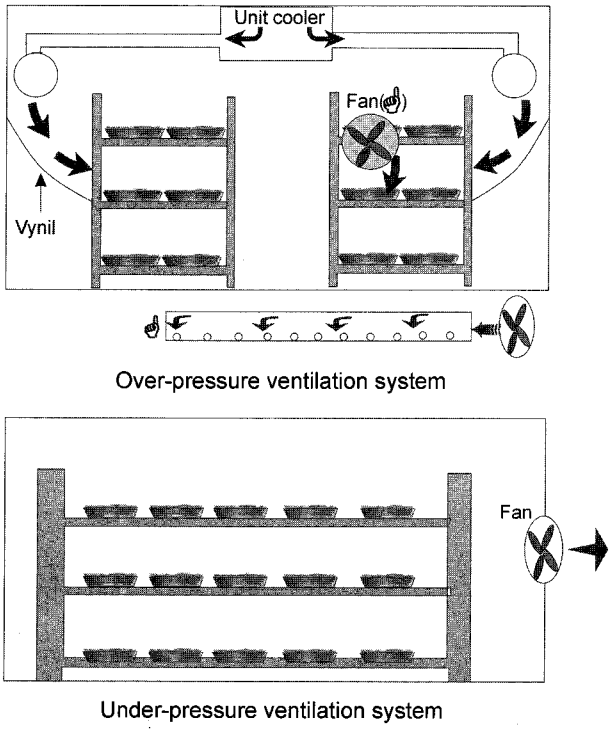


Fig. 3. The profile for the experiment by the different ventilation system.

Characteristics of fruitbody morphology of *P. ostreatus* on different watering frequencies and amounts. To investigate favourable watering frequency and amount for the good form and yield of *P. ostreatus*, the watering of 0, 1, 3 times a day and amount 0, 0.8, 2 *l*, 4 *l*/3.3 m^2 per watering time were done. And the yield and morphological characteristics of *P. ostreatus* on different watering times a flush, watering after 1~2 flush, and conventional watering were investigated.

Results and Discussion

Mycelial growth of *P. ostreatus* by CO_2 levels. The effects of CO_2 level on the mycelial growth of *P. ostreatus* are shown in Table 1. The mycelial growth of *P. ostreatus* measured good at 95 mm for 15 days on the paddy straw substrates in the CO_2 concentration of 0.1% and was moderate to be 90 mm for 15 days on the cotton substrates in 0.5% CO_2. On the paddy straw substrates, the mycelial growth was increased to 0.1% CO_2 level and decreased afterwards, while the mycelial growth on the cotton waste substrates was increased to 5% of CO_2 level (Table 1). Antonio (1972) reported that the optimum CO_2 concentration required for hyphal growth was between 0.1 and 0.5% CO_2 in *Agaricus bisporus*. It was suggested that the optimal CO_2 concentration on the mycelial growth of *P. ostreatus* should be higher than one in *Agaricus bisporus*.

Yield and morphological characteristics of *P. ostreatus* in the CO_2 concentrations. Cap size and yield of fruitbodies, as indicated in Table 2, decreased in opposition to the increase of CO_2 concentration. In the CO_2 concentration of 0.5%, the cap size was severely decreased. Stipe length increased in contrast with cap size and yield except 0.5% level. This result agrees with those of Kinugawa (1994). The stipe was elongated from 46 mm to 67 mm as long as CO_2 concentrations was increased to 0.03 to 0.3%, but was inhibited to 25 mm when CO_2 concentration of 0.5%. In 0.03 and 0.1% CO_2 concentration, the ratio of cap diameter to stipe length was 1:0.7 (65 mm:46 mm) and 1:2 (34 mm:68), respectively, to characterize normal types, while the ratio was 1:4 (6 mm:25 mm) to characterize elongation type of stipe in 0.3% CO_2 concentration. In 0.5% CO_2 concentration, the growth cap and stipe were inhibited and the shape of stipe was grown to jar types in abnormal (Fig. 4). The yield of *P. ostreatus* was high in the lowest CO_2 concentration of 0.03% to 289 g per 1,000 m*l* bottle, but was a tendency to decrease with the increase of CO_2 concentrations (Table 2).

Table 1. Mycelial growth of *P. ostreatus* on rice straw and cotton waste substrates in the CO_2 concentrations

Substrate	CO_2 concentration (%)			
	0.03	0.1	5	10
Rice straw	88[a]	95	73	68
Cotton waste	81	86	90	85

[a]The length (mm) of mycelial growth in the test tube (ϕ3 cm × 20 cm) for 15 days on apparatus described in the material and methods.

Table 2. Characteristics of fruitbody morphology and yield of *P. ostreatus* in the growth chambers adjusted to different CO_2 concentrations

Morphological characteristics and yield	CO_2 concentration (%)			
	0.03	0.1	0.3	0.5
Cap size	65[a]	34	24	6
Stipe length	46[a]	68	67	25
Yields	289[b]	251	201	53

[a]unit; mm.
[b]unit; g per bottle.

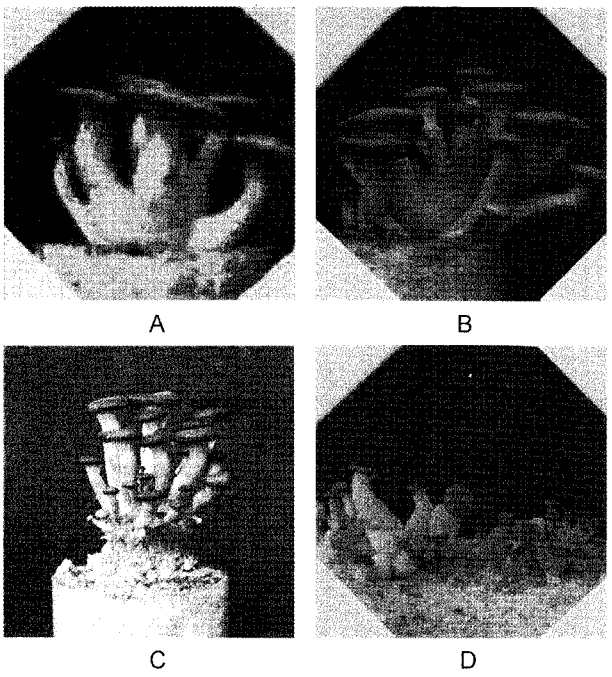


Fig. 4. Fruitbody morphology of *P. ostreatus* in the different CO_2 concentrations. A; 0.03%, B; 0.1%, C; 0.3%, D; 0.5%.

Morphological characteristics of *P. ostreatus* on different temperatures and relative humidity. Cap size and individual weight were 5.6 cm and 14.9 g, respectively, at the cultivation house controlled in the 13~16°C and 80% or more relative humidity, On the other hand, the cap size and stipe thickness decreased with 16°C and 80% or more relative humidity. Stipe thickness decreased severely with decrease of relative humidity, and individual weight was the lowest at 16°C or more and 60% or less relative humidity (Table 3). Deformed fruitbodies, of which color was pale gray and crumbly, appeared at 16°C or more and 60% or less relative humidity (Fig. 5).

Table 3. Fruitbody morphological characteristics of *P. ostreatus* on different cultivation conditions

Cultivation conditions		Morphological characteristics				Individual weight (g)
Temperature (℃)	Humidity (%)	Stipe (cm)		Cap (cm)		
		Length	Thickness	Size	Thickness	
excess 16	80 or more	5.4	1.1	2.6	2.6	13.7
	less 60	4.2	1.2	4.7	1.3	7.2
13~16	80 or more	5.3	1.3	5.6	1.9	14.9
	60~80	3.9	1.2	4.6	0.6	9.3
	less 60	4.1	1.5	4.9	0.2	13.4

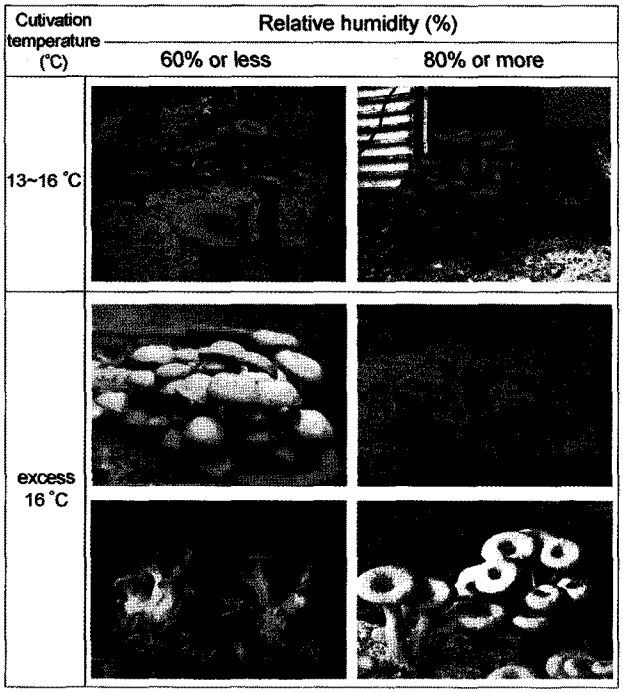
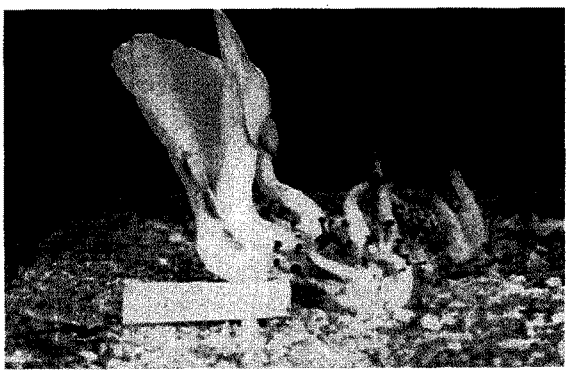


Fig. 5. Fruitbody morphology of *P. ostreatus* on different cultivation conditions.



Inpouring ventilation system

Expouring ventilation system

Fig. 6. Fruitbody morphology of *P. ostratus* on different ventilation system.

It was suggested that the optimal temperature and relative humidity for typical morphology and productivity of *P. ostreatus* to be suitable Korean preference were 13~16℃ and relative humidity of 80% or more.

Characteristics of fruitbody morphology of *P. ostreatus* on different ventilation systems. Variation of wind velocity and CO_2 concentration in the IVS was higher than those in the EVS, while EVS gave higher yields than

Table 4. Fruitbody morphological characteristics and yield of *P. ostratus* on different air ventilation system

Ventilation system	Wind velocity (fpm)[a]	Individual weight (g)	Expansion ratio (°)[b]	body yields (g/box)	CO_2 concentration (ppm)
IVS[α]	01	15.3	146	155	1850
	50 or more	10.7	164	250	1361
EVS[β]	0~0.2	8.8	110	530	530
	0.2~0.5	8.9	114	601	590
CVS[γ]	0.35	10.5	112	423	631

[a]fpm: feet per minute, [α]IVS: Inpouring ventilation system, [β]EVS: Expouring ventilation system, [γ]CVS: Conventional ventilation system.
[b]Expansion ratio: the angle of the stipe and cap.

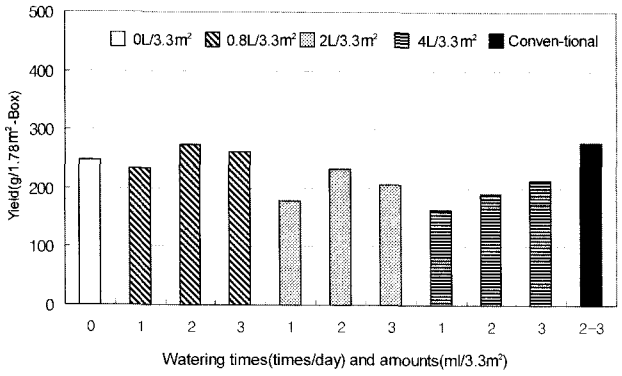


Fig. 7. Fruitbody yields of *P. ostratus* on different watering frequencies and amounts.

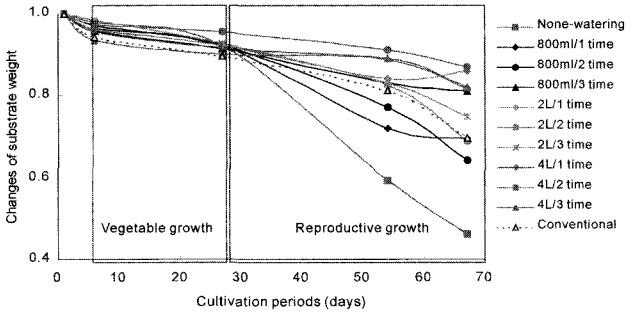


Fig. 8. Changes of substrate weight on different watering frequencies and amounts a day during the cultivation periods.

other systems. In the EVS, fruitbody appeared good shape, However, IVS caused the trumpet-like deformation of the cap, with expansion ratio of 146~164°, due to high CO_2 concentration and irregular wind velocity (Fig. 6). Kinugawa (1994) reported that pileus expansion was heavily damaged by trumpet-like deformation and yield decreased when the fruitbodies were exposed to high CO_2 concentration. So, it is recommended that the morphogenesis and yield of *P. ostratus* were influenced more by the wind velocity and CO_2 concentration than ventilation system in the cultivation house.

Characteristics of fruitbody morphology of *P. ostreatus* on different watering frequencies and amounts. When the cultures were sprinkled at the watering amount

Table 5. Fruitbody morphological characteristics of *P. ostreatus* on different watering frequencies after flush

Treatments	Cap(cm)		Stipe(cm)		Individual weight(g)	Total yield (g/1.76 m²)
	Size	Thickness	Length	Thickness		
No watering	4.9	0.2	4.5	1.4	8.3	821
Watering after 1flush	5.2	0.2	4.3	1.5	9.2	745
Watering after 2flush	4.7	0.2	4.3	1.5	11.9	833
Conventional	4.6	0.2	4.1	1.5	10.5	1,101

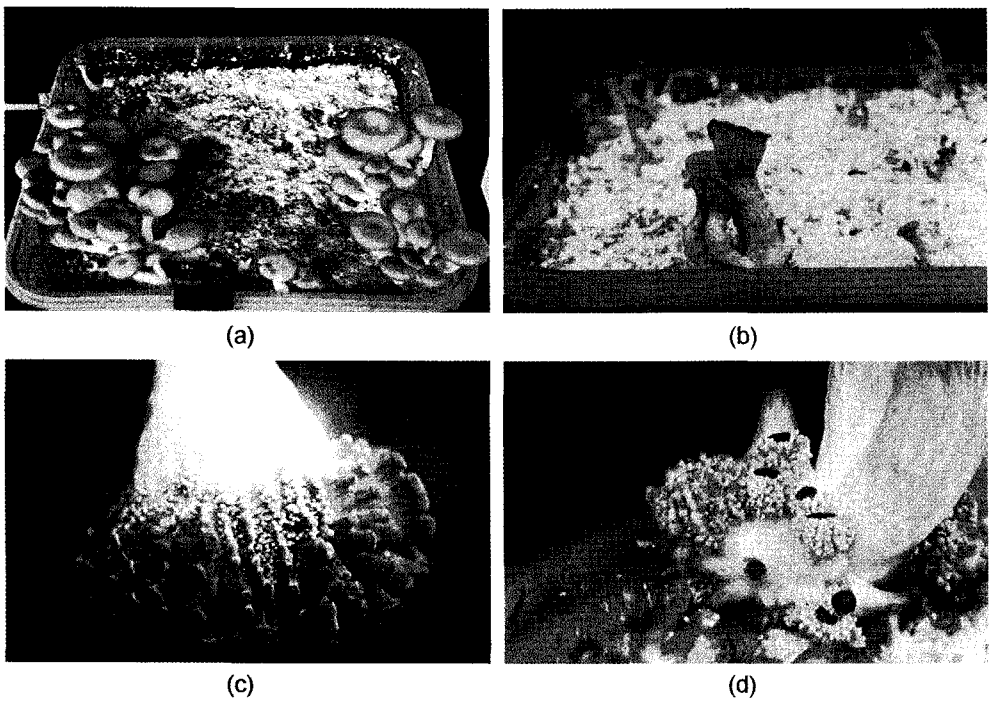


Fig. 9. Various deformation of Fruitbody of *P. ostreatus* on without watering during the cultivation periods.

of 0.8 *l*/day, the yield was intensive and intend to decrease with increase watering amounts. When the cultures were sprinkled at the watering of once a day, yield was the worst, But the conventional watering gave higher yields than other watering (Fig. 7).

There was no significant difference in morphological characteristics of *P. ostratus* by watering frequency and amount (data not shown). This result could be considered because bodies were harvested in one flush only. When the substrate weights were measured during vegetable growing periods, there were significant variations. But the weights decreased during reproductive growing periods. Specially, the weight of none-watering substrates decreased suddenly and it was observed that their substrates were separated from those inside of culturing box (Fig. 8, 9a).

Table 5 indicated no significant morphological characteristics in different watering frequencies after flush. When change of substrate weight had measured on different watering frequencies after flush, conventional watering substrates had higher yields than other and none-watering ones produced less yields than others (Table 5).

But individual weight of none-watering was lesser than other watering substrates including conventional watering. Stipe of some fruitbodies became brownish by drying (Fig. 9b) and regeneration of secondary fruitbody formation occurred (Fig. 9c, d) in none-watering substrates. This result suggested that the optimum watering of *P. ostreatus* should sprinkling at fruitbody before drying of substrates regardless of watering frequencies and amounts during the cultivation periods.

References

Antonio, J. P. S. and Thomas, R. L. 1972. Carbon dioxide stimulation of hyphal growth of the cultivated mushroom, *Agaricus bisporus* (Lange) sing. *Mushroom Science* **8**(1): 623-629.

Cha, D. Y., You, C. H. and Kim, K. P. 1989. New technology of mushroom cultivation. Sangrok press. pp. 1-19.

Donald, J. N. 1963. Role of carbon dioxide in the control of ing of *Schizophyllum commune*. *J. Bacteriol.* **85**: 1300-1308.

Lambert, E. B. 1933. Effect of excess carbon dioxide on growing mushrooms. *J. of Agricultural Research* **47**(8): 599-608.

Flegg, P. B. 1978. Effect of temperature on sporophore initiation and development in *Agaricus bisporus*. *Mushroom Science* **10**(1): 595-602.

Kinukawa, K. and Akira, S. 1994. Effect of concentrated carbon dioxide on the ing cultivated basidiomycetes (II). *Mycoscience* **35**: 345-352.

_____ and Tanesaka, T. 1990. Changes in the rate of CO_2 release from cultures of three basidiomycetes during cultivation. *Trans. Mycol. Soc. Japan* **31**: 489-500.

_____ and Takamatsu, Y. 1986. Effect of concentrated carbon dioxide on the ing cultivated basidiomycetes (I). *Trans. Mycol. Soc. Japan* **27**: 327-340.

Long, P. E. and Jacobs, L. 1969. Some observations on carbon dioxide and sporophore initiation in the cultivated mushroom. *Mushroom Science* **7**: 373-384.

Raudakoski, M. and Viitamen, H. 1982. Effect of aeration and light on body induction in *Schizophylum commune*, *Trans. Br. Mycol. Soc.* **78**(1): 89-96.

Ministry of Agriculture & Forestry. 2003. The actual production of industrial crop. pp. 8-9.

Paul, S. and Chilton, J. S. 1983. Chapter environmental factors: sustaining the mushroom crop in the mushroom cultivator. pp. 149-157.

Sohi, H. S. and Upadhyay, R. C. 1989. Effect of temperature on mycelial growth of *Pleurotus* species and their yield performance on selected substrates. *Mushroom Science* **12**(2): 49-56.

Yoo, Y. B., You, C. H. and Cha, D. Y. 1993. Strain improvement of the genus *Pleurotus* by protoplast fusion. *Korean. J. Mycol.* **21**(3): 200-211.