A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

CO_2 Control Strategies for Sustainable Cultivation of King Oyster Mushrooms and Romaine Lettuces in a Closed Production System

밀폐형 생산시스템 내 새송이 버섯과 로메인 상추의 지속적 재배를 위한 이산화탄소 제어 전략

BY

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FEBRUARY, 2016

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CO_2 Control Strategies for Sustainable Cultivation of King Oyster Mushrooms and Romaine Lettuces in a Closed Production System

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SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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FEBRUARY, 2016

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ABSTRACT

Although the research of life support systems is getting active, it is difficult to perform actual experiments because various internal components in the system react complicatedly. Therefore modeling and simulation approaches have increased in the research of the life support system. A concept of supplying the CO_2 generated by mushrooms to lettuces by simultaneous cultivation is important in both of the life support system and crop production. The objectives of this study were to establish a CO_2 emission rate model for mushroom respiration, a photosynthetic rate model for lettuce photosynthesis, and to develop a simultaneous cultivation system of lettuce and mushroom. The CO_2 emission rate of mushrooms and photosynthetic rate of lettuces were measured using closed acrylic chambers. The coefficients of the models were statistically determined. By using the established models, the simultaneous cultivation system was constructed for lettuce and mushroom, and the CO_2 concentration in each chamber was predicted by simulation. And the difference between measured and estimated values was verified. R^2 values of the CO_2 emission and photosynthetic rate models were 0.64 and 0.94, respectively. The CO_2 concentrations in the simultaneous cultivation system were controlled within allowable

ranges. With the simultaneous cultivation system, CO_2 emission into the atmosphere could be reduced to 80.6% of the total CO_2 emitted from mushrooms by respiration. Based on this study, it is possible to control the CO_2 concentration for optimum cultivation, and to reduce the CO_2 emitted into the atmosphere during mushroom cultivation. Finally, it will help us to find out the appropriate gas circulation strategies in the life support system researches.

Additional key words: Closed cultivation system, CO_2 exchange, CO_2 model, Lettuce canopy photosynthesis, Mushroom respiration

Student Number: 2014-20016

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GENERAL INTRODUCTION

Entering the 21st century, development of science and technology has been rapidly growing with an exponential increase of population, and many impossible things have become possible. However human beings are still not satisfied and trying to find something new. As a result, the interest in science fiction (SF) is getting hotter day by day. SF has been expressed as various formats, such as movie. Sunshine (2007), Snowpiercer (2013), Gravity (2013), Interstellar (2014), and Martian (2015) released in 10 years are good examples of the SF movies. Peoples appeared in the films want to go where cannot go or want to live where cannot survive. The films showed detail apparatus enable them alive. For example, in the Sunshine oxygen generated from the garden is supplied to the crew and a certain train compartment grows plants in the Snowpiercer.

The life support system can be divided largely into three parts of open-, semi-closed- and closed-loop systems according to the path and movement of resources. The open-loop system has an advantage that can be easily used to create, but a disadvantage in poor efficiency of long-term missions. The closed-loop system has a good efficiency in the long-term missions, but complex interactions among internal components (Barta and Henninger, 1994; Eckart, 1996). The regeneration techniques used in the closed-loop way include the physio-chemical regeneration and biological regeneration methods (Shaw, 2014). The biological regeneration method has been studied in some experiments such as Biosphere 2. It is considered as the only way that a massive ecosystem can be maintained over a long period of time (Nelson et al., 1994).

However, the actual design of closed-loop life support systems has many difficulties in demands of al lot of money for large-scale experimental equipment and biomasses as well as a long time to design. Therefore modeling and simulation techniques can be a good solution. Through creating a model for an actual system, expressing the physical phenomenon by mathematical form, and obtaining a hermeneutical solution by simulation, it is possible to derive the results of the experiments which are difficult to perform actually (Sokolowski and Banks, 2010).

Production and consumption of the CO_2 as elements in the life support system are suitable for the researches utilizing modeling and simulation techniques. There was an attempt to supply the CO_2 generated during mushroom cultivation to the lettuce cultivation (Kitaya et al., 1994). However, in this study, the amounts of CO_2 emitted by mushroom respiration and consumed by lettuce photosynthesis were not quantified. For efficient management of total cultivation system for mushrooms and lettuces, it is necessary to quantify and to establish the models of the CO_2 emission by mushrooms and consumed by lettuces.

The objectives of this study were to establish a CO_2 emission rate model for mushroom respiration and a CO_2 consumption rate model for lettuce photosynthesis, compare the actual and estimated CO_2 concentrations in the mushroom and lettuce chambers, and to develop a simultaneous cultivation system of lettuce and mushroom.

LITERATURE REVIEW

Mushroom Respiration Model

In mushroom cultivation, CO_2 is emitted during sporophore initiation, and mycelium and sporophore growth. The amount of CO_2 emitted depends on the specific strain, weight and moisture content of the medium, incubation temperature, and growth period (Jang et al., 2007). Zadražil (1975) reported that the mycelium growth of *Pleurotus* mushrooms is suppressed when exposed to high CO_2 concentrations of 22-28%. In addition, the expansion of sporophore pileus is inhibited at a CO_2 concentration of 3,000-9,000 μmol·mol^{-1} (Kinugawa et al., 1994), while the best quality of the mushroom sporophores are optimal at 2,400 μmol·mol^{-1} (Ryu et al., 2005).

There are existing mathematical models used to express the separate growth of medium and mycelium and sporophore mushroom parts. Likewise, the respiration rate of each of these parts can be represented using dry weight models of the mycelium and sporophores (Chanter and Thornley, 1978). The rates of respiration of the mycelium and sporophores can be derived from the respiration model developed by Thornley (1970).

$$R = M * W + P * \frac{dW}{dt}$$

where R is the CO_2 emission rate or respiration rate, W is the dry weight of mushroom, M is the maintenance coefficient, and P is the CO_2 production coefficient. And the mathematical model for mycelial growth and the initiation and growth of sporophores in

the mushroom crop is extended to allow for harvesting of the sporophores (Chanter, 1979). The model is used to demonstrate the importance of using regular harvesting schedules in mushroom cropping experiments.

Yang et al. (2011) reported that the effect of different temperature and humidity on mycelium growth was analyzed by using the GIS data acquisition tools. It is considered that the optimal temperature and relative humidity conditions for King Oyster mushroom growth were 16℃ and 95%.

Plant Photosynthesis Model

Explanatory models allow for testing hypotheses and synthesizing knowledge and facilitate comprehension of complex systems. Most explanatory models are photosynthesis based models (Marcelis et al., 1998). For leaves the light response curve of gross photosynthesis can be generalized to:

$$P_g = P_{g,max} \times f(x)$$

where $P_{g,max}$ is the maximum rate of leaf photosynthesis and x is the dimensionless parameter. To represent the function $f(x)$, one of the most general functions that express the photosynthesis is the non-rectangular hyperbola:

$$f(x) = \frac{1 + x - \sqrt{(1+x)^2 - 4x\theta}}{2\theta}$$

where parameter θ regulates the shape of this function.

In the biochemical approach of Farquhar et al. (1980) the photosynthetic properties of the leaf are expressed in the basic characteristics of Rubisco enzyme kinetics and electron

transport capacity. This approach can be allowed for description of the temperature and CO_2 dependence of photosynthesis. Thus, the FvCB model is consisted of Rubisco limited part and RuBP regeneration limited part:

$$\mathrm{A} = \begin{cases} V_{max}\dfrac{C_c - \Gamma^*}{K_c\left(1+\dfrac{O}{K_o}\right)+C_c} - R_d : \text{Rubisco limited} \\ \dfrac{J}{4}\dfrac{C_c - \Gamma^*}{C_c + 2\Gamma^*} - R_d : \text{RuBP regeneration limited} \end{cases}$$

where, V_{max} is the Rubisco activity, Γ^* is the CO_2 compensation point in the absence of dark respiration, K_c is the Michaelis-Menten constant for carboxylation by Rubisco, O is the partial pressure of oxygen within the chloroplast, K_o is the Michaelis-Menten constant for oxygenation by Rubisco, R_d is the rate of dark respiration in the light and J is the rate of electron transport.

However, in order to measure the canopy photosynthetic rate, the closed types of chamber are mainly used. The closed type chambers have a difficulty in measuring the steady state photosynthesis (Bugbee, 1992). Thus, the dynamic model for photosynthesis is required to express the canopy photosynthesis. Perhaps the equation that is most commonly used to describe steady-state photosynthetic response to light and CO_2 is the rectangular hyperbola:

$$P_g = \frac{\alpha I \beta C}{\alpha I + \beta C}$$

where P_g is the steady-state photosynthetic rate, I is the light-flux density, C is the CO_2 concentration and α and β are constants. However, the equation above can be modified through the solving simple differential equations to be used in the situation in which the

light fluctuation and CO_2 fluctuation (Kaitala et al. 1982; Thornley, 1974).

Life Support Systems

Astronauts are surrounded by the hostile environment of space. Only the life support system is dedicated explicitly to the survival of the crew. Complicated interactions exist between a lot of life support system technologies and components (Hendrickx et al., 2006; Volk and Rummel, 1987).

The life support system can be divided largely into three parts according to the path and movement of resources. There is an open-loop way that consumed resources by crew and discharged to waste, and a closed-loop way that regenerates all the resources to reuse. And a semi-closed-loop way is a compromise between the open-and closed-loop ways. The open-loop system has an advantage that can be easily used to create, but a disadvantage in poor efficiency of long-term missions. The closed-loop system has a good efficiency in the long-term missions, but complex interactions among internal components (Barta and Henninger, 1994; Eckart, 1996).

According to the Environmental Controlled Life Support System (ECLSS) roadmap released by NASA, the regeneration techniques used in the closed-loop way include the physio-chemical regeneration and biological regeneration methods (Metcalf et al., 2011). Shaw (2014) summarized that all the life support system can be classified systematically depending on the resources regeneration methods. The physicochemical regeneration life support system has a characteristic that relies on physical or chemical processes to transform or relocate resources. It mainly used in relatively short term space missions or

situations that easily supplied. On the other hand, the biological regeneration life support system has a characteristic that relies on biological processes to transform resources. In this case, living organism such as plant, bacteria or others are actively and intentionally used for the regeneration of the resources. The Earth is an entire biological regeneration life support system.

Biological regeneration method has been studied in some experiments such as Biosphere 2. It is considered as the only way that a massive ecosystem can be maintained over a long period of time (Nelson et al., 1994). Biological regenerative technologies developed by the Biosphere 2 project may find applications in the initial and near-term life support systems. Recently, the Virtual Habitat Project (V-HAB) was initialized to develop an engineering tool allowing dynamic simulations of different life support system architectures, enabling optimization of the life support system design in early stages of development (Olthoff et al., 2014). V-HAB project contains the plant model based on modified energy cascade model. The plant model consisted of two plant chambers; one containing wheat, soybean, potato and rice cultures and the other containing lettuce and beet.

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CHAPTER I

Estimation of CO_2 Emission Rate of King Oyster Mushroom with Temperature and Growth Stage

ABSTRACT

Temperature and CO_2 are important controllable factors in mushroom cultivation. CO_2 is produced during mushroom respiration, and accumulation of high concentrations of CO_2 can induce specific disorders in the fungi. The objective of this study was to quantify the CO_2 emission rate of King Oyster mushrooms (*Pleurotus eryngii* (DC.) Quél) as a function of temperature and growth stage. The CO_2 emission rates of the substrate and sporophores were separately measured in closed acrylic chambers (1.0m x 0.8m x 0.5m) at 8, 12, and 15 days after scratching at temperatures of 16℃, 18℃, 20℃, 22℃, and 25℃. Regression analysis was performed using the measured CO_2 emission rates to obtain respiration models. Finally, CO_2 emission rate models consisting of the substrate and sporophores were developed for the growth periods before and after thinning. The CO_2 emission rates estimated by the model were compared with the actual rates and showed an agreement of $R^2 = 0.642$. Finally, using the established model, the optimal CO_2 conditions for mushroom growth were obtained.

Additional key words: Model, Respiration, Sporophore, Substrate, Thinning

INTRODUCTION

Commercial cultivation of mushrooms has increased sharply over the last few decades with the development of cultivation techniques. Among mushrooms such as *Agaricus*, *Lentinus*, *Pleurotus*, *Auricularia*, *Volcariella*, *Flammulina*, and *Tremella* (Kües and Liu, 2000), Oyster mushrooms (*Pleurotus* genus) are widely cultivated in East Asia regions. The bottle cultivation methods developed since the 1950s have enabled large-scale production for commercial purposes (Iwase, 1997; Kües and Liu, 2000; Yamanaka, 2011). However, because King Oyster mushrooms (*P. eryngii*) are typically cultivated in bottles, adequate control of environmental factors such as temperature, humidity, and CO_2 concentration is required (Lee et al., 2003).

In mushroom cultivation, CO_2 is emitted during mycelium growth, sporophore initiation, and sporophore growth. The amount of CO_2 emitted depends on the specific strain, weight and moisture content of the medium, incubation temperature, and growth period (Jang et al., 2007). Mycelium growth and sporophore quality are negatively affected by accumulation of CO_2. Indeed, Zadražil (1975) reported that the mycelium growth of *Pleurotus* mushrooms is suppressed when exposed to high CO_2 concentrations of 22-28%. In addition, the expansion of sporophore pileus is inhibited at CO_2 concentrations of 3,000-9,000 μmol·mol^{-1} (Kinugawa et al., 1994), while the quality of the mushroom is optimal at 2,400 μmol·mol^{-1} (Ryu et al., 2005). To prevent physiological disorders during mycelium growth and sporophore formation in high CO_2 concentrations, levels should be adequately

controlled (Jang et al., 2007; Jang et al., 2009; Ryu et al., 2005). Quantitative analysis of mushroom CO_2 emissions is thus very important, although it has been rarely performed.

There are existing mathematical models used to express the separate growth of medium and mycelium and sporophore mushroom parts. Likewise, the respiration rate of each of these parts can be represented using dry weight models of the mycelium and sporophores (Chanter and Thornley, 1978). However, it is difficult to separate the medium from the mycelium under conditions of bottle cultivation. Thus, in order to establish the CO_2 emission rate model of the mushroom, it is necessary to determine the amount of mycelium in the substrate and use growth models reflecting the growth characteristics of sporophores. In order to estimate the mycelium abundance, an image processing method utilizing photographs of sections of medium was developed, in which the percentage change in mycelium as a function of temperature and humidity is expressed by a quadratic equation model (Yang et al., 2012). However, it is not possible to determine the change in respiration rate of different mushroom parts according to environmental conditions using existing models.

Repeated harvesting of sporophores from medium may reduce the number and weight of sporophores (Burton and Noble, 1993; Flegg and Smith, 1982). For practical purposes, a method involving cutting all but 1-2 large sporophores, called thinning, is widely used to improve the quality of King Oyster mushroom cultivars (Ryu et al., 2005). Therefore, models of the CO_2 emission rate of sporophores should include the thinning process. The objectives of this study were to separately measure the CO_2 emission rates of substrate and

sporophore and to establish a CO_2 emission rate model of mushrooms as a function of temperature and growth stage.

MATERIALS AND METHODS

Incubation and Growth Conditions of Mushrooms

King Oyster mushrooms (*Pleurotus eryngii* (DC.) Quél) cultivated in Ain-Farm (Geumgwangmyeon, Anseong, Korea) were used in this study. The disclosure strain was Sae song-ee No. 2, developed by Gyeonggi Agricultural Research Institute, Korea. The substrate used for mushrooms consisted of a 1:1:2 mixture of sawdust:corn cob:additive with a water content of 65-68%. The additives included beet pulp, soybean meal, wheat bran, and dried bean curd. For cultivation, 950 mL polypropylene bottles were filled with 570 g of substrate and sterilized for 60-90 min at 121℃. Inoculated mycelia were incubated for 26-28 days and post-incubated for 5 or 6 days in an incubation room maintained at a temperature of 21-22℃ and relative humidity of 65-70%. After scratching, the temperature and relative humidity in the incubation room were gradually changed from 18 to 15℃ and from 95 to 80-85%, respectively.

Measurement of the Mycelium Ratio in Bottles according to Growth Stage

In order to analyze the growth of mycelium and sporophore according to days after scratching (DAS), fresh and dry weights of mycelium and substrate were measured at DAS 8, 12, and 15. To estimate the percentage of mycelium in the substrate, photographs of five cross-sections of cylindrical bottles (at 2 cm intervals) were taken in three replications. Photographs were converted to binary images using Image J (US National Institutes of Health, Bethesda, MD, USA), and the ratios corresponding to the percentages of substrate and mycelium were obtained by calculating the number of pixels.

Measurement of CO_2 Emission Rate of Substrate and Sporophores according to Temperature and Growth Stage

To measure the CO_2 emission rates of substrate and sporophores, mushrooms were placed in closed acrylic chambers (1.0 m × 0.8 m × 0.5 m) maintained at a constant internal temperature (Fig. 1). CO_2 concentrations were measured using an infrared CO_2 sensor (LI-820, LI-COR, Lincoln, NE, USA) controlled by a data logger (CR1000, Campbell Scientific, Logan, UT, USA). Mushrooms at DAS 8, 12, and 15 were used for measurement of CO_2 emission rates. At DAS 12, all but the two largest sporophores of 2.5 to 3 cm were removed. Three mushroom bottles corresponding to each growth period were then placed in the chamber, and the CO_2 emission rates were measured in triplicate. The CO_2 emission rate of a single mushroom bottle was calculated by measuring the CO_2 concentration in the closed chamber every 5 sec. After removing sporophores, the CO_2 emission rate of the substrate was measured again using the same method. The CO_2 emission rates of the

mushrooms at DAS 8, 12, and 15 were measured at temperatures of 16℃, 18℃, 20℃, 22℃, and 25℃.

CO_2 Emission Models of the Substrate and Sporophore according to Temperature and Growth Stage

To establish a CO_2 emission model of the mushrooms, the measured CO_2 emission rates were regressed using the statistical program SPSS (SPSS Inc., Chicago, IL, USA). The CO_2 emission rate of the substrate as a function of temperature was expressed using Eq. I-1. The symbols used in the models are summarized in Table I-1.

$$R_m = a_1 * T^2 + a_2 * T + a_3 \quad \text{Eq. I-1}$$

Regression analysis was conducted at DAS 8 before thinning and at DAS 12 and 15 after thinning.

Although sporophore growth is known to follow the Gompertz equation (Chanter and Thornley, 1978), the sporophores of King Oyster mushroom in this study were harvested before reaching the stationary phase and were thus evaluated using an exponential function (Fig. I-2). Specifically, when the relative growth rate k is assumed to be constant after thinning, it is possible to express sporophore growth by substituting W_0 for W_T as follows.

$$W_s = W * e^{k*t} \quad \text{Eq. I-2}$$

Using the measured dry weights of the sporophores at DAS 8, 9, 11, 12, 13, and 15, regression analysis was performed with respect to time t.

The respiration rate of sporophores, divided into growth respiration and maintenance respiration, was determined by Eq. I-3, which consists of the dry weight and its rate of change (Cannell and Thornley, 2000; Chanter and Thornley, 1978; Thornley, 1970). By substituting Eq. I-2 into Eq. I-3, the following model of sporophore respiration was obtained (Eq. I-4).

$$R = M * W + P * \frac{dW}{dt} \quad \text{Eq. I-3}$$

$$R_s = M * W * e^{k*t} + P * W * k * e^{k*t} \quad \text{Eq. I-4}$$

The maintenance coefficient and CO_2 production coefficient in Eq. I-4 were defined as follows:

$$M = a_4 * T^2 + a_5 * T + a_6 \quad \text{Eq. I-5}$$

$$P = a_7 * T^2 + a_8 * T + a_9 \quad \text{Eq. I-6}$$

The regression coefficients in Eqs. I-5 and I-6 were determined using the measured CO_2 emission rates of the sporophores substituted into Eq. I-4. The model was expressed using two variable equations that included the growth period and temperature.

Validation of the CO_2 Emission Model

At DAS 7, 9, 11, 13, and 15, the CO_2 emission rates of the bottle-cultivated mushrooms at a temperature of 24℃ were measured in the closed chamber. The model was validated using linear regression analysis by comparing the measured CO_2 emission rates and those estimated from the model.

RESULTS

Mycelium Ratio and CO_2 Emission Rate of the Substrate with Growth Stage

The mycelium ratio of the substrate was 28.01±3.64%, 27.23±2.62%, and 32.08±3.91% at DAS 8, 12, and 15, respectively, and showed no significant difference as a function of time (Fig. I-2). Conversely, the CO_2 emission rates of the substrate before and after thinning were significantly different (Table I-2). Using the parameters in Eq. I-1, obtained by regression analysis, the CO_2 emission rates of the substrate before and after thinning were described using Eqs. I-7 and I-8, respectively. The value of R^2 in both cases was approximately 0.79, indicating the high reliability of the models.

$$R_{mb} = -1.313 * 10^{-5} * T^2 + 2.412 * T + 0.117 \quad \text{Eq. I-7}$$

$$R_{ma} = 0.027 * T^2 + 5.108 * T - 30.732 \quad \text{Eq. I-8}$$

Growth and CO_2 Emission Rate of Sporophores

The dry weight of the sporophores increased exponentially with time (Fig. I-3). The initial dry weight of sporophores before thinning (W_0) was 0.009 g, and the relative growth rate k according to Eq. I-2 was 0.549. The initial dry weight of sporophores after thinning (W_T) was 0.002, which was which obtained from Eq. I-2 using regression analysis. The value of R^2 in the two cases was estimated as 0.790 and 0.881, respectively. The CO_2 emission rates of the sporophores before and after thinning in Eq. I-4 were described using Eqs. I-9 and I-10, respectively.

$$R_{sb} = (0.009 * M_b * +0.00494 * P_b) * e^{0.549*t}$$ Eq. I-9

$$R_{sa} = (0.002 * M_a * +0.00110 * P_a) * e^{0.549*t}$$ Eq. I-10

Using the regression analysis with temperature, the values of M_b, M_a, P_b, and P_a were derived (Fig. I-4).

Total CO_2 Emission Rate of One Bottle of King Oyster Mushroom

The CO_2 emission rate models for a mushroom cultivation bottle before and after thinning were then described as Eqs. I-11 and I-12, respectively. Eqs. I-7 and I-9 before thinning and Eqs. I-8 and I-10 after thinning were applied to generate these models.

$$R_b = (9.670 * 10^{-5} * T^2 - 5.062 * 10^{-3} * T + 0.0789) * e^{0.549*t} - 1.313 * 10^{-5} * T^2 + 2.412 * T + 0.117$$ Eq. I-11

$$R_a = (9.670 * 10^{-5} * T^2 - 4.363 * 10^{-4} * T + 8.089 * 10^{-3}) * e^{0.549*t} + 0.0270 * T^2 + 5.108 * T - 30.732$$ Eq. I-12

These two models were described in a three-dimensional coordinate space (Fig. I-5), with the x, y, and z axes representing growth period, temperature, and CO_2 emission rate, respectively. The total CO_2 emission rate of mushroom cultivation bottles increased exponentially as the growth stage progressed and linearly as the temperature increased.

Validation of the CO_2 Emission Models

The CO_2 emission rates estimated by the models were in good agreement with those measured (Fig. I-6). Before the thinning stage, the CO_2 emission rate estimated by the model was close to 60 $\mu gCO_2 \cdot s^{-1}$, while actual values ranged from 40 to 110 $\mu gCO_2 \cdot s^{-1}$.

After the thinning stage, the estimated values were in the range of 130 to 150 $\mu gCO_2 \cdot s^{-1}$, but the actual values ranged between 100 and 200 $\mu gCO_2 \cdot s^{-1}$.

DISCUSSION

Mycelial growth in a limited volume of growth media can be represented by logistic curves that are dependent on the carrying capacity of K^+ (Koch, 1975). In a previous study, cross-sectional images of the substrate consisting of growth media and mycelia were used to identify a stationary phase in which the ratio of mycelia to the substrate was constant. The presence of this stationary phase suggested that the mycelia of the mushrooms used in our experiments were in the latter part of the logistic growth phase. This observation is consistent with previous research showing that mycelia enter into a stationary phase after six days of incubation (Yang et al., 2011). On the other hand, the mycelial dry weights of *Flammulina velutipes* are decreased after sporophore initiation (Kitamoto and Gruen, 1976). There are two possible explanations fir this observation. First, the nutrients in the medium may be reduced at the onset of the stationary phase as a result of mycelia feeding. Secondly, the nutrients may have moved to sporophores from the mycelia to facilitate sporophore growth (Chanter and Thornley, 1978). Indeed, the presence of an abundance of nutrients in the waste media of mushroom cultivation by-products has led to the use of such waste media as fodder in other applications (Kim et al., 2010). However, under the experimental conditions of the present study, the ratio of mycelium in the mixture was not decreased.

In a previous study based on image processing methods, the proportion of mycelium was determined to be greater than 60% during the entire growth period (Yang et al., 2011), while the present study identified a ratio of 30%. This difference may be due to the specific media used in the two studies: wood and corncob chips were used in the previous study, while the present study utilized sawdust. The difference in color between the media might have also affected our estimation. Therefore, it is necessary to provide a reference for estimating the proportion of mycelium when using image-based measurements. Soil respiration measurement systems can be used to measure the rate of CO_2 emission of mycelium in a given substrate (Bekku et al., 1995); however, it is difficult to measure the instantaneous respiration rate using this approach, as it relies on a blood collection syringe for gas sampling. Alternatively, respiration rates can be measured within a relatively short time by connecting the CO_2 analyzer and the chamber (Rochette et al., 1997). Likewise, since closed chamber systems are widely used for the measurement of photosynthesis to determine CO_2 consumption, an infra-red gas analyzer (IRGA) is useful to accurately measure CO_2 gas concentration (Takahashi et al., 2008).

During the thinning process, the base parts of the medium and sporophore suffer physical damage. Following these wounds, the mushroom respiration rate increases, and plant tissues age (Fonseca et al., 2002). Because the respiration of harvested mushrooms is closely related to their quality, this phenomenon has been quantified (Jacxsens et al., 2001). In this study, although there was no significant change in proportion of the mycelium (Fig. I-2), the CO_2 emission rate of the mycelium changed significantly (Table I-2). Based on this result, we inferred that the CO_2 emission rate increased due to thinning rather than

mycelium growth.

Utilizing the CO_2 emission rate models established in this study, it should now be possible to determine how CO_2 concentration increases in mushroom cultivation facilities. This should allow for development of a method for CO_2 control in mushroom cultivation, such as an optimum ventilation system. From a broader perspective, because CO_2 is one of the factors responsible for global warming, development of methods to minimize CO_2 emission will be useful for mushroom cultivation. For example, utilization of the CO_2 emitted during mushroom cultivation in plant photosynthesis has been studied (Jung et al., 2014; Kitaya et al., 1994; Tani et al., 1996).

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Table I-1. Parameters used in the models.

Parameter	Description
a_1~a_9	Regression parameters
k	Relative growth rate ($g \cdot g^{-1} \cdot d^{-1}$)
M	Maintenance coefficient (s^{-1})
M_a or M_b	Maintenance coefficient (s^{-1}) after or before thinning, respectively
P	CO_2 production coefficient [$gCO_2 \cdot$(g dry matter)$^{-1}$]
P_a or P_b	CO_2 production coefficient [$gCO_2 \cdot$(g dry matter)$^{-1}$] after or before thinning, respectively
R	CO_2 emission rate or respiration rate ($\mu gCO_2 \cdot m^{-2} \cdot s^{-1}$)
R_a or R_b	CO_2 emission rate of mushroom per bottle ($\mu gCO_2 \cdot m^{-2} \cdot s^{-1}$) after or before thinning, respectively
R_m	CO_2 emission rate of substrate ($\mu gCO_2 \cdot m^{-2} \cdot s^{-1}$)
R_{ma} or R_{mb}	CO_2 emission rate of substrate ($\mu gCO_2 \cdot m^{-2} \cdot s^{-1}$) after or before thinning, respectively
R_s	CO_2 emission rate of sporophores ($\mu gCO_2 \cdot m^{-2} \cdot s^{-1}$)
R_{sa} or R_{sb}	CO_2 emission rate of sporophores ($\mu gCO_2 \cdot m^{-2} \cdot s^{-1}$) after or before thinning, respectively
t	Days after scratching (DAS, d)
T	Temperature (℃)
W	Dry weight (g)
W_{at} or W_{bt}	Dry weight of sporophores (g) after or before thinning, respectively
W_O	Initial dry weight of sporophores (g)
W_T	Dry weight of sporophores (g) after thinning

Table I-2. CO_2 emission rate ($\mu gCO_2 \cdot s^{-1}$) of the substrate according to temperature and growth period.

Growth period (DAS[z])	Temperature (℃)					R^2
	16	18	20	22	25	
8	40.24±6.08[y]a*	40.13±1.14a	49.61±3.05a	54.43±4.31a	59.78±4.20a	0.795
12	61.36±10.49b	68.43±4.88b	77.54±5.63b	90.28±7.08b	115.42±10.08b	0.870
15	52.39±3.54ab	76.25±7.54b	83.48±4.00b	98.69±8.74b	112.45±11.91b	0.870

*Different letters indicate a statistically significant difference (ANOVA/Duncan) ($P<0.05$)

[z]DAS, days after scratching

[y]Mean±SD (n = 3).

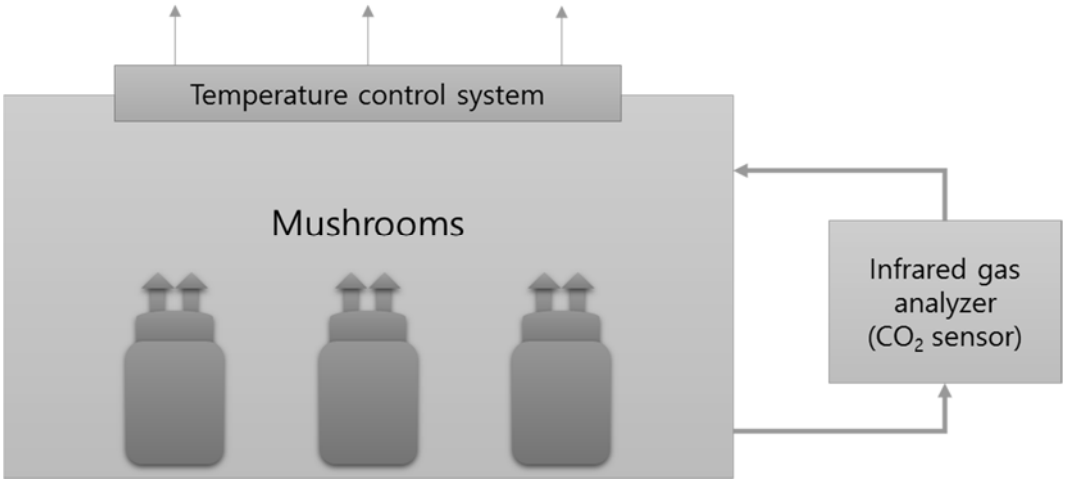

Fig. I-1. Measurement of the CO_2 emission rates of King Oyster mushrooms using a closed acrylic chamber (1.0m x 0.8m x 0.5m) in which the internal temperature was controlled within a range of 16-25℃.

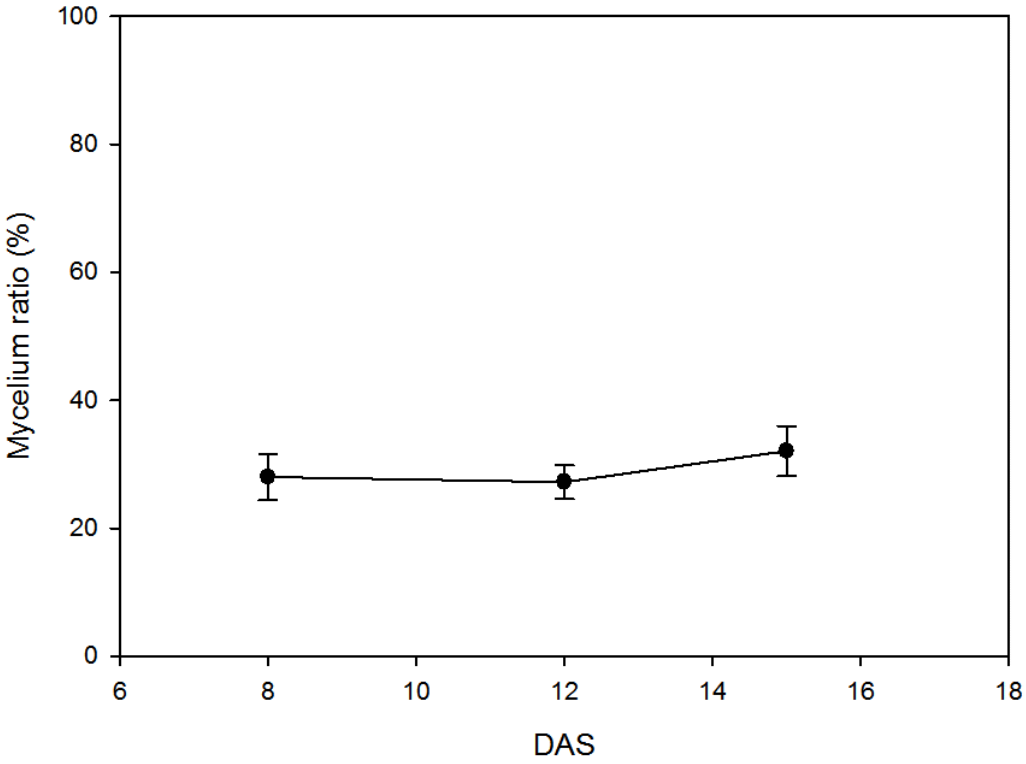

Fig. I-2. Change in the mycelium proportion of King Oyster mushrooms as a function of days after scratching (DAS). Data were collected by image processing. Vertical bars represent the mean ± SD (n = 15).

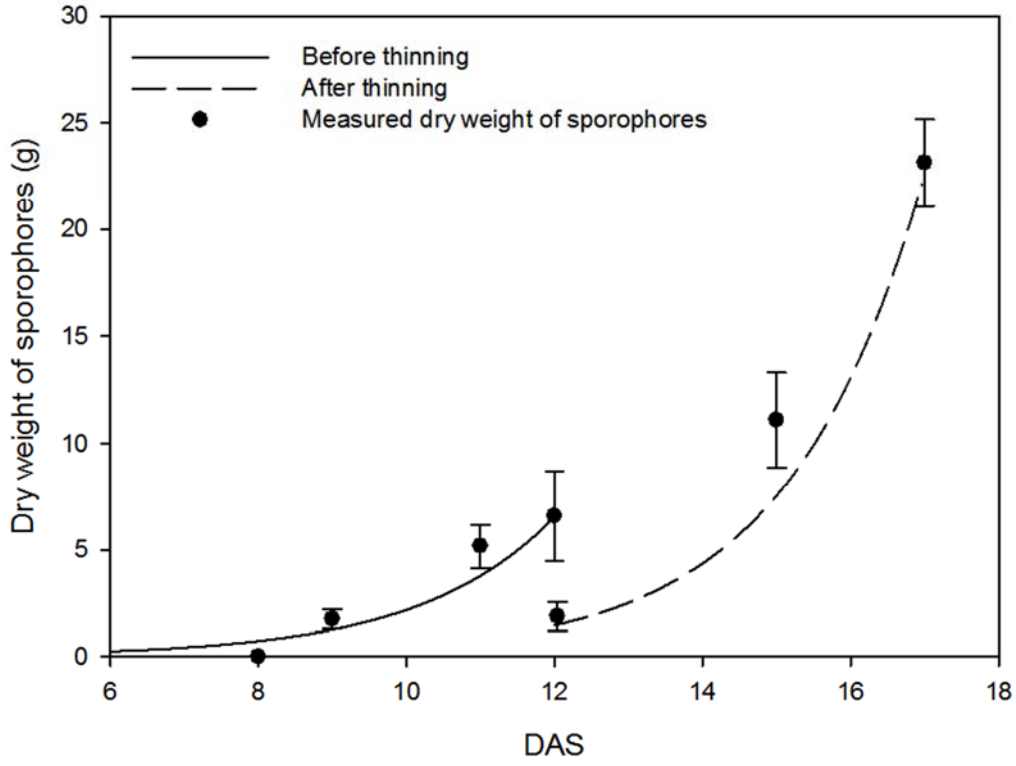

Fig. I-3. Change in the dry weight of King Oyster mushroom sporophores as a function of days after scratching (DAS). At DAS 12, all of the small sporophores were thinned, leaving only two large sporophores. Vertical bars represent the mean ± SD (n = 15).

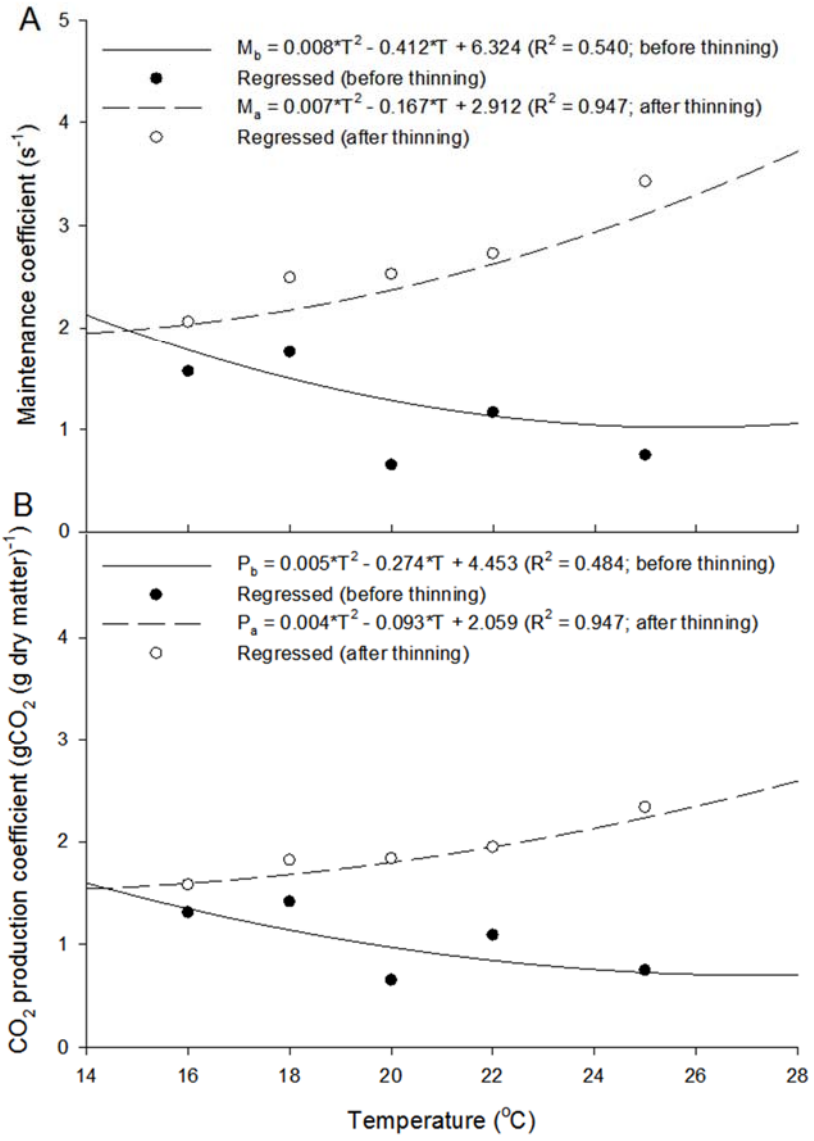

Fig. I-4. Quadratic equation regression results of the maintenance (A) and CO_2 production (B) coefficients as a function of temperature before and after thinning.

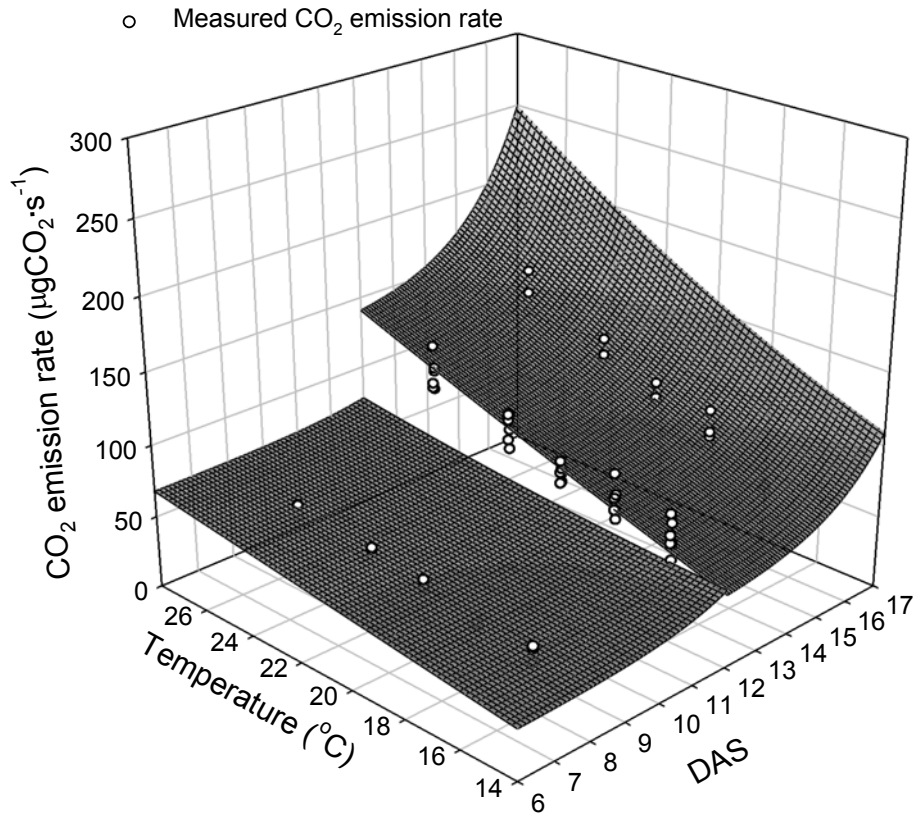

Fig. I-5. Three-dimensional diagram of the CO_2 emission rates of King Oyster mushroom cultivation bottles according to temperature and days after scratching (DAS).

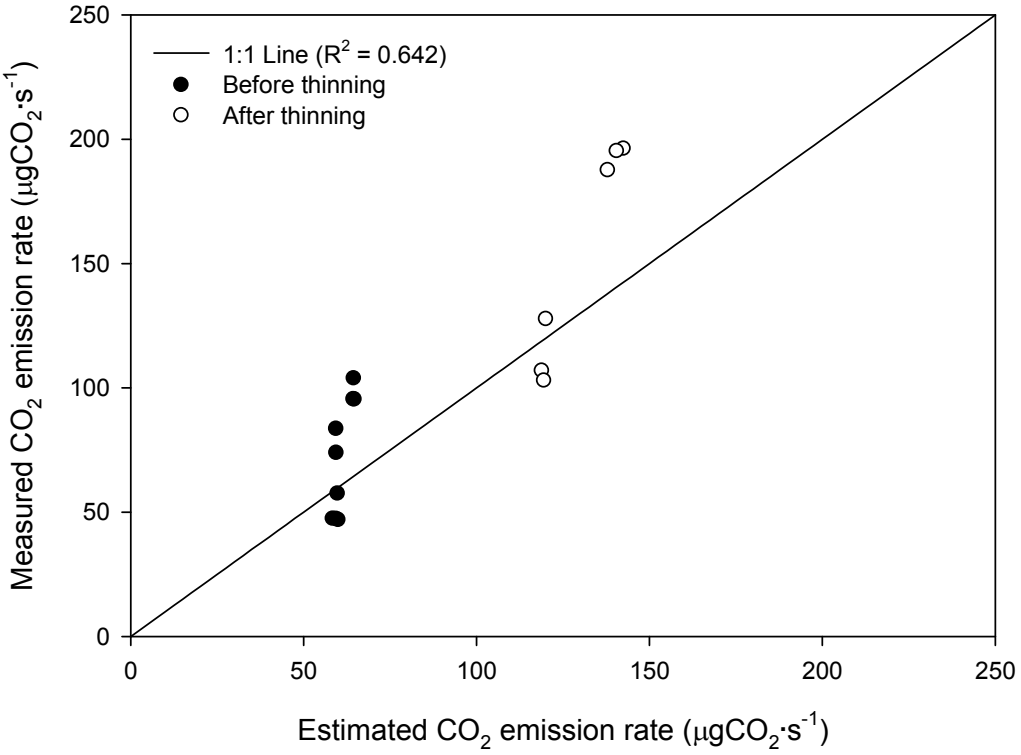

Fig. I-6. Validation of the estimated and measured CO_2 emission rates in King Oyster mushroom cultivation bottles.

CHAPTER II

Estimation of Canopy Photosynthetic Rate of Heuk Romaine Lettuce with CO_2 Concentration and Growth Stage

ABSTRACT

Since plants absorb the CO_2 in the atmosphere for photosynthesis, it is important to maintain adequate level CO_2 concentration for enhancement of crop production. To satisfy this purpose, changes in CO_2 concentration should be estimated by using photosynthetic rate models are required. The objective of this study was to estimate the canopy photosynthetic rate of romaine lettuce (*Lactuca sativa* L. 'Asia Heuk romaine') with CO_2 concentration and growth stage. Canopy photosynthetic rates of the plants were measured at 4, 7, 14, 21, and 28 days after transplanting by using closed acrylic chambers (1.0m×0.8m×0.5m), in which temperature and light intensity were maintained at 24℃ and 200 μmol·m^{-2}·s^{-1} with an 8:1:1 ratio of RBW light-emitting diodes, respectively. And 2000 μmol·mol^{-1} of initial CO_2 level were applied. By calculating the CO_2 consumption rate with time canopy photosynthetic rate of lettuce was determined. The canopy photosynthetic rate became saturated as the CO_2 concentration increased, and also showed exponential decreases with growth stage. Thornley model was suitable to express the canopy photosynthetic rate with R^2 = 0.985. The canopy photosynthetic rates estimated by the model were compared with those actually measured ones and showed a good agreement

with R^2 = 0.939. The established model can be helpful to determine the enrichment level of CO_2 concentration by growth stage and to calculate the reduced CO_2 amounts throughout cultivation period.

Additional key words: CO_2 consumption, Light-emitting diode, Light intensity, Thornley model

INTRODUCTION

As CO_2 concentration in the atmosphere becomes higher, global warming is continuously going. Since plants have abilities to fix CO_2 through photosynthesis, cultivation of plants helps to reduce the CO_2 concentration in the atmosphere. Particularly it can be better to maintain adequate level CO_2 concentration for enhancement of crop production (Leadley et al., 1999; McMurtrie and Wang, 1993). For investigating the increase in photosynthesis affected by environmental factors, it is useful to use a modeling techniques (Medina-Ruíz et al., 2011; Noe and Giersch, 2004).

Up to now, most of studies on photosynthetic model were to find out the photosynthetic reactions to environmental factors. In fact, it was difficult to make accurate models expressing photosynthesis under the complex effects of many environmental factors (Johnson et al., 2010). Thus, multivariable models have been recently used for

representing interactions of the variables in a simple multiplication form because of easiness and simplicity (Jones et al., 1991; Medina-Ruíz et al., 2011).

A model commonly used to express photosynthetic responses to light and CO_2 is a rectangular hyperbola model, showing that the photosynthetic rate increases like a saturation curve with increase of light intensity or CO_2 concentration. Thronley set a simple chemical formula for the light and dark reactions of photosynthesis (1974). By applying differential equations for each chemical formula, a rectangular hyperbola model empirically used was obtained (Kaitala et al., 1982). This model could express the change in photosynthetic rate with light intensity and CO_2 concentration, but could not express the change with growth stage. Because the light use efficiency is exponentially decreased with increase of leaf area index as plants grow (Green, 1987; Leadly et al., 1990), it is necessary to modify the model according to the growth stage.

Lettuce is a kind of leafy vegetable and easily used as a model crop for the research of plant factory. Several models were already well established for estimation of photosynthesis and growth in various cultivation systems (Caporn 1989; Shimizu et al., 2008). However, up to now, there were no attempts to apply the Thornley's multivariable model to estimate of the photosynthesis of lettuces. The objective of this study was to estimate the canopy photosynthetic rate of romaine lettuce (*Lactuca sativa* L. 'Asia Heuk romaine') with CO_2 concentration and growth stage.

MATERIALS AND METHODS

Plant and Growth Conditions

For measuring the canopy photosynthetic, Heuk romaine lettuces (*Lactuca sativa* L. 'Asia Heuk Romaine') were hydroponically grown in a plant factory module of Seoul National University. Yamazaki's nutrient solutions with an electrical conductivity (EC) of 1.2 $dS \cdot m^{-1}$ were supplied to the plants. Inside temperature and light intensity were maintained at 24℃ and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 8:1:1 ratio of RBW light-emitting diodes (LEDs), respectively. The lettuces were transplanted 3 weeks after sowing, and grown for 4 weeks with a planting density of 25 cm×25 cm.

Measurement of Canopy Photosynthetic Rate according to CO_2 Concentration and Growth Stage

The canopy photosynthetic rates were measured by using closed acrylic chambers (1.0 m×0.8 m×0.5 m) at five growth stages (4, 7, 14, 21, and 28 days after transplanting; DAT), when 24, 24, 12, 8, and 8 lettuces were grown in each stage, respectively. Planting density of the plants in the chamber was set to the same during the experiment. Temperature and relative humidity were maintained at 24℃ and 75%, respectively. Light intensity was 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 8:1:1 ratio of RBW LEDs. Initial CO_2 concentration was set at 2000 $\mu mol \cdot mol^{-1}$. Changes in CO_2 concentration was measured by an infrared gas analyzer (LI-

840A, LI-COR, Lincoln, NE, USA) and controlled by a data logger (CR1000, Campbell Scientific, Logan, UT, USA). Using three chambers were used for 3 replicates. Shoot fresh and dry weights, and leaf area of the plants were measured at each growth stage.

Estimation of Intercellular CO_2 concentration

In order to express the A/Ci curve by using measured CO_2 concentration in the chambers, it is necessary to know the relationship between the atmospheric and intercellular CO_2 concentrations. Eight models express the exchange of CO_2 between atmosphere and leaves of plants (Katul et al., 2000). Among them, the Ball-Berry model has been widely used because of its simple form as follows:

$$\frac{C_i}{C_a} = 1 - \frac{1}{m} * \frac{1}{RH} \qquad \text{Eq. II-1}$$

where, C_i and C_a are the intercellular and atmospheric CO_2 concentrations ($\mu mol \cdot mol^{-1}$), respectively. RH is the relative humidity and m is an empirical parameter. In fact, m ranges from 3 to 10 for various plant species, but has not been reported for lettuce (Leuning, 1995). The symbols used in the models were summarized in Table II-1. To estimate the m value of lettuce, C_i, C_a and RH values were measured by an using leaf photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) and regressed with Eq. II-1.

Establishment of Canopy Photosynthetic Rate Model according to CO_2 Concentration and Growth Stage

Using atmospheric CO_2 concentration measured over time in the chambers, its moving average of 12 minutes was calculated. The CO_2 concentrations measured in each growth stage were converted to the intercellular CO_2 concentration by using Eq. II-1

Photosynthetic rate with CO_2 concentration was obtained by using Eq. II-2 (Thornley, 1974).

$$P = \frac{a*b*PPFD*C_i}{a*PPFD+b*C_i} + c \quad \text{Eq. II-2}$$

where, P means the photosynthetic rate ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$) and $PPFD$ is the photosynthetic photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$). a, b, and c are the photochemical efficiency ($\mu molCO_2 \cdot \mu mol^{-1}$), carboxylation conductance (s^{-1}), and dark respiration ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$), respectively. Because the light use efficiency is exponentially reduced, a decrease in photosynthetic rate with growth stage could be expressed as follows:

$$P = p * e^{-q*DAT} \quad \text{Eq. II-3}$$

where, DAT is the days after transplanting, and p and q are regression parameters.

A simple multiplication model (Eq. II-4) was developed by multiplying Eqs. II-12 and II-3 as follows:

$$P = \left(\frac{a*b*PPFD*C_i}{a*PPFD+b*C_i} + c\right) * (p * e^{-q*DAT}) \quad \text{Eq. II-4}$$

By inserting Eq. II-1 to Eq. II-4 a photosynthetic rate model (Eq. II-5) was obtained as follows:

$$P = \frac{p_1*p_2*e^{-(q_1+q_2)*DAT}*PPFD*C_i}{p_1*e^{-q_1*DAT}*PPFD+p_2*e^{-q_2*DAT}*C_i} + p_3 * e^{-q_3*DAT} \quad \text{Eq. II-5}$$

All the regression parameters in the simple multiplication model and Thornley model were analyzed using a statistical program SPSS (IBM, New York, NY, USA).

Validation of the Simple Multiplication Model and Thornley Model

The simple multiplication model and Thornley model were validated with linear regression analysis by comparing the measured and estimated canopy photosynthetic rates. For validation, the plants grown in the same period were used when the models established.

RESULTS

Shoot Fresh and Dry Weights, and Leaf Area according to Growth Stage

Shoot fresh and dry weight, and leaf area increased exponentially according to growth stage (Fig. II-1). The shoot fresh and dry weights, and leaf area were 154.58±56.79 g, 5.40±1.95 g, and 2595.57±770.65 cm^2 at DAT 28, respectively. Especially, the leaf area (LA) was regressed as following exponential model:

$$LA = 64.76 * e^{0.132*DAT} \quad \text{Eq. II-6}$$

The value of R^2 in the model was estimated to be 0.849.

Intercellular CO_2 Concentration

Intercellular CO_2 concentration increased linearly according to atmospheric CO_2 concentration (Fig. II-2). As the atmospheric CO_2 concentration increased from 100 to 1000 μmol·mol^{-1}, the intercellular CO_2 concentration increased from 80 to 800 μmol·mol^{-1}, respectively. The m value in Eq. II-1 was estimated to be 5.041 at a RH of 75%. The value of R^2 in the Ball-Berry model (Eq. II-1) was estimated to be 0.968.

Canopy Photosynthetic Rate Models according to Intercellular CO_2 Concentration and Growth Stage

Canopy photosynthetic rate of the plants according to intercellular CO_2 concentration increased showing a saturation curve (Fig. II-3). The CO_2 compensation points were estimated to be 70 μmol·mol^{-1} in all the growth stage. The maximum canopy photosynthetic rates were 26, 22, 16, 8, and 5 μmolCO_2·m^{-2}·s^{-1} at DAT 4, 7, 14, 21, and 28, respectively. The maximum canopy photosynthetic rates decreased exponentially as growth stage progresses. The canopy photosynthetic rate was saturated over an intercellular CO_2 concentration of 1200 μmol·mol^{-1} at DAT 4 and 7, 1000 μmol·mol^{-1} at DAT 14, and 800 μmol·mol^{-1} at DAT 21 and 28.

A regression analysis result for the simple multiplication model was shown in Fig. II-5 A. The canopy photosynthetic rate decreased exponentially as growth stage progresses. And the canopy photosynthetic rate increased as a saturation curve as intercellular CO_2 concentration increases. By regression analysis, the parameters of a, b, c, p and q in

Eq. II-4 were estimated to be 0.688, 0.904, -42.694, 0.406 and 0.055, respectively. The simple multiplication model was expressed as follows:

$$P = (\frac{124.3904 * C_i}{137.6 + 0.904 * C_i} - 42.694) * 0.406 * e^{-0.055 * DAT} \quad \text{Eq. II-7}$$

The value of R^2 in the model was estimated to be 0.985.

The photochemical efficiency, carboxylation conductance, and dark respiration in Eq. II-2 were regressed with high R^2 values according to growth stage (Table II-2). The photochemical efficiency and carboxylation conductance decreased exponentially, while the dark respiration increased exponentially (Fig. II-4). As results, Thronley model could be expressed as follows (Fig. II-5 B).

$$P = \frac{21.812 * e^{-0.107 * DAT} * C_i}{57.4 * e^{-0.057 * DAT} + 0.380 * e^{-0.050 * DAT} * C_i} - 18.608 * e^{-0.056 * DAT} \quad \text{Eq. II-8}$$

The value of R^2 in the model was estimated to be 0.985.

Validation of the Simple Multiplication Model and Thornley Model

The canopy photosynthetic rates estimated by the simple multiplication model and Thornley model showed good agreements with measured ones (Fig. II-6). The values of R^2 in the two models were estimated to be the same value of 0.939. However in early stages of DAT 4 and 7, the measured canopy photosynthetic rates were slightly lower than the estimated ones.

DISCUSSION

It is preferred to use CO_2 flux for expressing leaf photosynthesis. However for several plants in the chamber, canopy photosynthesis can be expressed as CO_2 absorption per plant. Thus, the leaf area model as Eq. II-6 can be used when the photosynthetic rate of one plant is required in the canopy.

Various types of chamber are used for the measurement of photosynthesis (Mitchell, 1992), and the closed types are mainly used for the measurement of canopy photosynthesis. The closed systems have an advantage in its simple equipment, but a disadvantage of difficulty in measuring the steady state photosynthesis (Bugbee, 1992). Thus, the Thornley's dynamic photosynthesis model is more appropriate to use measured canopy photosynthetic rates.

In measuring the photosynthetic rates of willow plants at a light intensity of 220 $\mu mol \cdot m^{-2}$, the photochemical efficiency and carboxylation conductance were estimated to be 0.00028 $\mu mol^{-1} \cdot m^2$ and 0.001053 (kg CO_2 $m^{-3}) \cdot s^{-1}$, respectively (Kaitala et al., 1982). And the photochemical efficiency of *Atriplex hastata*, was estimated to be 0.177 $\mu molCO_2 \cdot \mu mol^{-1}$ (Marshall and Biscoe, 1980). However, these studies did not track the changes in photochemical efficiency and carboxylation conductance with growth stage. In this study, the photochemical efficiency and carboxylation conductance of the lettuce tended to exponentially decrease as growth stage progresses.

A simple multiplication model could be easily applied because of its simplicity in the form. However, in order to express the changes in photochemical efficiency and

carboxylation conductance according to growth period, Thornley model can be better because it reflects the physiological reactions of the plants. The R^2 values of the simple multiplication model and Thornley model were 0.985 when regressed and 0.939 when validated. Although the both R^2 values were reliable, it is better to use Thornley model because it reflects the physiological reactions.

From many studies on various plant species, it was known that CO_2 enrichment is effective to increase the plant biomass (Leadley et al., 1999). Quantification of the CO_2 consumption through photosynthesis will help to establish the efficient CO_2 enrichment strategies in plant production. It can also be coupled with microclimate models reflecting environmental conditions in greenhouse or plant factory for adequate management of crop cultivation.

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Table II-1. Parameters used in the models.

Parameter	Description
C_i	Intercellular CO_2 concentration ($\mu mol \cdot mol^{-1}$)
C_a	Atmospheric CO_2 concentration ($\mu mol \cdot mol^{-1}$)
RH	Relative humidity
m	An empirical parameter
P	Canopy photosynthetic rate ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)
$PPFD$	Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
a	Photochemical efficiency ($\mu molCO_2 \cdot \mu mol^{-1}$)
b	Carboxylation conductance (s^{-1})
c	Dark respiration ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)
DAT	Days after transplanting (d)
p and q	Regression parameters
LA	Leaf area (cm^2)

Table II-2. Regression results of photochemical efficiency, carboxylation conductance, dark respiration, and R^2 values.

DAT	Photochemical efficiency ($\mu molCO_2 \cdot \mu mol^{-1}$)	Carboxylation conductance (s^{-1})	Dark respiration ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)	R^2
4	0.235	0.333	-15.967	0.970
7	0.182	0.258	-12.004	0.995
14	0.146	0.225	-9.564	0.995
21	0.085	0.134	-5.825	0.992
28	0.054	0.070	-3.374	0.997

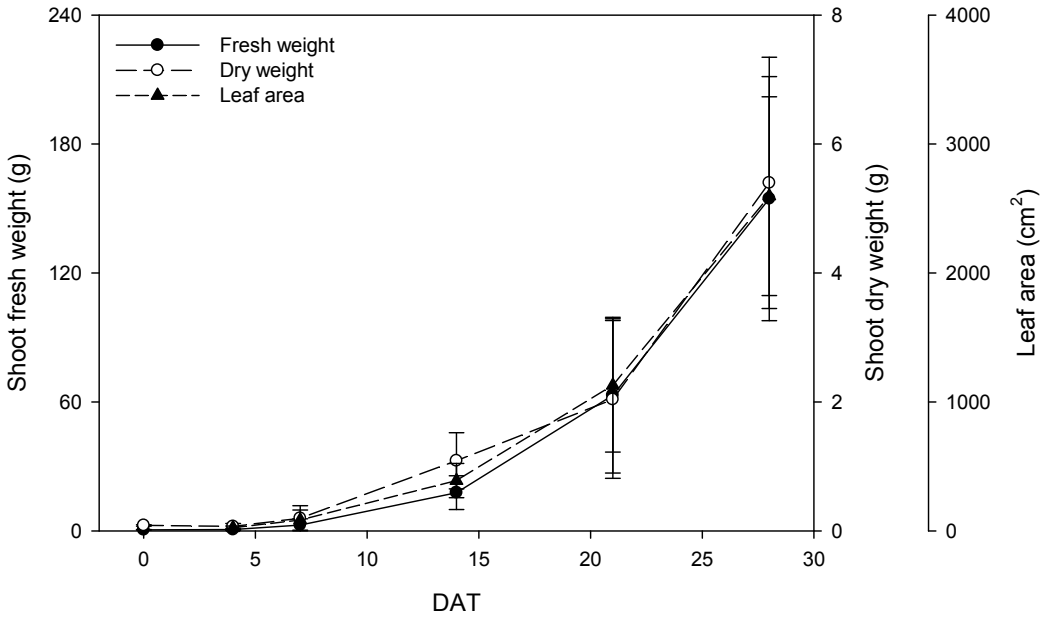

Fig. II-1. Measured shoot fresh (solid circle and solid line) and dry (void circle and medium dash) weights, and leaf area (solid triangle and short dash) of the lettuce with growth stage. Vertical bars represent the mean ± SD (n = 15).

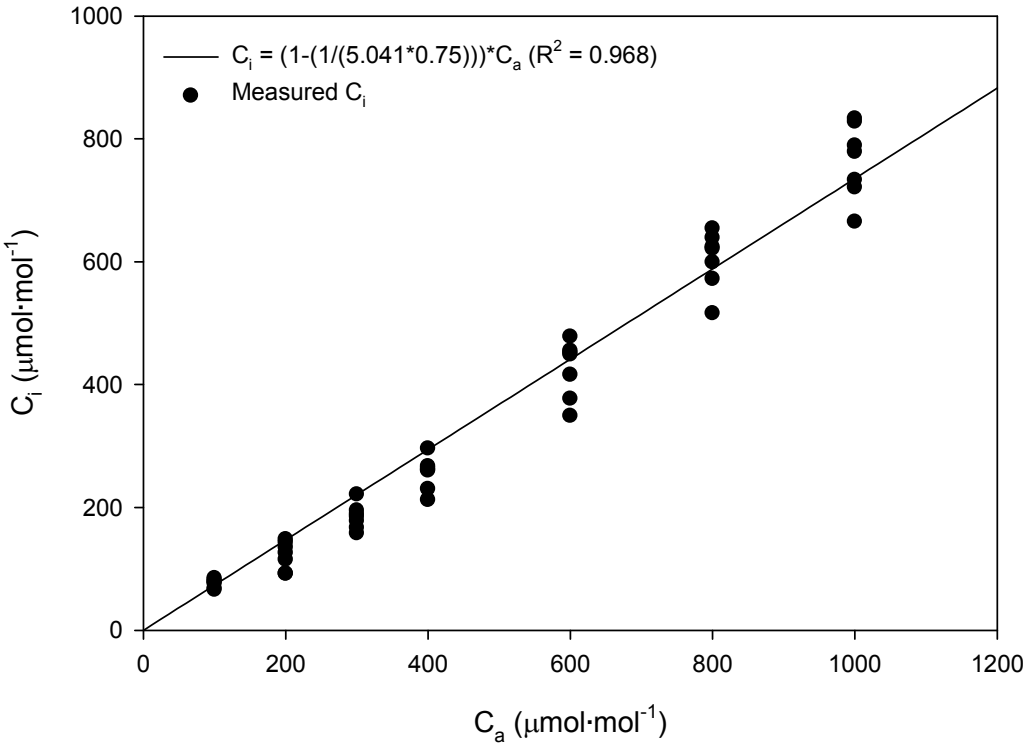

Fig. II-2. Relationship between atmospheric (C_a) and intercellular (C_i) CO_2 concentrations in the lettuce leaves. The m value in the Ball-Berry model was used for C_i.

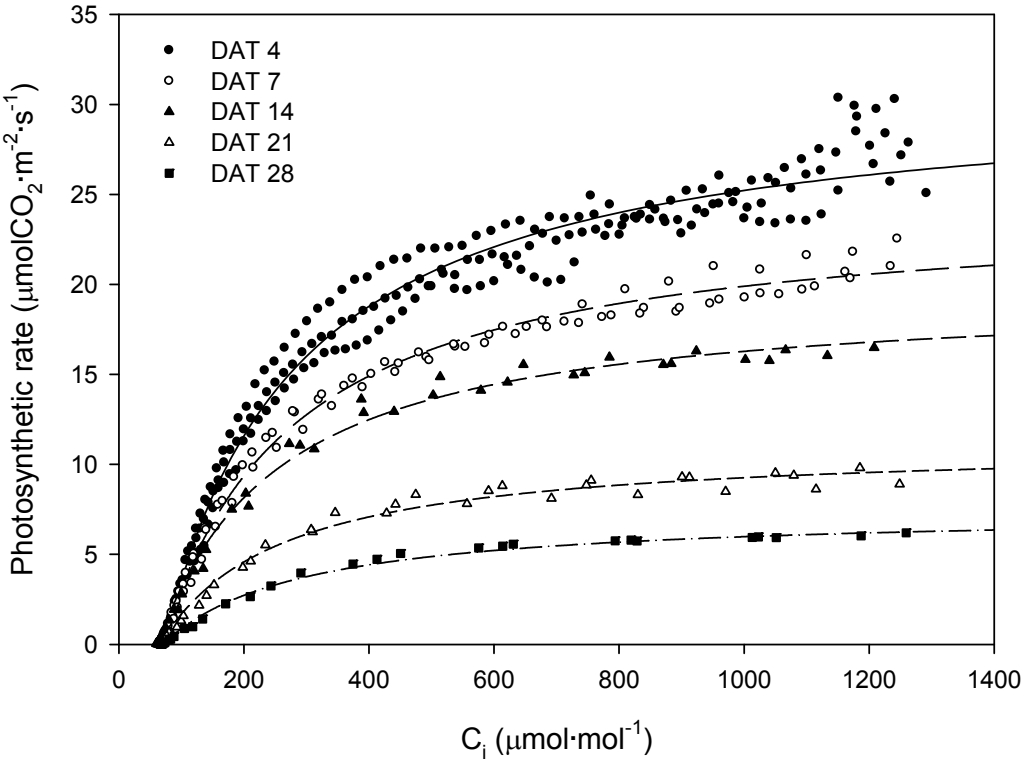

Fig. II-3. Regressed A/Ci curves at difference growth stages of DAT 4 (solid), 7 (long dash), 14 (medium dash), 21 (short dash) and 28 (dash-dot). The symbols mean the measured photosynthetic rates of the lettuce at DAT 4 (solid circle), 7 (void circle), 14 (solid triangle), 21 (void triangle) and 28 (solid square).

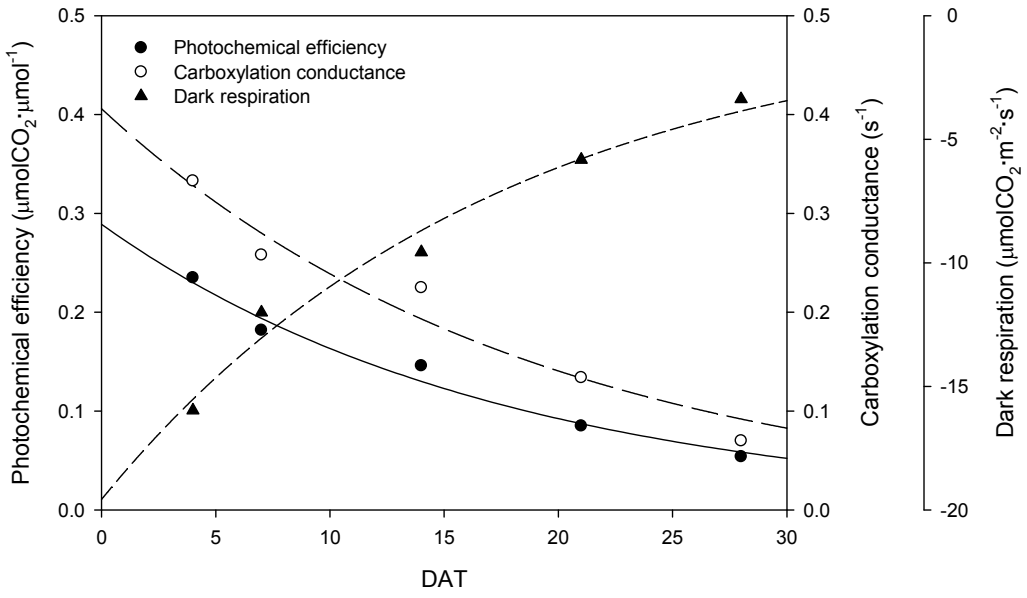

Fig. II-4. Regressed results of photochemical efficiency (solid circle), carboxylation conductance (void circle), and dark respiration (solid triangle).

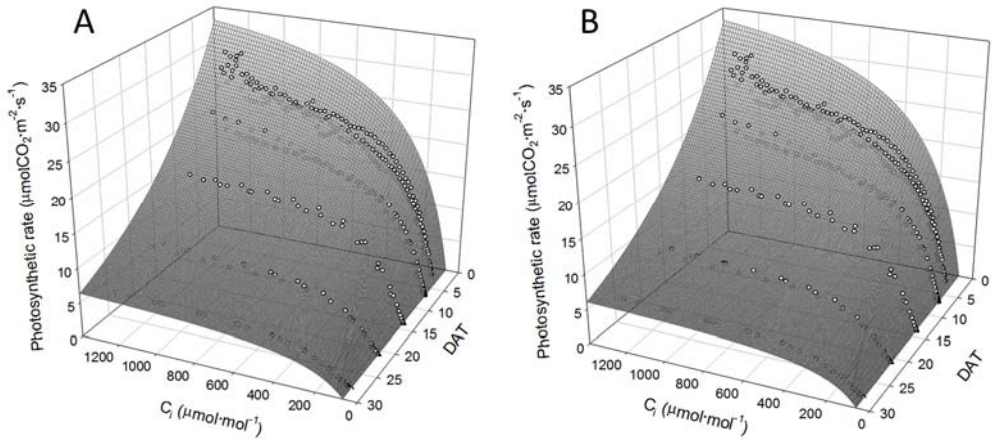

Fig. II-5. Regressed results of the simple multiplication model (A) and Thornley model (B) of canopy photosynthetic rates according to intercellular CO_2 concentration and growth stage. White circles mean the measured canopy photosynthetic rates of the lettuce.

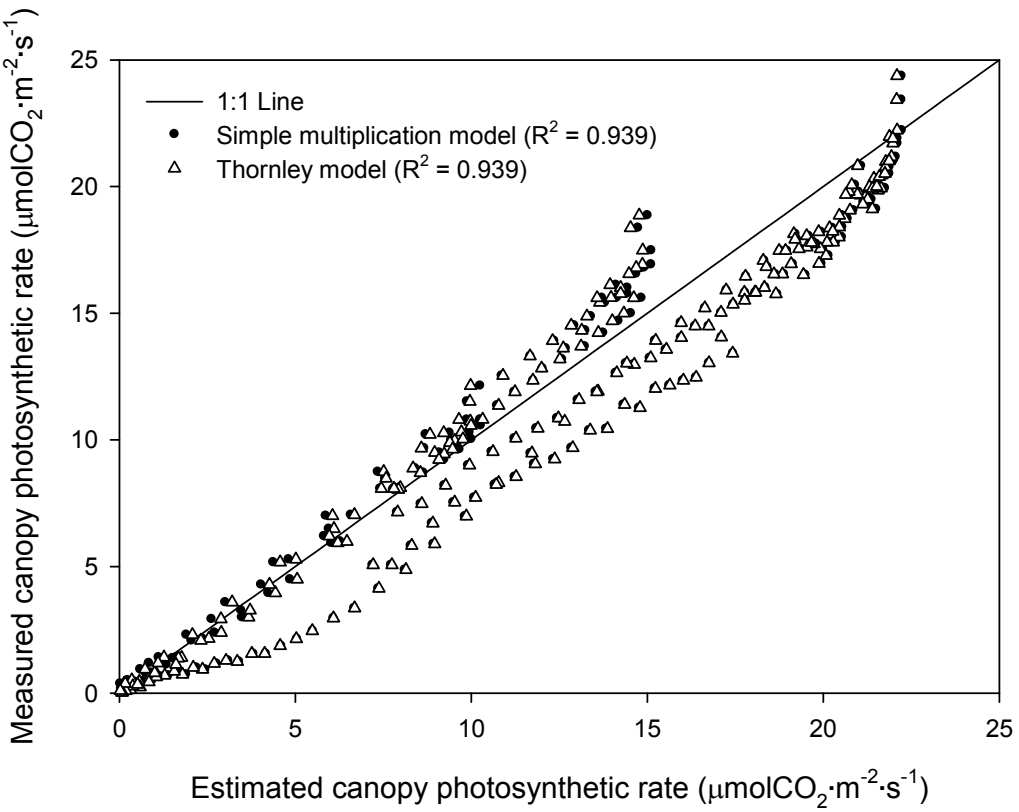

Fig. II-6. Validation of canopy photosynthetic rates of the simple multiplication model and Thornley model.

CHAPTER III

Continuous Cultivation Strategies with CO_2 Control for a Closed Production of King Oyster Mushrooms and Romaine Lettuces

ABSTRACT

Since a large amount of CO_2 gas emitted from mushrooms is useful for plant photosynthesis and plant growth, more systematic approach for mixed cultivation of mushrooms and plants is required. The objective of this study was to establish an appropriate cultivation strategy for CO_2 balance in a closed production of mushroom and lettuce by using a CO_2 emission rate model of mushroom and a photosynthetic rate model of lettuce. A closed plant production system consisting of three lettuce chambers, one mushroom chamber, and one gas-mixing chamber were established. Simulations were conducted at two cultivation conditions in the closed plant production system by using MATLAB: One was to put all the lettuces and mushrooms into the chambers and the other was to put them step by step. The lettuce and mushroom chambers were stably maintained over 1000 μmol·mol^{-1} and under 2000 μmol·mol^{-1}, respectively, in the continuous cultivation. During the 5 days, shoot fresh weights increased about 20 to 30% at all growth stages. Compared to the single cultivation of mushrooms, the mixed cultivation of mushrooms and lettuces could reduce the CO_2 emissions into the atmosphere by 80.6%. It is concluded that the simultaneous cultivation strategy of mushroom and lettuce will reduce

the greenhouse gas emissions and applied to the closed plant production such as space agriculture.

Additional key words: Canopy photosynthesis, CO_2 balance, CO_2 emission, Mushroom respiration, Simulation

INTRODUCTION

Since mushrooms are aerobic fungi, a large amount of CO_2 gas in mushroom cultivation are emitted by respiration into the atmosphere as greenhouse gas (Thavivongse and Buppachat, 2013). In addition, CO_2 gases of high concentration suppress the growth of mushrooms (Jang et al., 2009), but is useful for enhancement of plant biomass production (Leadley et al., 1999).

In the research of Controlled Ecological Life Support System (CELSS), plants were designed to absorb the CO_2 generated by human respiration (Daunicht, 1997). However, these studies differ from the plant production systems conducting an efficient crop growth through an active usage of CO_2 gas (Son et al., 1999). There was an attempt to supply the CO_2 generated by mushrooms during cultivation to lettuces (Kitaya et al., 1994). However the amount of mushrooms was not quantified because bag cultures were used. For an accurate analysis of CO_2 behaviors, the simultaneous cultivation of lettuces and mushrooms

for a long cultivation periods are required. Jung et al. (2014) just showed the changes in CO_2 concentration with growth stage and the ratio of lettuces and mushrooms. For sustainable cultivation, the amounts of CO_2 emitted and consumed by lettuces and mushrooms, respectively, should be equal over time.

These problems can be solved by continuous (conveyer) cultivations where plants at several growth stage groups are simultaneously plated to the cultivation systems (Gitelson et al., 2003). The continuous cultivation method has an advantage of smaller fluctuations in CO_2 concentration than the method to input all the plants at a time. However, these cultivation systems are actually difficult to manage because it includes various environmental variables as well as it is difficult to track the interaction of each variable (Hendrickx et al., 2006). For adequate investigation of such complicated systems, simulation methods are essentially required (Volk and Rummel, 1987). In this study, a simultaneous cultivation system of lettuce and mushroom was analyzed by simulation methods.

The objectives of this study were to find a cultivation method to archive equilibrium concentration of CO_2 by circulating a gas between the cultivation chambers and confirm the difference between the CO_2 concentration which measured actually and predicted by the simulation method.

MATERIALS AND METHODS

Cultivation Conditions

Heuk romaine lettuces (*Lactuca sativa* L. 'Asia Heuk Romaine') at DAT 0, 5, 10, 15, and 20 were used for the experiment. Yamazaki's nutrient solutions with an electrical conductivity (EC) of 1.2 $dS \cdot m^{-1}$ were supplied to the plants. Inside temperature and light intensity were maintained at 24℃ and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 8:1:1 ratio of RBW light-emitting diodes (LEDs), respectively. Three lettuces were used at each growth stage in each chamber. Photoperiods in the lettuce chambers were shown in Table III-2. King Oyster mushrooms (*Pleurotus eryngii* (DC.) Quél) at DAS 3, 7, 11, and 15 were used for the experiment. Three mushroom bottles at each growth stage were placed in the mushroom chamber.

Cultivation Systems

For simulation and verification of simultaneous cultivations of lettuces and mushrooms, a system consisted of three lettuce chambers, one mushroom chamber, and one mixing chamber was constructed (Fig. III-1). The size of the lettuce and mushroom chambers was 400 L (1.0 m × 0.8 m × 0.5 m) and that of the mixing chamber was 125 L (0.5 m × 0.5 m × 0.5 m). Each chamber was made by acryl plates and completely sealed during experimental periods. Temperatures in the lettuce and mushroom chambers were maintained at 24℃ and 18℃, respectively. Air in the chambers was circulated at a flow

rate of 62 L·min^{-1} between chambers by a diaphragm pump (Boxer 7004, Uno International Ltd., London. UK). CO_2 generated in the mushroom chamber did not going directly to the lettuce chambers, and it moved through the mixing chambers. The mixing chamber was designed to allow gas exchanges with the external air, because it prepare for the case of CO_2 concentration in the total system excessed or depleted.

CO_2 Measurement

CO_2 concentration in each chamber was measured every two minutes using an infrared CO_2 sensor (LI-820, LI-COR, Lincoln, NE, USA) controlled by a data logger (CR1000, Campbell Scientific, Logan, UT, USA). The pumps between lettuce and mixing chamber, between the mushroom and mixing chamber, and between the mixing chamber and the external air were operated when the CO_2 concentrations in each lettuce chamber became below 1000 μmol·mol^{-1}, when in the mushroom chamber over 2000 μmol·mol^{-1}, and in the mixing chamber over 2000 μmol·mol^{-1}. AC/DC controllers (SDM-CD16AC, Campbell Scientific Inc., Logan, UT, USA) were used to control the diaphragm pumps.

Simulation Models

Following governing equations were used to express the CO_2 behavior in the cultivation systems:

$$V * \frac{dC_{L1}}{dt} = (C_X - C_{L1}) * Q_{L1} - LN1 * P1 \quad \text{Eq. III-1}$$

$$V * \frac{dC_{L2}}{dt} = (C_X - C_{L2}) * Q_{L2} - LN2 * P2 \quad \text{Eq. III-2}$$

$$V * \frac{dC_{L3}}{dt} = (C_X - C_{L3}) * Q_{L2} - LN3 * P3 \quad \text{Eq. III-3}$$

$$V * \frac{dC_M}{dt} = (C_X - C_M) * Q_M + MN * R \quad \text{Eq. III-4}$$

$$V * \frac{dC_X}{dt} = (C_{L1} - C_X) * Q_{L1} + (C_{L2} - C_X) * Q_{L2} + (C_{L3} - C_X) * Q_{L3} + (C_M - C_X) * Q_M + (C_O - C_X) * Q_X \quad \text{Eq. III-5}$$

The symbols used in the models are summarized in Table III-1. Initial CO_2 concentrations in the lettuce, mushroom, and mixing chambers was 1000, 500, and 1000 μmol·mol^{-1}, respectively. Ambient CO_2 concentration was assumed as 500 μmol·mol^{-1}.

Simulation Conditions

Accumulated photosynthetic rates in the lettuce chambers ($P1 \sim P3$) were calculated using Eqs. II-6 and II-8. And accumulated CO_2 emission rates in the mushroom chamber (R) was calculated using Eqs. I-11 and I-12. Three lettuces were added to the lettuce chamber every 5 days, and three mushroom bottles were added to the mushroom chamber every 4 days (Fig. III-2). The pumps among the lettuce chambers and mixing chamber (Q_{L1}~Q_{L3}) were operated when the CO_2 concentration in each lettuce chamber became below 1000 μmol·mol^{-1}, while the pumps between the mushroom chamber and mixing chamber (Q_M) operated when the CO_2 concentration in the mushroom chamber became over 2000 μmol·mol^{-1}. The pumps between the mixing chamber and the external air (Q_X) were operated when the CO_2 concentration in the mixing chamber became over 2000 μmol·mol^{-1}. The photoperiod in the lettuce chambers were shown in Table III-2. Simulations were conducted to estimate the CO_2 concentration in each chamber for 3 days

by using MATLAB (Mathworks, Natick, MA, USA). CO_2 emissions from the mixing chamber to the external air were calculated.

Experimental Conditions for Verification

Verification experiments were carried out for 5 days. Shoot fresh weight and leaf area of the lettuces at each growth stage were measured before and after the experiment. Also, in order to compare the growth of the lettuce used in the experiment, lettuces were grown in the chambers that the other environmental conditions were the same except for the CO_2 concentration. And the accumulated CO_2 amounts from the mixing chamber to the external air were calculated. All the experimental conditions were the same as described in the simulation.

RESULTS AND DISCUSSION

CO_2 Concentrations in Lettuce and Mushroom Chambers

The measured and estimated CO_2 concentrations in the lettuce and mushroom chambers were compared (Fig. III-3). The CO_2 concentration in the lettuce chambers were controlled by the set point of 1000 $\mu mol \cdot mol^{-1}$ or higher (Fig. III-3 A ~ C). However, the CO_2 concentration of the lettuce chambers falls below the set point (Fig. III-3 D). This phenomenon is considered to occur when the pumps are operating at a lower CO_2

concentration of the mixing chambers (Fig. III-3 E). It can be confirmed from the period of the CO_2 concentration in the mixing chamber becomes lower to the period of the CO_2 concentration in the lettuce chambers becomes lower is consistent. In addition, the respiration rates of the lettuces in the verification experiment are lower than the respiration rate of the lettuces in the simulation.

From the results, it is confirmed that the gas exchange also took place due to the malfunction of pumps during dark period in the lettuce chambers, while the CO_2 concentration in the mushroom chamber was well controlled below the set point of 2000 $\mu mol \cdot mol^{-1}$. When the CO_2 concentration in the mushroom cultivation facilities is below 2400 $\mu mol \cdot mol^{-1}$, marketable values of King Oyster mushrooms appeared much higher (Ryu et al., 2005). Therefore, when applying the simultaneous cultivation method, the CO_2 concentration should be maintained at an appropriate level.

The shoot fresh weight and leaf area of the lettuces grown for significantly increased 5 days except for leaf area at DAT 25 (Table III-3). Particularly, the shoot fresh weight increased about 20 to 30% at all growth stages.

Accumulated CO_2 Emission into the Atmosphere

The accumulated CO_2 emission from the mixing chamber into the atmosphere was estimated to be 35 g for 3 days, while actual value was 48 g (Fig. III-4). Because the CO_2 was emitted only when the pumps are operating, the accumulated CO_2 emission with time appeared as a step-like. If the only mushrooms are cultivated, the simulation results might be 180 g of CO_2 emission during the 3 days. Therefore, CO_2 emission into the atmosphere

could be reduced to 80.6% of the total CO_2 emitted from mushrooms during mushroom cultivation in this study.

This result is a method that can achieve a CO_2 reduction when construct the plant cultivation system. These studies can also be utilized in the life support systems research. From 2014, NASA has been trying to consume the CO_2 generated by astronauts and to supply fresh vegetables using small plant cultivation systems in the International Space Station (ISS) (Stutte et al., 2011; Zabel et al., 2014). If astronauts have to stay over a long period in the space, the life support systems require biological reproduction. In conclusion, the simultaneous cultivation system of lettuces and mushrooms designed in this experiment will reduce the CO_2 emission and improve the plant production.

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Table III-1. Parameters used in the models.

Parameter	Description
V	Volume of the chamber (L)
C_{L1}~ C_{L3}	CO_2 concentration in the lettuce chambers (μmol·mol^{-1})
C_M	CO_2 concentration in the mushroom chamber (μmol·mol^{-1})
C_X	CO_2 concentration in the mixing chamber (μmol·mol^{-1})
C_O	CO_2 concentration in the external air (μmol·mol^{-1})
Q_{L1}~Q_{L3}	Flow rate of the diaphragm pumps between lettuce and mixing chambers (L·min^{-1})
Q_M	Flow rate of the diaphragm pumps between mushroom and mixing chambers (L·min^{-1})
Q_X	Flow rate of the diaphragm pumps between mixing chamber and external air (L·min^{-1})
$LN1$~ $LN3$	Number of lettuces at particular growth stage
MN	Number of mushrooms at particular growth stage
$P1$~ $P3$	Accumulated photosynthetic rate in the each lettuce chambers (μmolCO_2·mol^{-1}·s^{-1})
R	Accumulated CO_2 emission rate in the mushroom chamber (μmolCO_2·mol^{-1}·s^{-1})

Table III-2. Photoperiod in the lettuce chambers.

Period (h)	Chamber 1	Chamber 2	Chamber 3
00 : 00 ~ 08 : 00	Light	Light	Dark
08 : 00 ~ 16 : 00	Dark	Light	Light
16 : 00 ~ 00 : 00	Light	Dark	Light

Table III-3. Fresh weight and leaf area of the lettuces before and after experiments.

Growth stage (DAT[z])	Before	After
	Shoot fresh weight (g)	
0	0.25±0.14[y]a[*]	-
5	1.41±0.43ab	2.33±0.51ab
10	4.30±0.60bc	5.58±1.12c
15	10.10±1.35d	14.05±0.48e
20	25.07±2.91f	32.48±2.40g
25	54.58±1.99h	58.07±5.32i
	Leaf area (cm^2)	
0	11.37±2.37a	-
5	45.31±16.42a	74.37±10.04ab
10	147.20±16.36bc	165.26±17.30c
15	331.50±38.34d	414.37±16.89e
20	693.61±83.64f	935.55±104.78g
25	1510.88±74.20h	1533.15±84.23h

[*]Different letters indicate statistically significant differences (ANOVA/Duncan) (P<0.05)

[z]Days after transplanting.

[y]Mean±SD (n = 6).

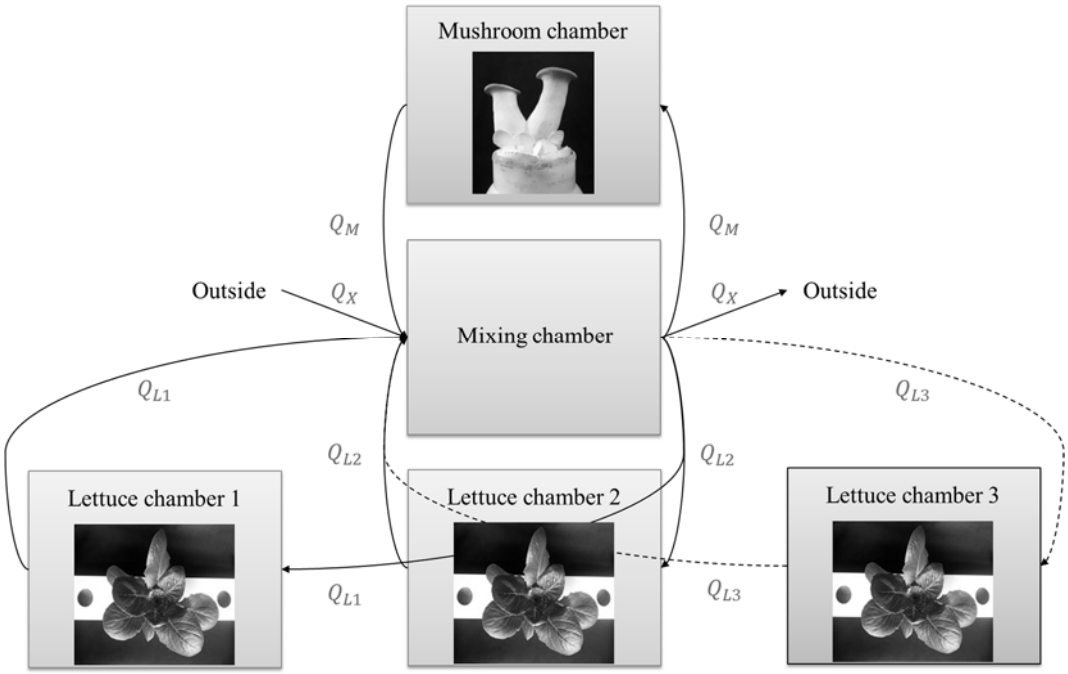

Fig. III-1. Conceptual diagram of a system consisting of three lettuce chambers, one mushroom chamber, and one CO_2 mixing chamber. Arrow and symbol mean air flow air flow rate, respectively. Solid and dashed arrows mean pump-on and off, respectively.

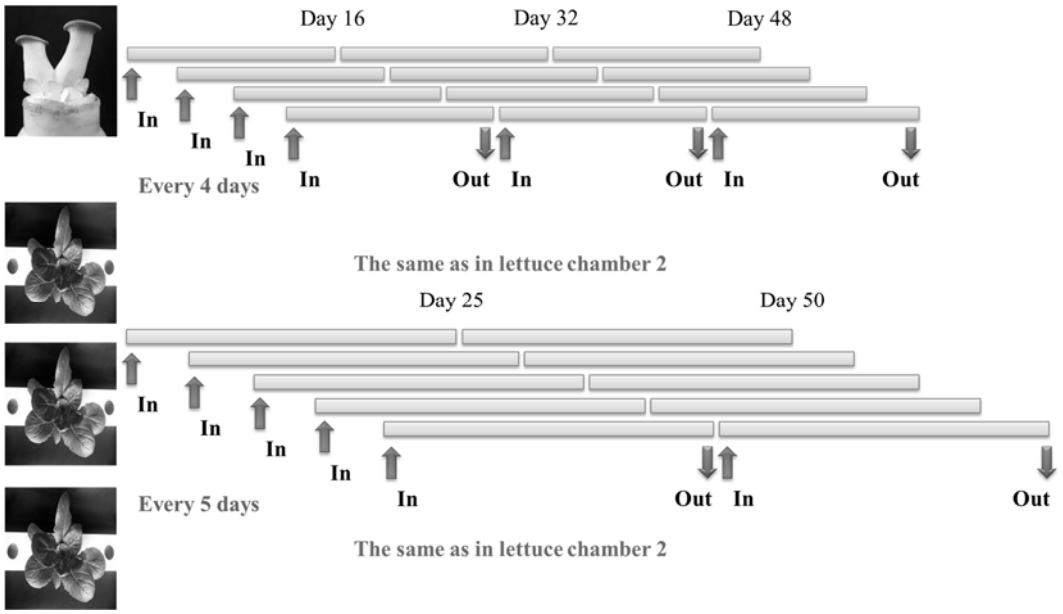

Fig. III-2. Schematic diagram of the continuous cultivation. Three bottles of new mushrooms put into the chamber every 4 days and three lettuces into each chamber every 5 days. Total number of lettuces and mushrooms in each chamber at a certain time are 15 and 12, respectively.

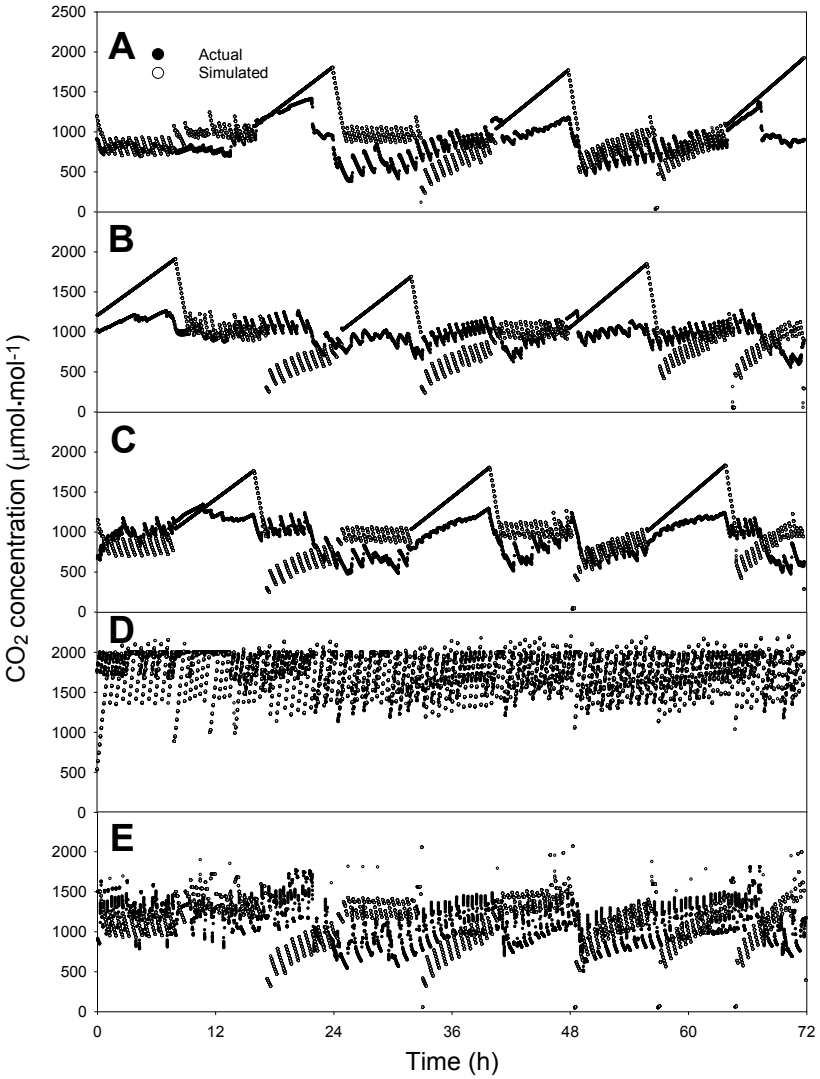

Fig. III-3. Actual and simulated CO_2 concentrations in each chamber for 3 days; lettuce chamber 1 (A), lettuce chamber 2 (B), lettuce chamber 3 (C), mushroom chamber (D), and mixing chamber (E). Solid and void circles mean the actual and simulated CO_2 concentrations.

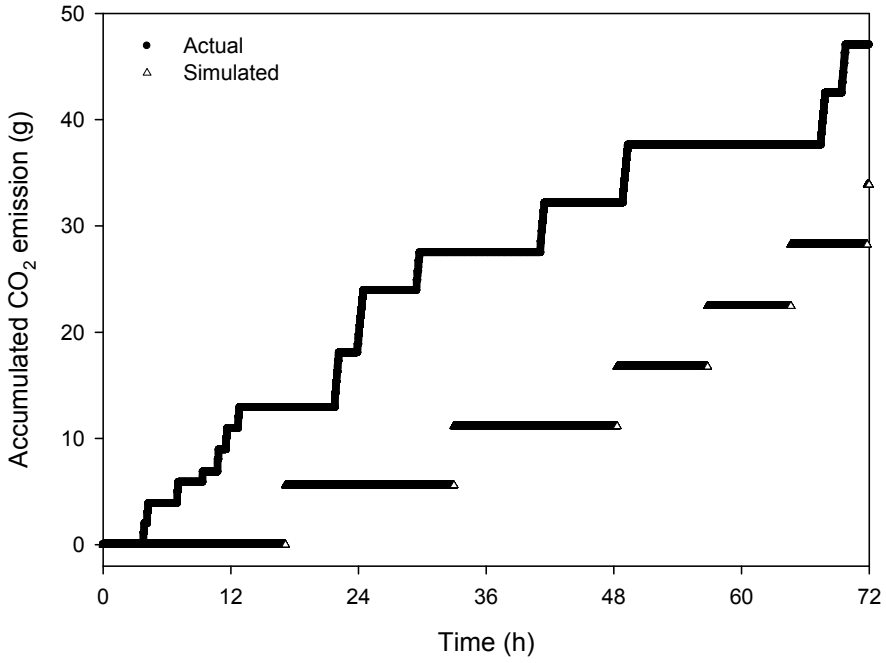

Fig. III-3. Accumulated CO_2 emission into the atmosphere for 3 days. Solid and void circles mean the actual simulated CO_2 emissions.

CONCLUSION

The CO_2 emission rate models of mushrooms before and after thinning were developed as Eqs. I-11 and I-12, respectively. The CO_2 emission models showed good agreements with measured data. And it is possible to estimate the increase in CO_2 concentration in the mushroom cultivation facilities. Also the photosynthetic rate models of lettuces were developed as Eqs. II-7 and II-8. Thornley's dynamic model for photosynthesis was used to express the canopy photosynthesis because this model reflects physiological reactions of plants well. Using simulation methods, CO_2 concentrations in the lettuce and mushroom chambers could be accurately estimated in the simultaneous cultivation and enabled to design the efficient cultivation systems. CO_2 concentrations in the lettuce and mushroom chambers were verified and shown to be controlled within allowable ranges. With the simultaneous cultivation system, CO_2 emission into the atmosphere could be reduced to 80.6% of the total CO_2 emitted from mushrooms by respiration. Because CO_2 is one of the factors responsible for global warming, development of the methods to minimize CO_2 emission will be useful for mushroom cultivation. These studies can also be utilized to the life support systems in which reuse the CO_2 caused by the crew for photosynthesis of crops. In conclusion, the simultaneous cultivation system of lettuces and mushrooms designed in this experiment enables to reduce the CO_2 emission as well as improve the plant production.

APPENDICES

Simulation Codes for Lettuce and Mushroom Simultaneous Cultivation

```
clear all
clc

% Time unit (5 second), simulation for 3 days

STARTTIME = 0;
STOPTIME = 51840;
DT = 1;
Duration = (STOPTIME - STARTTIME)/DT + 1;

% Used variables

P1(Duration,1) = 0;
P2(Duration,1) = 0;
P3(Duration,1) = 0;
R(Duration,1) = 0;

P11(Duration,1) = 0;
P21(Duration,1) = 0;
P31(Duration,1) = 0;

P12(Duration,1) = 0;
P22(Duration,1) = 0;
P32(Duration,1) = 0;

P13(Duration,1) = 0;
P23(Duration,1) = 0;
P33(Duration,1) = 0;

P14(Duration,1) = 0;
P24(Duration,1) = 0;
P34(Duration,1) = 0;

P15(Duration,1) = 0;
P25(Duration,1) = 0;
P35(Duration,1) = 0;

R1(Duration,1) = 0;
R2(Duration,1) = 0;
```

```
R3(Duration,1) = 0;
R4(Duration,1) = 0;

CL1(Duration,1) = 0;
CL2(Duration,1) = 0;
CL3(Duration,1) = 0;
CM(Duration,1) = 0;
CX(Duration,1) = 0;

CL1(1,1) = 1200;
CL2(1,1) = 1200;
CL3(1,1) = 1200;
CM(1,1) = 500;
CX(1,1) = 1500;

QL1I(Duration,1) = 0;
QL1O(Duration,1) = 0;
QL2I(Duration,1) = 0;
QL2O(Duration,1) = 0;
QL3I(Duration,1) = 0;
QL3O(Duration,1) = 0;
QMI(Duration,1) = 0;
QMO(Duration,1) = 0;
VXI(Duration,1) = 0;
VXO(Duration,1) = 0;

LN1(Duration,1) = 3;
LN1(1,1) = 3;
LN2(Duration,1) = 3;
LN2(1,1) = 3;
LN3(Duration,1) = 3;
LN3(1,1) = 3;

MN(Duration,1) = 3;
MN(1,1) = 3;

COE(Duration,1) = 0;
COE(1,1) = 0;

COEV(Duration,1) = 0;
COEV(1,1) = 0;

CO = 500;

TIME = (STARTTIME:DT:STOPTIME)';

% Iteration start
```

```
for time = STARTTIME+1:STOPTIME/DT-1

   % Timer for mushroom, lettuce and pumps

   ClockM = mod(time, 276480);

   ClockL1 = mod(time, 17280);
   ClockL2 = mod(time-5760, 17280);
   ClockL3 = mod(time-11520, 17280);

   LI840Clock = mod(time, 120);

   % Lettuce Photosynthesis Set 1

   if (ClockL1 <= 11520)
      P11(time,1) = 5*(((21.812*exp(-0.107*(mod(time,
432000)/17280))*(0.735502*CL1(time, 1)))/(57.4*exp(-
0.057*(mod(time, 432000)/17280))+0.38*exp(-0.05*(mod(time,
432000)/17280))*(0.735502*CL1(time, 1))))-18.608*exp(-
0.056*(mod(time, 432000)/17280)))*0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846;
   else
      P11(time,1) = -5*4.735*exp(-0.065*(mod(time,
432000)/17280))*0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846;
   end

   if (ClockL2 <= 11520)
      P21(time,1) = 5*(((21.812*exp(-0.107*(mod(time,
432000)/17280))*(0.735502*CL2(time, 1)))/(57.4*exp(-
0.057*(mod(time, 432000)/17280))+0.38*exp(-0.05*(mod(time,
432000)/17280))*(0.735502*CL2(time, 1))))-18.608*exp(-
0.056*(mod(time, 432000)/17280)))*0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846;
   else
      P21(time,1) = -5*4.735*exp(-0.065*(mod(time,
432000)/17280))*0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846;
   end

   if (ClockL3 <= 11520)
      P31(time,1) = 5*(((21.812*exp(-0.107*(mod(time,
432000)/17280))*(0.735502*CL3(time, 1)))/(57.4*exp(-
0.057*(mod(time, 432000)/17280))+0.38*exp(-0.05*(mod(time,
432000)/17280))*(0.735502*CL3(time, 1))))-18.608*exp(-
0.056*(mod(time, 432000)/17280)))*0.0064755*exp(0.132*(mod(time,
```

```
432000)/17280))*44*0.000553846;
    else
        P31(time,1) = -5*4.735*exp(-0.065*(mod(time,
432000)/17280))*0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846;
    end

    % Lettuce Photosynthesis Set 2

    if (ClockL1 <= 11520)
        P12(time,1) = 5*(((21.812*exp(-0.107*(mod(time-86400,
432000)/17280))*(0.735502*CL1(time, 1)))/(57.4*exp(-
0.057*(mod(time-86400, 432000)/17280))+0.38*exp(-0.05*(mod(time-
86400, 432000)/17280))*(0.735502*CL1(time, 1))))-18.608*exp(-
0.056*(mod(time-86400,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846;
    else
        P12(time,1) = -5*4.735*exp(-0.065*(mod(time-86400,
432000)/17280))*0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846;
    end

    if (ClockL2 <= 11520)
        P22(time,1) = 5*(((21.812*exp(-0.107*(mod(time-86400,
432000)/17280))*(0.735502*CL2(time, 1)))/(57.4*exp(-
0.057*(mod(time-86400, 432000)/17280))+0.38*exp(-0.05*(mod(time-
86400, 432000)/17280))*(0.735502*CL2(time, 1))))-18.608*exp(-
0.056*(mod(time-86400,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846;
    else
        P22(time,1) = -5*4.735*exp(-0.065*(mod(time-86400,
432000)/17280))*0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846;
    end

    if (ClockL3 <= 11520)
        P32(time,1) = 5*(((21.812*exp(-0.107*(mod(time-86400,
432000)/17280))*(0.735502*CL3(time, 1)))/(57.4*exp(-
0.057*(mod(time-86400, 432000)/17280))+0.38*exp(-0.05*(mod(time-
86400, 432000)/17280))*(0.735502*CL3(time, 1))))-18.608*exp(-
0.056*(mod(time-86400,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846;
    else
        P32(time,1) = -5*4.735*exp(-0.065*(mod(time-86400,
432000)/17280))*0.0064755*exp(0.132*(mod(time-86400,
```

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432000)/17280))*44*0.000553846;
    end

    % Lettuce Photosynthesis Set 3

    if (ClockL1 <= 11520)
        P13(time,1) = 5*(((21.812*exp(-0.107*(mod(time-172800,
432000)/17280))*(0.735502*CL1(time, 1)))/(57.4*exp(-
0.057*(mod(time-172800, 432000)/17280))+0.38*exp(-0.05*(mod(time-
172800, 432000)/17280))*(0.735502*CL1(time, 1))))-18.608*exp(-
0.056*(mod(time-172800,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846;
    else
        P13(time,1) = -5*4.735*exp(-0.065*(mod(time-172800,
432000)/17280))*0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846;
    end

    if (ClockL2 <= 11520)
        P23(time,1) = 5*(((21.812*exp(-0.107*(mod(time-172800,
432000)/17280))*(0.735502*CL2(time, 1)))/(57.4*exp(-
0.057*(mod(time-172800, 432000)/17280))+0.38*exp(-0.05*(mod(time-
172800, 432000)/17280))*(0.735502*CL2(time, 1))))-18.608*exp(-
0.056*(mod(time-172800,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846;
    else
        P23(time,1) = -5*4.735*exp(-0.065*(mod(time-172800,
432000)/17280))*0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846;
    end

    if (ClockL3 <= 11520)
        P33(time,1) = 5*(((21.812*exp(-0.107*(mod(time-172800,
432000)/17280))*(0.735502*CL3(time, 1)))/(57.4*exp(-
0.057*(mod(time-172800, 432000)/17280))+0.38*exp(-0.05*(mod(time-
172800, 432000)/17280))*(0.735502*CL3(time, 1))))-18.608*exp(-
0.056*(mod(time-172800,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846;
    else
        P33(time,1) = -5*4.735*exp(-0.065*(mod(time-172800,
432000)/17280))*0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846;
    end

    % Lettuce Photosynthesis Set 4
```

```
   if (ClockL1 <= 11520)
      P14(time,1) = 5*(((21.812*exp(-0.107*(mod(time-259200,
432000)/17280))*(0.735502*CL1(time, 1)))/(57.4*exp(-
0.057*(mod(time-259200, 432000)/17280))+0.38*exp(-0.05*(mod(time-
259200, 432000)/17280))*(0.735502*CL1(time, 1))))-18.608*exp(-
0.056*(mod(time-259200,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846;
   else
      P14(time,1) = -5*4.735*exp(-0.065*(mod(time-259200,
432000)/17280))*0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846;
   end

   if (ClockL2 <= 11520)
      P24(time,1) = 5*(((21.812*exp(-0.107*(mod(time-259200,
432000)/17280))*(0.735502*CL2(time, 1)))/(57.4*exp(-
0.057*(mod(time-259200, 432000)/17280))+0.38*exp(-0.05*(mod(time-
259200, 432000)/17280))*(0.735502*CL2(time, 1))))-18.608*exp(-
0.056*(mod(time-259200,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846;
   else
      P24(time,1) = -5*4.735*exp(-0.065*(mod(time-259200,
432000)/17280))*0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846;
   end

   if (ClockL3 <= 11520)
      P34(time,1) = 5*(((21.812*exp(-0.107*(mod(time-259200,
432000)/17280))*(0.735502*CL3(time, 1)))/(57.4*exp(-
0.057*(mod(time-259200, 432000)/17280))+0.38*exp(-0.05*(mod(time-
259200, 432000)/17280))*(0.735502*CL3(time, 1))))-18.608*exp(-
0.056*(mod(time-259200,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846;
   else
      P34(time,1) = -5*4.735*exp(-0.065*(mod(time-259200,
432000)/17280))*0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846;
   end

   % Lettuce Photosynthesis Set 5

   if (ClockL1 <= 11520)
      P15(time,1) = 5*(((21.812*exp(-0.107*(mod(time-345600,
432000)/17280))*(0.735502*CL1(time, 1)))/(57.4*exp(-
```

```
0.057*(mod(time-345600, 432000)/17280))+0.38*exp(-0.05*(mod(time-
345600, 432000)/17280))*(0.735502*CL1(time, 1))))-18.608*exp(-
0.056*(mod(time-345600,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846;
   else
      P15(time,1) = -5*4.735*exp(-0.065*(mod(time-345600,
432000)/17280))*0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846;
   end

   if (ClockL2 <= 11520)
      P25(time,1) = 5*(((21.812*exp(-0.107*(mod(time-345600,
432000)/17280))*(0.735502*CL2(time, 1)))/(57.4*exp(-
0.057*(mod(time-345600, 432000)/17280))+0.38*exp(-0.05*(mod(time-
345600, 432000)/17280))*(0.735502*CL2(time, 1))))-18.608*exp(-
0.056*(mod(time-345600,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846;
   else
      P25(time,1) = -5*4.735*exp(-0.065*(mod(time-345600,
432000)/17280))*0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846;
   end

   if (ClockL3 <= 11520)
      P35(time,1) = 5*(((21.812*exp(-0.107*(mod(time-345600,
432000)/17280))*(0.735502*CL3(time, 1)))/(57.4*exp(-
0.057*(mod(time-345600, 432000)/17280))+0.38*exp(-0.05*(mod(time-
345600, 432000)/17280))*(0.735502*CL3(time, 1))))-18.608*exp(-
0.056*(mod(time-345600,
432000)/17280)))*0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846;
   else
      P35(time,1) = -5*4.735*exp(-0.065*(mod(time-345600,
432000)/17280))*0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846;
   end

   % Mushroom Respiration Set 1

   if (ClockM <= 207360)
      R1(time,1) = 5*(0.0191365*exp(0.549*mod(time-51840,
276480)/17280)+43.5288)*0.000538928;
   else
      R1(time,1) = 5*(0.0061971*exp(0.549*mod(time-51840,
276480)/17280)+62.0868)*0.000538928;
   end
```

```
    % Mushroom Respiration Set 2

    if (ClockM <= 207360)
        R2(time,1) = 5*(0.0191365*exp(0.549*mod(time-120960,
276480)/17280)+43.5288)*0.000538928;
    else
        R2(time,1) = 5*(0.0061971*exp(0.549*mod(time-120960,
276480)/17280)+62.0868)*0.000538928;
    end

    % Mushroom Respiration Set 3

    if (ClockM <= 207360)
        R3(time,1) = 5*(0.0191365*exp(0.549*mod(time-190080,
276480)/17280)+43.5288)*0.000538928;
    else
        R3(time,1) = 5*(0.0061971*exp(0.549*mod(time-190080,
276480)/17280)+62.0868)*0.000538928;
    end

    % Mushroom Respiration Set 4

    if (ClockM <= 207360)
        R4(time,1) = 5*(0.0191365*exp(0.549*mod(time-259200,
276480)/17280)+43.5288)*0.000538928;
    else
        R4(time,1) = 5*(0.0061971*exp(0.549*mod(time-259200,
276480)/17280)+62.0868)*0.000538928;
    end

    % Differential equation solving (Using Euler's method)

    CL1(time + 1,1) = CL1(time,1) + DT*(CX(time,1)*QL1I(time,1) -
CL1(time,1)*QL1O(time,1) - LN1(time,1)*(P11(time,1) + P12(time,1)
+ P13(time,1) + P14(time,1) + P15(time,1)));

    CL2(time + 1,1) = CL2(time,1) + DT*(CX(time,1)*QL2I(time,1) -
CL2(time,1)*QL2O(time,1) - LN2(time,1)*(P21(time,1) + P22(time,1)
+ P23(time,1) + P24(time,1) + P25(time,1)));

    CL3(time + 1,1) = CL3(time,1) + DT*(CX(time,1)*QL3I(time,1) -
CL3(time,1)*QL3O(time,1) - LN3(time,1)*(P31(time,1) + P32(time,1)
+ P33(time,1) + P34(time,1) + P35(time,1)));

    CM(time + 1,1) = CM(time,1) + DT*(CX(time,1)*QMI(time,1) -
CM(time,1)*QMO(time,1) + MN(time,1)*(R1(time,1) + R2(time,1) +
```

```
R3(time,1) + R4(time,1)));

    CX(time + 1,1) = CX(time,1) + DT*(CL1(time,1)*QL1O(time,1) +
CL2(time,1)*QL2O(time,1) + CL3(time,1)*QL3O(time,1) +
CM(time,1)*QMO(time,1) - CX(time,1)*QL1I(time,1) -
CX(time,1)*QL2I(time,1) - CX(time,1)*QL3I(time,1) -
CX(time,1)*QMI(time,1) - CX(time,1)*VXO(time,1) + CO*VXI(time,1));

    COE(time + 1,1) = COE(time, 1) +
DT*(CX(time,1)*0.00180555*VXO(time,1));

    COEV(time + 1,1) = (CX(time, 1)*0.00180555/125)*VXO(time, 1);


    % Fan ON/OFF Conditions


    if(LI840Clock >= 0 && LI840Clock < 24)
        if(CL1(time+1,1) <= 1000)
            QL1I(time+1,1) = 0.3;
        elseif(CL1(time+1,1) > 1000 && abs(CL1(time+1,1) -
CL1(time,1)) < 0.01)
            QL1I(time+1,1) = 0;
        end
    else
        QL1I(time+1,1) = QL1I(time,1);
    end

    if(LI840Clock >= 0 && LI840Clock < 24)
        if(CL1(time+1,1) <= 1000)
            QL1O(time+1,1) = 0.4;
        elseif(CL1(time+1,1) > 1000 && abs(CL1(time+1,1) -
CL1(time,1)) < 0.01)
            QL1O(time+1,1) = 0;
        end
    else
        QL1O(time+1,1) = QL1O(time,1);
    end


    if(LI840Clock >= 24 && LI840Clock < 48)
        if(CL2(time+1,1) <= 1000)
            QL2I(time+1,1) = 0.3;
        elseif(CL2(time+1,1) > 1000 && abs(CL2(time+1,1) -
CL2(time,1)) < 0.01)
            QL2I(time+1,1) = 0;
        end
```

```
    else
        QL2I(time+1,1) = QL2I(time,1);
    end

    if(LI840Clock >= 24 && LI840Clock < 48)
        if(CL2(time+1,1) <= 1000)
            QL2O(time+1,1) = 0.4;
        elseif(CL2(time+1,1) > 1000 && abs(CL2(time+1,1) -
CL2(time,1)) < 0.01)
            QL2O(time+1,1) = 0;
        end
    else
        QL2O(time+1,1) = QL2O(time,1);
    end


    if(LI840Clock >= 48 && LI840Clock < 72)
        if(CL3(time+1,1) <= 1000)
            QL3I(time+1,1) = 0.3;
        elseif(CL3(time+1,1) > 1000 && abs(CL3(time+1,1) -
CL3(time,1)) < 0.01)
            QL3I(time+1,1) = 0;
        end
    else
        QL3I(time+1,1) = QL3I(time,1);
    end

    if(LI840Clock >= 48 && LI840Clock < 72)
        if(CL3(time+1,1) <= 1000)
            QL3O(time+1,1) = 0.4;
        elseif(CL3(time+1,1) > 1000 && abs(CL3(time+1,1) -
CL3(time,1)) < 0.01)
            QL3O(time+1,1) = 0;
        end
    else
        QL3O(time+1,1) = QL3O(time,1);
    end


    if(LI840Clock >= 72 && LI840Clock < 96)
        if (CM(time+1,1) >= 2000)
            QMI(time+1,1) = 0.3;
        elseif(CM(time+1,1) < 2000 && abs(CM(time+1,1) -
CM(time,1)) < 0.1)
            QMI(time+1,1) = 0;
        end
    else
        QMI(time+1,1) = QMI(time,1);
```

```
    end

    if(LI840Clock >= 72 && LI840Clock < 96)
        if (CM(time+1,1) >= 2000)
            QMO(time+1,1) = 0.5;
        elseif(CM(time+1,1) < 2000 && abs(CM(time+1,1) -
CM(time,1)) < 0.1)
            QMO(time+1,1) = 0;
        end
    else
        QMO(time+1,1) = QMO(time,1);
    end


    if(LI840Clock >= 96 && LI840Clock < 120)
        if (CX(time+1,1) >= 2000)
            VXI(time+1,1) = 0.4;
        elseif(CX(time+1,1) < 2000 && abs(CX(time+1,1) -
CX(time,1)) < 0.1)
            VXI(time+1,1) = 0;
        end
    else
        VXI(time+1,1) = VXI(time,1);
    end


    if(LI840Clock >= 96 && LI840Clock < 120)
        if (CX(time+1,1) >= 2000)
            VXO(time+1,1) = 1.5;
        elseif(CX(time+1,1) < 2000 && abs(CX(time+1,1) -
CX(time,1)) < 0.1)
            VXO(time+1,1) = 0;
        end
    else
        VXO(time+1,1) = VXO(time,1);
    end


    % Number of mushrooms and lettuces

    LN1(time+1,1) = LN1(time,1);
    LN2(time+1,1) = LN2(time,1);
    LN3(time+1,1) = LN3(time,1);
    MN(time+1,1) = MN(time,1);


    % Photosynthesis and Respiration in each chambers
```

```
   P1(time,1) = (P11(time,1)/(0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846) +
P12(time,1)/(0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846) +
P13(time,1)/(0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846) +
P14(time,1)/(0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846) +
P15(time,1)/(0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846));
   P2(time,1) = (P21(time,1)/(0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846) +
P22(time,1)/(0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846) +
P23(time,1)/(0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846) +
P24(time,1)/(0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846) +
P25(time,1)/(0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846));
   P3(time,1) = (P31(time,1)/(0.0064755*exp(0.132*(mod(time,
432000)/17280))*44*0.000553846) +
P32(time,1)/(0.0064755*exp(0.132*(mod(time-86400,
432000)/17280))*44*0.000553846) +
P33(time,1)/(0.0064755*exp(0.132*(mod(time-172800,
432000)/17280))*44*0.000553846) +
P34(time,1)/(0.0064755*exp(0.132*(mod(time-259200,
432000)/17280))*44*0.000553846) +
P35(time,1)/(0.0064755*exp(0.132*(mod(time-345600,
432000)/17280))*44*0.000553846));
   R(time,1) = (R1(time,1) + R2(time,1) + R3(time,1) +
R4(time,1))/0.00184278;


end

% Plotting graphs

figure;
plot(TIME,CL1,TIME,CL2,TIME,CL3,TIME,CM,TIME,CX);

figure;
plot(TIME,COE);
```

Simulation Codes for Mushroom Only Cultivation

```
clear all
clc

% Time unit (5 second), simulation for 3 days

STARTTIME = 0;
STOPTIME = 51840;
DT = 1;
Duration = (STOPTIME - STARTTIME)/DT + 1;

% Used variables


R(Duration,1) = 0;

R1(Duration,1) = 0;
R2(Duration,1) = 0;
R3(Duration,1) = 0;
R4(Duration,1) = 0;


CM(Duration,1) = 0;
CM(1,1) = 500;

VXI(Duration,1) = 0;
VXO(Duration,1) = 0;

MN(Duration,1) = 3;
MN(1,1) = 3;

COE(Duration,1) = 0;
COE(1,1) = 0;

COEV(Duration,1) = 0;
COEV(1,1) = 0;

CO(Duration,1) = 500;

TIME = (STARTTIME:DT:STOPTIME)';

% Iteration start

for time = STARTTIME+1:STOPTIME/DT-1
```

```
    % Timer for mushroom, lettuce and pumps

    ClockM = mod(time, 276480);

    LI840Clock = mod(time, 120);

    % Mushroom Respiration Set 1

    if (ClockM <= 207360)
        R1(time,1) = 5*(0.0191365*exp(0.549*mod(time-51840,
276480)/17280)+43.5288)*0.000538928;
    else
        R1(time,1) = 5*(0.0061971*exp(0.549*mod(time-51840,
276480)/17280)+62.0868)*0.000538928;
    end

    % Mushroom Respiration Set 2

    if (ClockM <= 207360)
        R2(time,1) = 5*(0.0191365*exp(0.549*mod(time-120960,
276480)/17280)+43.5288)*0.000538928;
    else
        R2(time,1) = 5*(0.0061971*exp(0.549*mod(time-120960,
276480)/17280)+62.0868)*0.000538928;
    end

    % Mushroom Respiration Set 3

    if (ClockM <= 207360)
        R3(time,1) = 5*(0.0191365*exp(0.549*mod(time-190080,
276480)/17280)+43.5288)*0.000538928;
    else
        R3(time,1) = 5*(0.0061971*exp(0.549*mod(time-190080,
276480)/17280)+62.0868)*0.000538928;
    end

    % Mushroom Respiration Set 4

    if (ClockM <= 207360)
        R4(time,1) = 5*(0.0191365*exp(0.549*mod(time-259200,
276480)/17280)+43.5288)*0.000538928;
    else
        R4(time,1) = 5*(0.0061971*exp(0.549*mod(time-259200,
276480)/17280)+62.0868)*0.000538928;
    end
```

```
    % Differential equation solving (Using Euler's method)

    CM(time + 1,1) = CM(time,1) + DT*(CO(time,1)*VXI(time,1) -
CM(time,1)*VXO(time,1) + MN(time,1)*(R1(time,1) + R2(time,1) +
R3(time,1) + R4(time,1)));

    COE(time + 1,1) = COE(time, 1) +
DT*(CM(time,1)*0.00180555*VXO(time,1));

    COEV(time + 1,1) = (CM(time, 1)*0.00180555/125)*VXO(time, 1);


    % Fan ON/OFF Conditions


    if (CM(time+1,1) >= 2000)
           VXI(time+1,1) = 62/400;
    elseif(CM(time+1,1) < 2000 && abs(CM(time+1,1) - CM(time,1)) <
3000)
           VXI(time+1,1) = 0;
    end


    if (CM(time+1,1) >= 2000)
           VXO(time+1,1) = 62/400;
    elseif(CM(time+1,1) < 2000 && abs(CM(time+1,1) - CM(time,1)) <
3000)
           VXO(time+1,1) = 0;
    end



    % Number of mushrooms and lettuces

    MN(time+1,1) = MN(time,1);
    CO(time+1,1) = CO(time,1);


    % Photosynthesis and Respiration in each chambers

    R(time,1) = (R1(time,1) + R2(time,1) + R3(time,1) +
R4(time,1))/0.00184278;
```

```
end

% Plotting graphs

figure;
plot(TIME,CM);

figure;
plot(TIME,COE);
```

ABSTRACT IN KOREAN

최근 생명지원시스템에 대한 연구가 활발히 이루어지고 있지만, 다양한 내부 구성요소들이 상호작용하고 있기 때문에 실제로 실험을 수행하여 연구하기가 어렵다. 따라서 모델링과 시뮬레이션 방식을 이용한 생명지원시스템 연구가 점차 증가하고 있다. 버섯과 상추를 동시에 재배하여 버섯에서 발생한 CO_2 를 상추에 시비하는 개념은 생명지원시스템 연구와 작물 생산 연구 양쪽에서 중요한 역할을 한다. 본 연구에서는 버섯의 호흡에 의해 발생하는 CO_2 의 양과 상추가 광합성을 통해 소모하는 CO_2 의 양을 정량화 하여 모델을 확립하고, 두 작물을 밀폐형 생산시스템에서 동시에 재배하는 경우 내부의 CO_2 농도를 지속 가능한 재배를 위한 수준에서 유지할 수 있도록 하는 재배 전략을 확립하는 것을 목적으로 하였다. 버섯의 CO_2 발생 속도와 상추의 광합성 속도는 밀폐형 아크릴 챔버를 이용하여 측정하였으며, 통계 프로그램을 이용하여 모델의 계수를 확정하였다. 확립된 모델을 이용하여 상추와 버섯을 동시에 재배하는 시스템을 구성하고 시뮬레이션하여 각 재배 챔버 내부의 CO_2 농도 변화를 예측하였다. 또한 실증 실험을 수행하여 시뮬레이션과 비교하여 어떤 차이가 있는 지 알아보았다. 버섯의 CO_2 발생 속도 모델과 상추의 광합성 속도 모델은 각각 R^2 값이 0.642 와 0.939 로 높은 신뢰성을 보였다. 상추와 버섯을 동시에 재배하는 시스템에서 시뮬레이션과 실증 실험 모두 각 챔버 내부의 CO_2 농도가 설정치 안에서 제어되는 것을 확인하였다. 또한 버섯을 단독으로 재배하는 것에 비해 대기 중으로 방출되는 누적 CO_2 양이 80.6% 감소하는 것을 확인하였다. 이러한 연구를 바탕으로 각 작물 재배 시 CO_2 농도를 제어할 수 있으며,

버섯 재배 시 대기 중으로 방출되는 CO_2 의 양을 줄일 수 있을 것이다. 최종적으로 생명지원시스템 연구 분야에서도 기체 순환 전략을 확립하는 데 도움이 될 것이다.

주요어: 밀폐형 생산시스템, 버섯 호흡, 상추 군락 광합성, 이산화탄소 교환, 이산화탄소 모델

학번: 2014-20016