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The Design of a Biochamber for Micropropagation of Banana Tissue Culture Plants and Controlling the Biochamber Process Using Programmable Logic Controller (PLC)

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ABSTRACT

Biochamber of banana tissue culture is one of the technology for plant propagation that using the Aeroponics System. The aeroponics method is applied because it requires sterile environment. The project is done in order to control the environment for the banana tissue culture propagation using a biochamber. It consists of researching the banana tissue culture propagation and the aeroponics method, surveying and analyzing the hardware requirement such as pump, valve, nozzle, Reverse Osmosis System, sensors and Laminar Flow Cabinet. The process is controlled by using the Programmable Logic Controller (PLC).

INTRODUCTION

The objective of this project is to design a biochamber using the aeroponics system for propagation of banana tissue culture from step V7 to V8 where the tissue culture roots start to grow. This chamber controls the environment for the tissue culture propagates as in actual soil environment. Besides of controlling any contamination that attacks the tissue culture, the chamber also can train the tissue culture to suit into actual soil environment fast by using the temperature, humidity and pH sensor. These sensors can control the amount of temperature, humidity and pH level for the tissue culture where it can maintain the environment as required for the tissue culture.

Programmable Logic Controller (PLC) is used as a controller for this biochamber where it is to control parameters such as pH, temperature and humidity of the chamber environment. A program will be installed in the PLC to control the water

and media spraying intervals. This is to ensure the banana tissue culture will get the best environment to make sure it is ready for actual soil environment.
There are 10 steps in production (V1 to V10) for the propagation of the banana tissue culture plant. The steps are shown in table below:

Table 1: Step production of banana tissue culture plant.

Step	Process
V1 to V2	Meristems process - banana tissue culture starts to meristem
V3 to V4	Duplicate stems process - banana tissue culture stem duplicate
V5 to V6	Regenerate process- banana tissue culture starts to generate
V7 to V8	Roots grow process - banana tissue culture roots grow
V9 to V10	Process of transfering to soil - banana tissue culture will be transferred to soil

METHODOLOGY

The method of this project is to design a biochamber for the micropropagation of banana tissue culture plants. This biochamber process and its requirement are simulated by using Programmable Logic Controller (PLC).

Biochamber Design

The object of the biochamber is to grow banana tissue culture plantlet in a controlled environment [1]. The biochamber itself is divided into 2 parts, the upper part is for spraying water; usually reverse osmosis water and the lower part is for media; normally Murashinge and Skoog. Murashinge and Skoog is a type of media which is used to feed the banana tissue culture plantlet. The temperature and humidity sensors are allocated at the upper part. The pH probe is allocated at the lower part where suitable for the pH level detection. The biochamber made from transparent polymer that allows observation and penetration of light in the chamber. The water and media are supplied using piping that can be connect and disconnect to the biochamber. This will allow the biochamber for easy displacement.

The system will also allow for the collection of used media for the purpose of investigating its composition [1].

The size of the biochamber should be fit into the standard laminar flow cabinet while the size of tray must be fit into autoclave for sterilization process.

The biochamber needs two trays and it will be placed in the middle of the biochamber where it can be spray from top and bottom. These trays also must be removable, so as to facilitate autoclave sterilization and the installation of the banana plantlets.

The size between holes should be fit to make sure the tissue culture cannot touch each other when it grows. The number of plant is achieved from the size of tray.

Before inserting the plant into the biochamber, the biochamber must be sterilized to make sure it is free from any contamination. There are three methods of sterilization. First method is by spraying with hot water, secondly by wiping internal surface using chemical (Ethanol) and lastly by using Ultra Violet Light (UV Light).

After the sterilizing process, the 6 liter media will be inserted into a media tank manually. The media tank is made from plastic with level sensor to detect the minimum level of the media. For program A, the media required to reach the root of the tissue culture is 800ml for 24 hours. The amount of media that can be applied is shown in table below:

Table 2: Number of Days the Media Tank can supply.

The amount of media/day	The number of days the tank can supply
800 ml	6 liter/800 ml

The minimum level that the sensor of media tank will sense is if the media is in the 400ml condition.

Water storage tank also can store 6 liter of reverse osmosis system. This tank is equipped with one maximum and minimum level sensor and a heater (60 Watt, 240 Volt). The heater will be controlled manually if needed and there will be an external wire to supply power to the heater. The time taken to fill water into water tank is referred to the flow rate of the reverse osmosis system can supply. For this Reverse Osmosis System, the flow rate is 26 gallons per day or 68.36ml per minute. The calculation of the time taken to fill the 6 liter tank is shown in table below:

Table 3: Time taken to fill 6 liter water tank.

The flow rate of reverse osmosis system	To fill 6 liter water tank
68.36 ml/minutes	6 liter/68.36 ml = 87.77 minutes = 1 hour 28 minutes

To standby the water in a tank, the Reverse Osmosis System must operate for 1 hour 28 minutes before starting the process of sterilization using hot water. After this process, the water needs minimum 1 hour to cool down the water temperature. This means that it needs minimum 2 ½ hours to standby before the process starts.

The amount of water that should be received by the tissue culture is 4 liters in 24 hours. That means, 2 liter in every 12 hours. To refill the 2 liter it needs 30 minutes. The best way to make the process run smoothly is to increase the minimum water level to 3 liter level. If the minimum level sensor detects minimum level of water, the process still can be run with a spare of 1 liter. Then, the water can be refilled to maximum level before it reaches for the second spray intervals.

For the propagation of the tissue culture, it depends on the success of the program where it might take 2 or 3 weeks before the tissue culture ready to be transferred in soil. Below is one of the programs to examine the tissue culture propagation:

Program A

Table 4: The feeding requirement for Banana Plantlets.

Media	Water
4 times in 24 hours (every 6 hours) 800 ml/4 hours = 200ml per hour Amount of Media standby = 6 liter	2 times in 24 hours (every 12 hours) 2 liter in 30 minutes Amount of Water standby = 6 liter

HARDWARE

Laminar Flow Cabinets

Laminar flow cabinet is a series of cabinets that are design to provide a high degree of protection for process products and apparatus in laboratory and production facilities [2].

Laminar Flow is used when inserting the tissue culture in the biochamber. Then, the biochamber will be placed into Laminar Flow Cabinet. This cabinet using HEPA filter that can produce sterilized environment of 99.997% clean air free from contamination.

Nozzle

Nozzle is a device that sprays droplets of liquid. Droplet size refers to the diameter of an individual spray droplet. The spray refers to the pressure applied to the nozzle, by a pump. The factors that affect the actual flow rate produced by this pressure are liquid viscosity, size of a pipe, pipe roughness (friction), turbulence of liquid and etc [3].

Eight nozzles are required for this purpose. The nozzles selected must withstand pressure more than 0.6 Mpa (maximum pressure can be supply by the pump). Model 1/4TG is a suitable nozzle for the biochamber application which can withstand maximum pressure of 2.0685 Mpa, more than three times of the pump pressure. In this project, the important part is to control the spray of media and water. After reviewing the type of spray, hollow cone spray is the most convenient.

pH Sensor

The pH value indicates the activity of the hydrogen ions in a solution. It describes the acidity or alkalinity of the solution. The pH range normally 0 to 14 where pH 7 is the normal or neutral stage. When the number of hydrogen ions is increasing, the solution becomes more acidic and pH value decreases [4]. On the other hand, as the number of hydrogen ions decreases (and the number of hydroxyl ion increases), the pH value increases and becomes more alkaline [4].

pH sensor HI 2910B/5 is the product to be used in the biochamber system. This product physically can reach near the root system where it would be more effective. Besides that, it can be used as pH measurer in liquid such as water. This model can be connected to 5m long. The disadvantage of this model is not suitable for liquid that is very acidic or acrylic because it uses cloth as a junction.

Pump

There are many types of pump that operate for different types of fluid. Fluid can be a liquid or gaseous state such as air, oil, water, oxygen and nitrogen [5]. There are two types of process pump automatically operated type, air operated type and built in solenoid valve [6].

In this project, built in solenoid pump is selected. Air-operated pump is not preffered because the air operated need more space to put its external solenoid and need more piping work to connect the solenoid. By using built in solenoid valve, the pump looks more compact and can save space.

For the discharge pressure, the requirement for the maximum discharge pressure is 0.6Mpa. This pressure must be applied more than the nozzle, valve and maximum pressure of the pipe in order to prevent leakage. The discharge flow rate must be in range of 0 to 2000ml/min. The selection of the pump discharge flow rate must be same to valve discharge flow rate to produce a better effect for the spray process. In addition, it is easy to estimate the amount of spray by applying calculation.

Reverse Osmosis System

Reverse Osmosis System is the method of purifying water by forcing it under pressure through a special synthetic material called semipermeable membrane [7].

For biochamber application, the process requires only 3.2 liter per day and the amount of water that can be supply from reverse osmosis system for home treatment is 26 gallon per day or 98.42 liter per day. This range, met the requirement for this project. The flow rate of this system is 68.36ml per minute and the time required to fill the 6-liter water tank is approximately 1 hour 28 minutes. This system needs maintenance every 3 month for cleaning and changing the filter if necessary.

Humidity and Temperature Sensor

The term of humidity is referred to the amount of water vapor present in the air or in other gasses. Normally, changes of temperature is closely related to the change of humidity where if the temperature is high, the reading of humidity is low and vise-versa.

The combination of temperature and humidity sensor is the best device to control the temperature and humidity environment. This device allows quick reading than a separate device.

The temperature and humidity sensor will measure the environment of the biochamber, where it will measure the humidity and temperature and control the environment using PLC. If the temperature decrease, the humidity will decrease, the PLC will give a signal to the valve and pump to operate spraying the water to maintain the environment required.

Valve

Valve is an important device that involved in many applications requiring control of fluid process [8].

For this project, there are three type of valves required. There are 2 way valve to control the spraying process, 3 way valve to separate the liquid mixture and 2 way manual ball valve to control the main pipe. The important valve to be concentrated is the 2 way valve where it uses to control the flow of liquid involves by certain duration of time. For examples, the pump provides a pressurized liquid (water and media) continuously to the valve. The valve supplies the flow rate of 3.33ml per minute for one hour to produce 200ml to the nozzle. If the spray process needs 800ml for one hour, the valve will opening the rate of 13.33ml per minute to get the result of 800ml in one hour. Minimum pressure that the valve can handle is 0.4Mpa where this is the pressure requirement for the system. However, from observation and survey, the maximum pressure of the valve can handle is up to 2.0Mpa or equal to 286.33 psi. To capture and understand on how the overall biochamber process run, please refer appendix for system block diagram – water and media delivery and system block diagram – flow rate and pressure applied in the system.

RESULTS

PLC for Program A

Program A is one of the basics program to spray water and media for the tissue culture. It can be change regarding to the requirement and type of research. The objective of Program A is to feed the banana tissue culture in proper schedule and stop the feeding when certain root length required is achieved. The tissue culture perform its root length depends on many factors such as feeding, environment, type of media and etc. In program A, the experiment needs to spray the media about two times in 12 hours and spray the water once in 12 hours. The flow rate for the media is 200ml per hour and the water is 4 liter per hour. This schedule will perform until the banana tissue culture root reach at certain length required. Application function for Program A can be seen in the appendix attached.

The overall biochamber process can be summarized in flowchart, discrete state process, sequence of events and ladder diagram. These parts are important in order to write a program for PLC. Below is the schematic diagram of a biochamber.

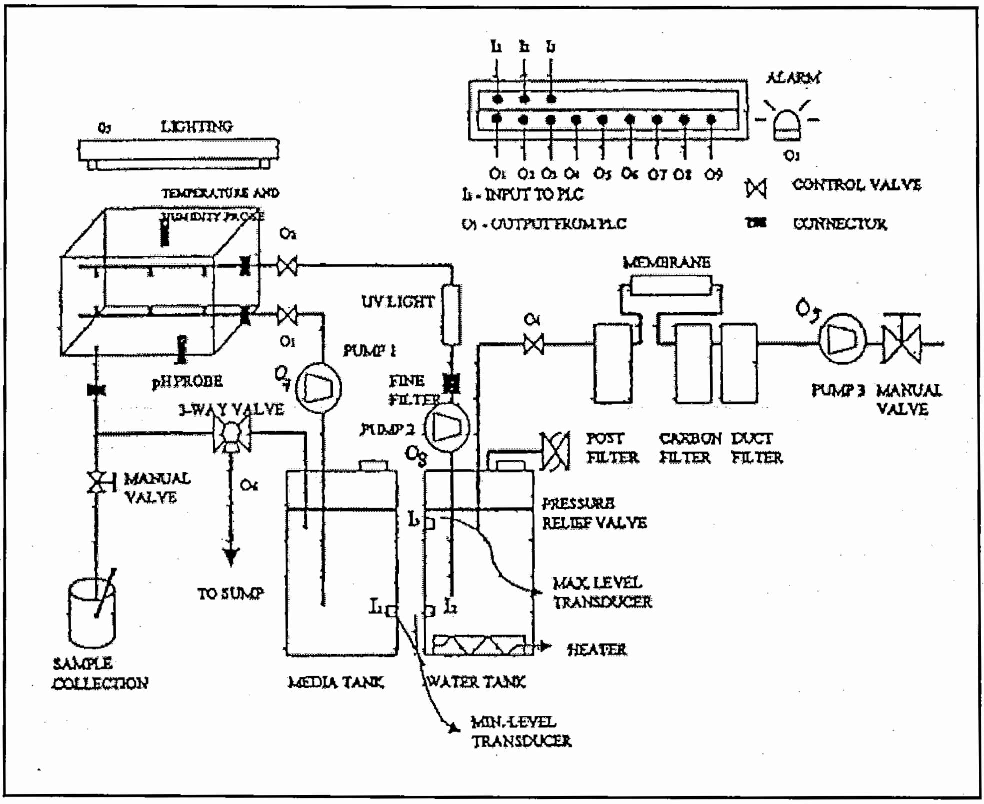

Figure 1: A Schematic Diagram of Biochamber.

Flowchart for Program A

The biochamber process can be simplified as in flowchart below.

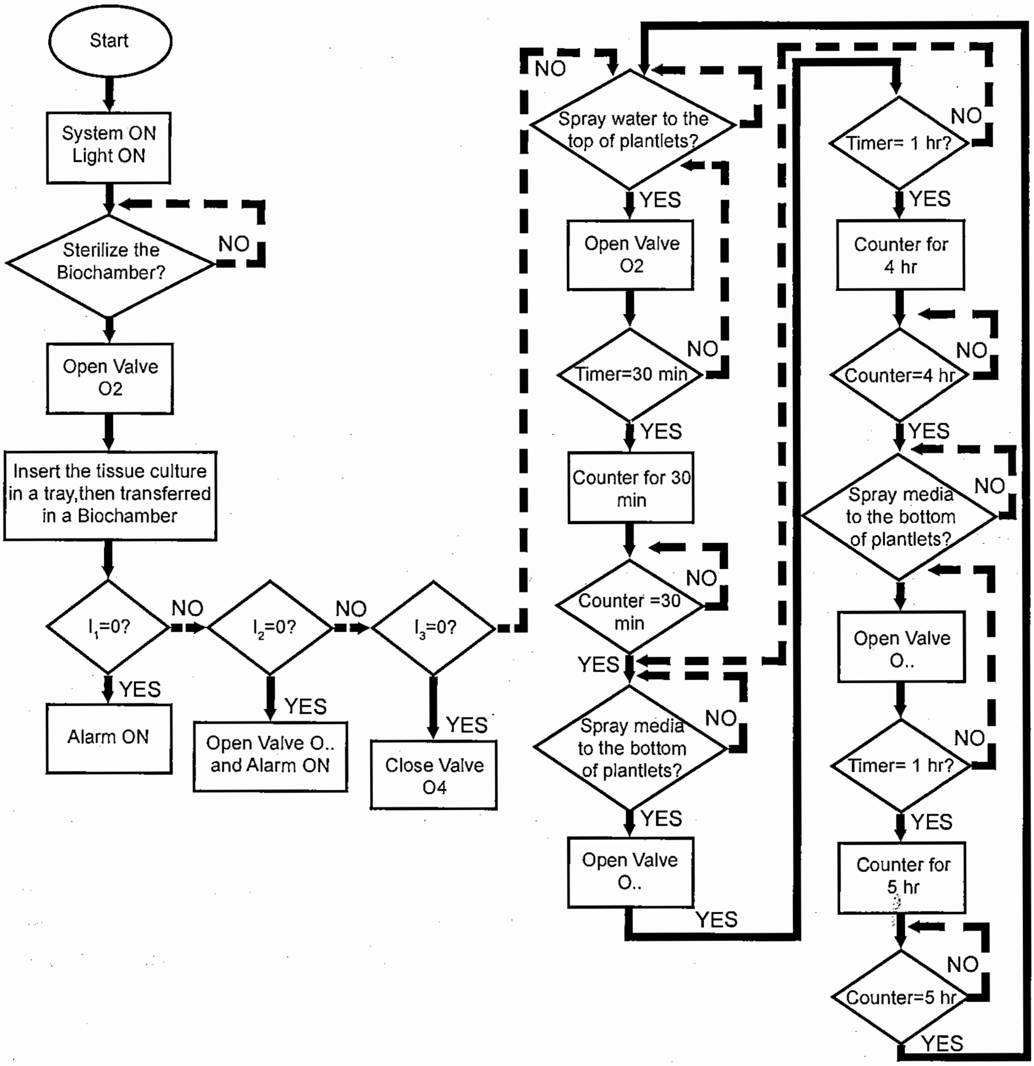

Figure 2: Flowchart for Program A.

Discrete State System

Table 5: Discrete State System.

State/Condition		Level transducer 1 (I_1)	Level transducer 2 (I_2)	Level transducer 3 (I_3)	Valve O_1	Valve O_2	Alarm O_3	Valve O_4	Light O_5	3-way valve (O_6)	Pump 1 (O_7)	Pump 2 (O_8)	Pump 3 (O_9)
State 1	Sterilize	OFF	OFF	OFF	OFF	ON	OFF	OFF	ON	OFF	OFF	ON	OFF
State 2	Media minimum	ON	OFF	OFF	OFF	OFF	ON	OFF	ON	OFF	OFF	OFF	OFF
State 3	Water minimum	OFF	ON	OFF	OFF	OFF	ON	ON	ON	OFF	OFF	OFF	ON
State 4	Water maximum	OFF	OFF	ON	OFF	OFF	OFF	OFF	ON	OFF	OFF	OFF	OFF
State 5	Spraying water	OFF	OFF	OFF	OFF	ON	OFF	OFF	ON	OFF	OFF	ON	OFF
State 6	Spraying media	OFF	OFF	OFF	ON	OFF	OFF	OFF	ON	OFF	ON	OFF	OFF

The table above shows the discrete-state process for the banana tissue culture plants. There are many states in this process that need to be considered. As in Table 5 above, the sequence process begin with sterilize the biochamber. Before sterilizing process, Pump 3, Reverse Osmosis System and Valve O_4 will start to generate after the 2-way manual valve is open. Then, the maximum level transducer I_3 will sense the water in maximum condition and the 2-way manual valve will be shut off.

In state 1, the biochamber is sterilized using hot water. The heater is ON in order to boil the water tank. After the water boils enough, the heater is shut off by manually. Then, Valve O_2, will be function, pump 2 will be ON and so as the light. After sterilize condition, the banana tissue culture plants are inserted in two trays and will be transferred into a biochamber.

In state 2, if the media is in minimum condition, the level transducer I_1 will detect it and automatically the alarm will be ON. The level transducer I_1 and the alarm will operate simultaneously. If the water is in minimum condition; state 3, the level transducer I_2 will detect it, an alarm will be ON and at the same time Pump 3 and Valve O_4 will be ON. This Pump 3 and Valve O_4 are ON because the water from Reverse Osmosis System is supplied into the water tank.

After the water has reached the maximum condition; state 4, the level transducer I_3 will detect it and automatically shut off the alarm, Pump 3 and Valve O_4. In order to feed the banana plantlets, the water is sprayed to the top of banana plantlets generally the plant leaves. At this state which is state 5, Valve O_2 will be function and Pump 2 will be ON. The water is sprayed for 30 minutes.

The next state; state 6, the media is sprayed to the roots of banana tissue culture. At this state, Valve O_1 will be open and Pump 1 will be ON. After the media is sprayed in about 1 hour, 4 hours is taken for rest time. Then, the media is sprayed again to the roots and this time, the media is sprayed for 1 hour and rest about 5 hours. So, total hour for feeding the banana plantlets is about 12 hours. In 24 hours, the media is sprayed about 4 times and the water is sprayed about 2 times. This shows that in a real condition the water is sprayed to the plants 2 times, in the morning and in the evening. This explanation can be seeing clearly in the sequence of events of biochamber process. (Please refer Appendix for Sequence of Events and PLC Ladder Diagram).

RECOMMENDATIONS

Before designing the chamber, understanding the aeroponics system is required. This knowledge will help to understand the requirement of the hardware for the biochamber. From observation, there are some important factors need to be considered:
1) Controlling the temperature of the biochamber by maintaining the temperature of the laboratory room where the biochamber will be located.
2) The biochamber should have a spare tank after the Reverse Osmosis System because R.O. System cannot supply water fast enough to the water tank.
3) The piping between water and media should be separated. If the media and water use the same pipe it will cause less concentration on the media and it will affected the result of the experiment.
4) When the prototype is successful and bigger prototype will be built, water atomizer is more suitable for spraying water than using a nozzle because it is effective in maintaining the temperature and humidity of the biochamber.
By using Programmable Logic Controller (PLC) as a controller, the simulation of biochamber process will be easy because PLC can make correction or changes in a programmed sequence of events.

CONCLUSION

This project determines the limitation of biochamber prototype and redesign the prototype to make it sure it suitable to the requirement. The application of Programmable Logic Controller (PLC) will give an advantage for the system where it can be programmed using ladder diagram. By using PLC, the design can be upgraded regarding to the process requirement easily.

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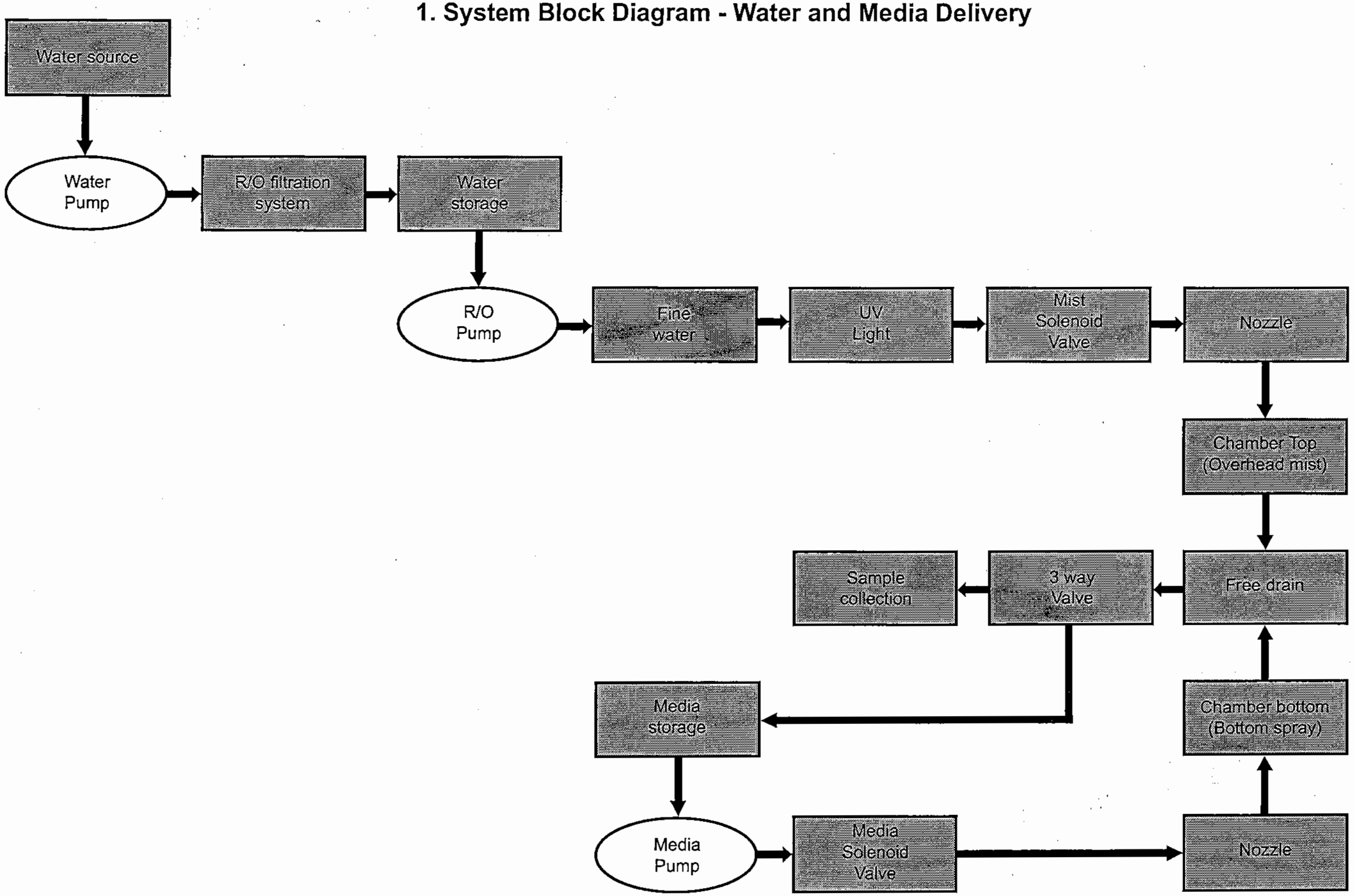
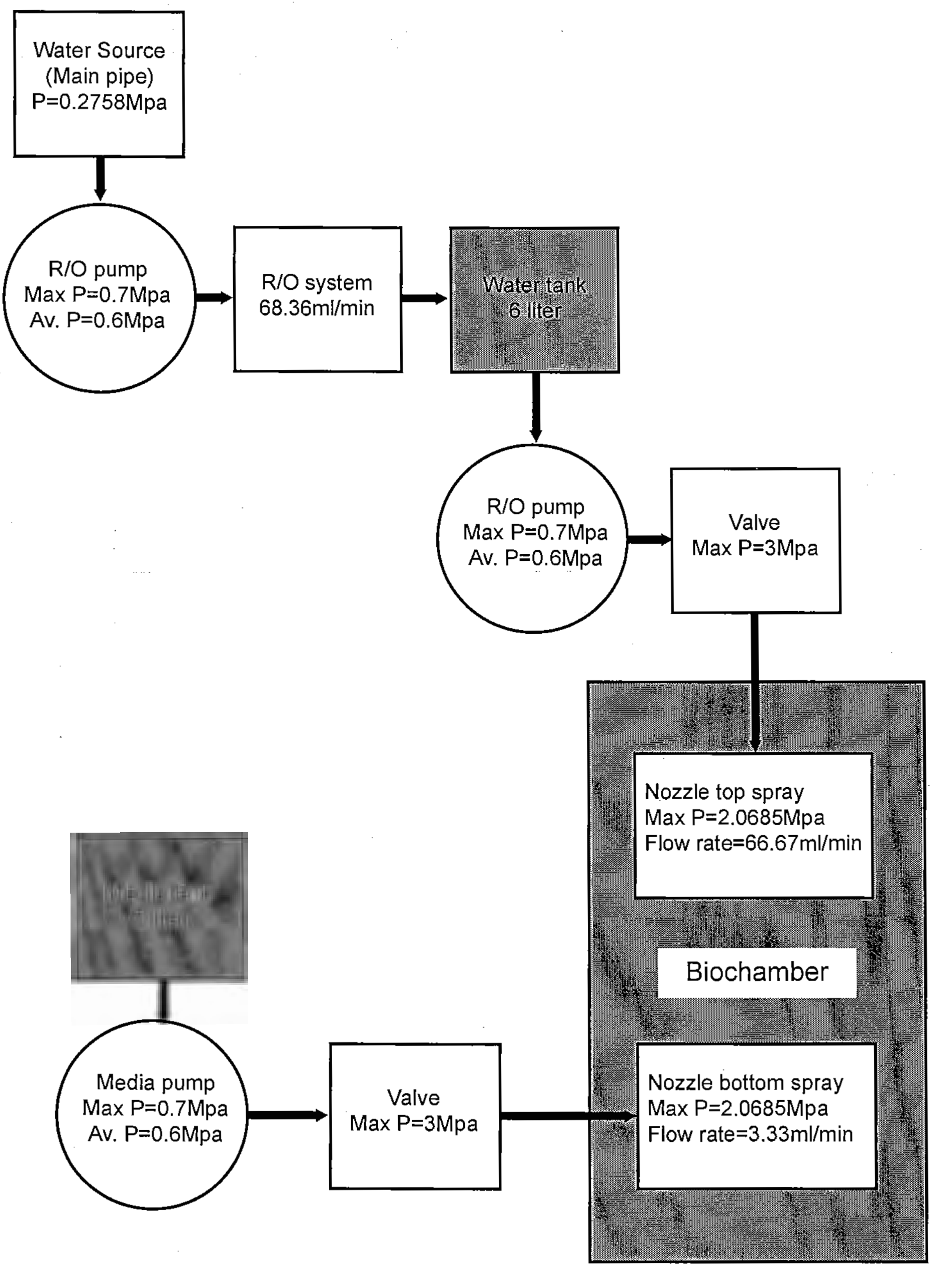
APPENDIX

1. System Block Diagram - Water and Media Delivery



2. System Block Diagram - Flow Rate and Pressure Applied in System

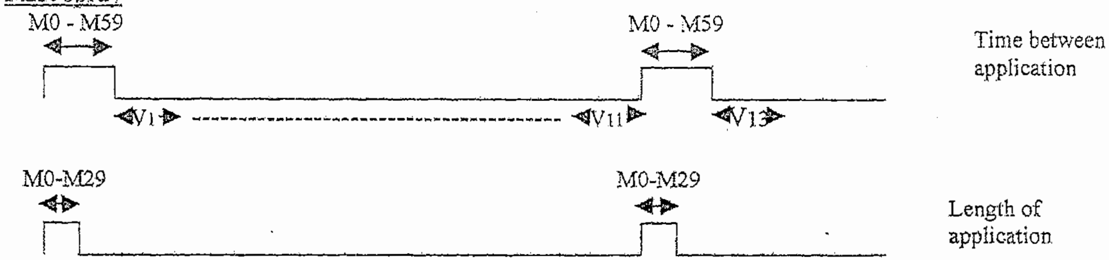


System Diagram - Application Function for Program A

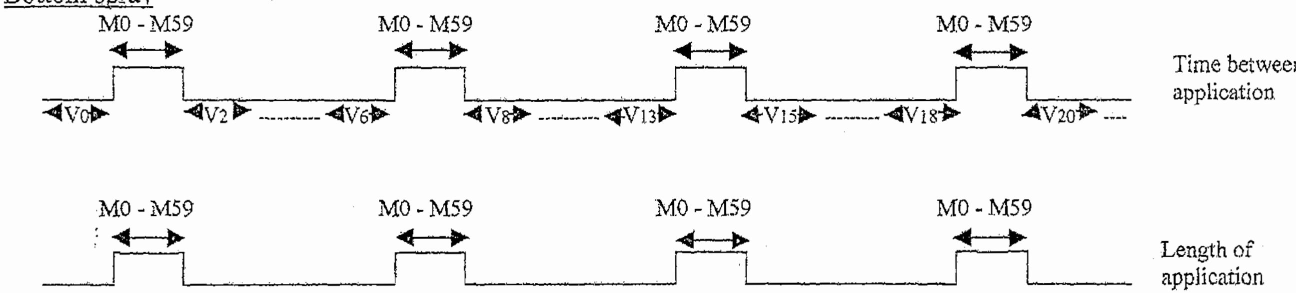
Reference:

V0 - V23	(Hour)
M0 - M59	(Minutes)
S0 - S59	(Second)

Mist spray

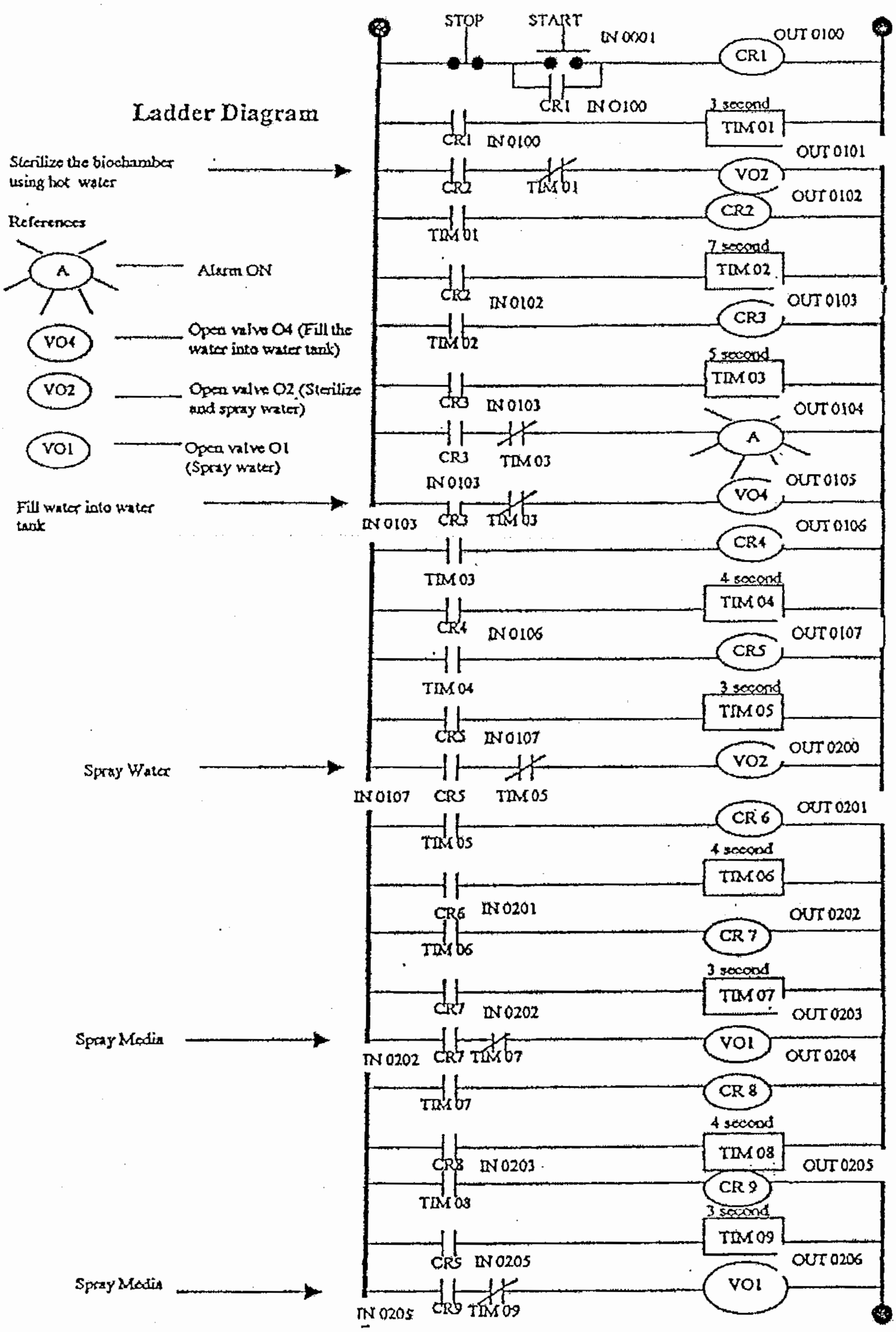


Bottom spray



Sequence of Events for Program A

1) System On and Lighting ON
2) Sterilize the biochamber
   a) No, check again
   b) Yes, open valve O2 and pump 2 enable
3) Insert the tissue culture in a tray and then transferred into a biochamber.
4) Check for media (I_1=0, minimum)
   a) No, next step
   b) Yes, alarm ON
5) Check the minimum level of water, I_2 (I_2=0, minimum level).
   a) No, next step
   b) Yes, open valve O4, pump 3 enable and alarm ON
6) Check the maximum level of water, I_3 (I_3=1, maximum level).
   a) No, next step
   b)Yes, close valve O4 and pump 3 disable
7) Spray water to the top of the banana plantlets.
   a) No, check again
   b) Yes, open valve O2 and pump 2 enable
8) Check the timer for 30 minutes.
   a) No, step 7)
   b) Yes, next step
9) Start the counter and spray water is OFF.
10) Check the counter for 30 minutes.
   a) No, check again
   b)Yes, next step
11) Spray the media to the bottom of the plantlets
   a) No, check again
   b) Yes, open valve O1 and pump 1 enable
12) Check the timer for 1 hour
   a) No, step 11)
   b) Yes, next step
13) Start the counter and spray media is OFF.
14) Check the counter for 4 hour
   a) No, check again
   b) Yes, next step
15) Spray media to the bottom of plantlets
   a) No, check again
   b) Yes, open valve O1
16) Check the timer for 1 hour
   a) No, step 15)
   b) Yes, next step
17) Start the counter and spray media is OFF.
18) Check the counter for 5 hour
   a) No, check again
   b) Yes, step 7)


Ladder Diagram
Sterilize the biochamber using hot water
References
A
Alarm ON
VO4
Open valve O4 (Fill the water into water tank)
VO2
Open valve O2 (Sterilize and spray water)
VO1
Open valve O1 (Spray water)
Fill water into water tank
Spray Water
Spray Media
Spray Media
STOP
START
IN 0001
OUT 0100
CR1
CR1 IN 0100
3 second
TIM 01
CR1 IN 0100
OUT 0101
VO2
CR2 TIM 01
OUT 0102
CR2
TIM 01
7 second
TIM 02
CR2 IN 0102
OUT 0103
CR3
TIM 02
5 second
TIM 03
CR3 IN 0103
OUT 0104
CR3 TIM 03
IN 0103
OUT 0105
VO4
IN 0103 CR3 TIM 03
OUT 0106
CR4
TIM 03
4 second
TIM 04
CR4 IN 0106
OUT 0107
CR5
TIM 04
3 second
TIM 05
CR5 IN 0107
OUT 0200
VO2
IN 0107 CR5 TIM 05
OUT 0201
CR 6
TIM 05
4 second
TIM 06
CR6 IN 0201
OUT 0202
CR 7
TIM 06
3 second
TIM 07
CR7 IN 0202
OUT 0203
VO1
IN 0202 CR7 TIM 07
OUT 0204
CR 8
TIM 07
4 second
TIM 08
CR8 IN 0203
OUT 0205
CR 9
TIM 08
3 second
TIM 09
CR9 IN 0205
OUT 0206
VO1
IN 0205 CR9 TIM 09