Design and development of temporary immersion bioreactor system controlled by microcontroller

A Woowong¹ and N Piladaeng²

¹Department of Electrical and Computer Engineering, Faculty of Engineering, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand.
²Research Unit for Computational Electromagnetics and Optical Systems (CEMOS), Faculty of Engineering, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand.

Abstract. This research aims to develop, design and construct a temporary immersion bioreactor (TIB) controlled by microcontroller. The designed TIB can control the time for plant feeding and the carbon dioxide (CO2) concentration via application. The TIB consists of 2 sets of the plant tissue culture containers. The TIB can define the feeding and the CO2 concentration control times up to 10 and 4 time periods per day, respectively. The system feeds the plants and controls the CO2 concentration during the defined time periods. The test results of the feeding time control illustrated that the TIB could set the feeding times conveniently and quickly. Moreover, the system could properly work following the set times. For the test results of the CO2 concentration control, it was found that the TIB could control the CO2 concentration in the containers during the set operating time. It took about 7, 28, 30, 36 and 21 minutes after the system started working to adjust the CO2 concentration in order to be at the set levels of <750, 1,500, 2,000, 2,500 and 3,000 ppm, respectively. The CO2 concentration control system could properly work with error less than 10%.

1. Introduction
Tissue culture is one of the techniques used for plant propagation. This technique can produce a large number of healthy plants which free from pests and diseases in a short time. This technique is now widely used for commercial plant propagation [1]. Solid culture and liquid culture are the conventional plant propagation systems that have been frequently used. However, there are many problems that happened with these systems such as slow-growing plants, hyperhydricity from long soaking in food, and contamination. Therefore, a temporary immersion bioreactor (TIB) has been studied and developed by many researchers [2-7]. Using the TIB system, the plants do not sink in liquid food all the time. This can reduce hyperhydricity in plant tissue culture and help plants grow faster. In addition, the TIB system can work easily, conveniently, and fast compared to a traditional tissue culture. The TIB system normally controls only a function of plant feeding which can determine the duration and number of the feeding times [8].

It's not just nutrients for plants, there is one factor that is essential for plant growth: photosynthesis. The external factors that affect plant photosynthesis include light intensity carbon dioxide, temperature, leaf age, water, and nutrients. If the light intensity and temperature are appropriate,
the rate of photosynthesis depends on the concentration of carbon dioxide (CO₂). The study of the CO₂ effect on the rate of photosynthesis found that the rate of photosynthesis decreases with decreasing the CO₂ concentration, while the rate of photosynthesis increases when the CO₂ concentration increases. However, when the concentration of the CO₂ increases to a certain value, the rate of photosynthesis will no longer increase [9]-[10]. Therefore, if the CO₂ concentration is controlled to a suitable level for each type of plant, it will increase the plant growth rate of that plant.

Currently, there are not many studies about the TIB system which control the concentration of carbon dioxide, Because of this, this research has developed the TIB system that is easy to set the plants feeding times. The plant feeding times can be set according to the user's needs and to suit each type of plant. It also includes the design and construction of the concentration of carbon dioxide control system. The system will be controlled by the microcontroller. All values and data are entered and displayed through the application.

2. Materials and Methods

A temporary immersion bioreactor (TIB) controlled by microcontroller consists of three sub-systems, including the feeding control system, the CO₂ concentration control system and a user interface in the web application. The designed TIB system as shown in figure 1 consists of a tissue culture unit and a control system for controlling feeding. The details are as follows:

2.1 Feeding control system design

2.1.1 Tissue culture unit. Tissue culture unit consists of plant tissue culture containers and food containers arranged vertically. All containers have lids and are connected together by rubber hoses. Plant tissues are placed in the top containers and liquid foods are contained in the below containers. Then, the air hoses are connected to the tissue culture unit.

2.1.2 A feeding control system. Feeding control system is controlled by microcontroller. Users can set the feeding time via the application. In figure 1, when reaching the set feeding start time, the microcontroller sends a signal to the relay to control the valves into the feeding state by opening the S3-S5 and S9 valves, and closing the S1-S2, S6-S8, S10 and S11 valves. Air from an air compressor flows through the S4 and S5 valves and passes the air filters into the food containers. Then, the foods are pushed up into the plant tissue culture containers. When the foods run out, the pump continuously compresses the air into the containers to make air bubbles in the plant tissue culture containers. This increases the oxygen supply to the plants. While feeding, airs in both plant tissue culture containers are ventilated outward through the air filters, S3 and S9 valves. At the end of the set feeding time, the microcontroller sends a signal to the relay to open the S3, S6, S9 and S10 valves and close the S1-S2, S4-S5, S7-S8 and S11 valves in order to pump air out. The foods will flow back to the food containers by gravity. When the foods are completely removed from the plant tissue culture containers, the microcontroller sends a signal to the relay for controlling all valves to standby state by closing all valves to prepare for further works.

2.2 Carbon Dioxide Concentration Control System Design

The carbon dioxide concentration control system as illustrated in figure 1 consists of a carbon dioxide concentration measurement system and a carbon dioxide concentration control system. The details are as follows:

2.2.1 Carbon dioxide concentration measurement system. In figure 1, the CO₂ concentration sensors are installed inside the plant tissue culture containers. The detected CO₂ values are sent to microcontroller for analyzing and then used for further CO₂ concentration control.
2.2.2 Carbon dioxide concentration control system. A microcontroller is used for analyzing the CO₂ concentrations obtained from the sensors in order to control the valves to add CO₂ or air into the plant tissue culture containers. Users can set the CO₂ concentrations and times via the application. If the CO₂ concentrations in the plant tissue culture containers are lower than the set values, the microcontroller sends signals to the relay to control the valves by opening the S3, S7-S9 and S11 valves and closing the S1-S2, S4-S6 and S10 valves to fill CO₂ into the containers. Carbon dioxide from a CO₂ tank flows through the S11 valve and passes S7-S8 valves and air filters into the plant tissue culture containers in order to increase the CO₂ concentration. If the CO₂ concentration is higher than the set value, the microcontroller sends a signal to the relay to control the valves by opening the S1-S3 and S9 valves and closing the S4-S8, S10 and S11 valves to pump air and carbon dioxide out. Air from an air compressor flows through the S1-S2 valve and the air filters into the plant tissue culture containers to reduce the concentration of CO₂. If the CO₂ concentration is within the appropriate range, all valves are in standby state, the microcontroller sends a signal to the relay to closes all valves to prepare for further operations.

![Diagram of temporary immersion bioreactor system](image)

**Figure 1.** Diagram of temporary immersion bioreactor system

2.3 User interface design

The design of the user interface in the web application consists of 3 sections; a feeding control, a CO₂ concentration control, and a display. For a feeding control, the user can set the times for the feeding control system as indicated in figure 2. The set times will be saved to the database and the recorded times can operate again without new setting. In the section of the CO₂ concentration control, the user can set the operating times and a value of the CO₂ concentration which is suitable for each plant as shown in figure 3. For a display part illustrated in figure 4, a display in the web application shows the set feeding time, the measured CO₂ concentration, the set value of the CO₂ concentration, and the current working status of the feeding and CO₂ concentration control systems. Moreover, the user can set the time to turn on and off the lamp in order to provide the light for photosynthesis of plants as illustrated in figure 5.
2.4 Experimental setup

The constructed TIB system is shown in figure 6. The TIB system is controlled by ESP32 Devkit V1 node MCU with built-in Wi-Fi. The control unit is shown in figure 7. Plant tissue culture containers are placed above food containers. Both sets of containers are connected together by hoses. Then, air hoses, valves, air filters, air compressor, CO₂ tank and control unit are connected to the tissue culture unit. The function of the feeding time setting system is tested by setting 10 feeding durations as indicated in table 1. The feeding system starts working at 8:00 a.m. and works every 2 hours. The duration of each feeding is 15 minutes and the time to take food out at the end of feeding is 120 seconds. The CO₂ concentration control system is experimented by controlling the CO₂ concentration at the levels of <750, 1,500, 2,000, 2,500 ppm and 3,000 ppm. The time for measuring and adjusting the CO₂ concentration to be at each determined level is an hour. Another test is to set 4 durations for measuring and adjusting the CO₂ concentration with different levels as indicated in table 2. All control systems are tested for a week.
3. Results and discussions
The experiment was conducted following the experimental setup in the previous section. The results are as follows:

3.1 Feeding time control system testing
From testing the operation of the feeding system at the time set according to table 1 for a week, it was found that the system was performed correctly within the set durations. Each state of the valves operated correctly as designed. In addition, the TIB system could feed food in and out properly as designed.
3.2 Carbon dioxide concentration control system testing

The CO$_2$ concentration in the tissue culture containers obtained from the sensors are displayed as graphs on the application, and the obtained data can be sent to the user's email. From the test, it was found that the CO$_2$ concentration control system could start and stop working properly as designed. The experimental results of the CO$_2$ concentration control are illustrated in figure 8 – 12.

From figure 8, the result showed that the system could adjust the CO$_2$ concentration in the plant tissue culture container to be at a level of <750 ppm within 10 minutes after the system started working.

![Figure 8. CO$_2$ concentration control at level of <750 ppm in 1 hour](image)

Figure 8 shows the control of the CO$_2$ concentration at a level of 1,500 ppm in 1 hour. The results showed that the system took about 28 minutes to adjust the concentration of CO$_2$ to the desired level and there was an error less than 10%. Figure 10 illustrates the control of the CO$_2$ concentration at a level of 2,000 ppm in 1 hour. The results indicated that the system took about 30 minutes to adjust the concentration of CO$_2$ to the desired level and there was an error less than 8%. From figure 11, the system could adjust the CO$_2$ concentration in the plant tissue culture container to be at a level of 2,500 ppm within 36 minutes after the system started working. At steady state of the CO$_2$ concentration, there was an error within 8%. The result of the last CO$_2$ concentration level is illustrated in figure 12. The system could adjust the CO$_2$ concentration in the plant tissue culture container to be at a level of 3,000 ppm within 21 minutes after the system started working with error less than 10%.
Figure 10. CO$_2$ concentration control at level of 2,000 ppm in 1 hour

From observing the concentration of CO$_2$ inside the plant tissue culture containers, it was found that the CO$_2$ concentration remained close to the set value after the system stopped working without new feeding even though 5 hours have passed. Moreover, the CO$_2$ concentration tends to increase after the control period has ended due to the remained CO$_2$ inside the pipes and the containers.

From a test of time setting to control the CO$_2$ concentration with 4 different time periods and different CO$_2$ concentrations as mentioned in table 2, it was found that the control system was correctly performed follows the set time and the system could correctly adjust the CO$_2$ concentration inside the plant tissue culture container as set. The obtained result is indicated in figure 13. When adjusting the CO$_2$ concentration from low level to high level, the system took about 10-20 minutes to adjust the concentration of CO$_2$ to the set level.
4. Conclusion
In this paper, a development, design and construction of a temporary immersion bioreactor (TIB) controlled by microcontroller has been reported. The constructed TIB was designed to control the time for plant feeding, light and the carbon dioxide concentration. The TIB system can define the feeding time up to 10 periods per day and also can set the time to control carbon dioxide concentration up to 4 periods per day. The results indicate that the constructed TIB system can set the feeding times conveniently and quickly. This system can properly work following the set times and also turn on/off a lamp as desired. Moreover, the TIB can properly control the carbon dioxide concentration in the containers during the set operating time periods and work with low error.

References
[1] Hussain A, Qarshi I A, Nazir H and Ullah I 2012 Plant Tissue Culture: Current Status and Opportunities, Agricultural and Biological Sciences. Recent Advances in Plant in vitro Culture (London: Intech Open)
[2] M Carvalho L S O, Ozudogru E A, Lambardi M and Paiva L V 2018 Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47 269-277
[3] Lyam P T, Musa M L, Jameleddine Z O, Okere U A and Odofin W T 2012 J. of Biology and Life Science 3 66-86
[4] Gueguim Kana E B, Oloke J K, Lateef A, Azanfack K R H, and Adeyemi A 2010 Biotechnology
& Biotechnological Equipment. 24 2149-2153
[5] Solórzano-Acosta R and Guerrero-Padilla M 2020 Trujillana Red. American J. of Plant Sciences. 11 1429-1442
[6] Loyola-González O, Medina-Pérez M, Hernández-Tamayo D, Monroy R, Carrasco-Ochoa J, and García-Borroto M 2019 Sensors 19 414
[7] Abdullahi B Y, Shuaibu M H and Ogbadu L J 2017 J. of Scientific and Engineering Research, 4 259-265
[8] Ruta C, De Mastro G, Ancona S, Tagarelli A, De Cillis F, Benelli C, and Lambardi M 2020 Plants 9 844
[9] Michael T, Dananjali G, Naoki H, Anke M and Saman S 2017 Frontier in Physiology 8 1-13
[10] Daniel R T 2010 Nature Education 3 10

Acknowledgement
This research was financially support by the Faculty of Engineering, Mahasarakham University, Maha Sarakam, Thailand. The authors would like to thank Asst. Prof. Dr. Sudarat Thanonkeo, director of Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakam, Thailand for valuable advice.