Duckweed (*Landoltia punctata*) production using an Arduino-based culturing chamber

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Abstract: Continued increase in energy demand, high energy prices and concerns about environmental pollution and climate change that are mainly driven by widespread use of fossil fuels have led to the growing concern about global energy security. *Landoltia punctata*, a local strain of duckweeds in the Philippines which possesses a great starch accretion ability which are needed for bioethanol was studied and cultured in an Arduino-based culturing chamber under three different culturing medium of swine effluent to water ratio for 6 days culturing period. The results showed that controlled light intensity, pH level, 16:8 photoperiod and 20ºC to 30ºC temperature favored the starch accumulation and biomass production. Moreover, the dilution ratio 2:1 (swine effluent: water) has the most significant starch increase, from 26% to 94%. Moreover, the biomass increased from 0.51 grams to 2.49 grams which is 388.24% of the starting weight. Furthermore, the results suggested that the more concentrated the culturing medium is, the higher the biomass production and starch accretion given that the optimum living condition was maintained. This study provides optimized duckweeds living condition for future industrial large-scale duckweed cultivation.

Keywords: duckweed production, Arduino-based culturing chamber, starch accumulation, biomass production

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1 Introduction

The continued increase in energy demand and the inevitable depletion of non-renewable sources sets off the exploration for alternative energy sources. Researchers are exerting significant efforts in searching for best alternatives to solve the global energy crisis. Solar, wind, and hydropower are some of the green energy sources that are being utilized and introduced to the public in attempt to aid the energy problem.

Philippines is facing a serious energy crisis as the Malampaya gas fields depletes which sustains the 30% of Luzon's energy consumption. It is expected to be completely depleted by the year 2024 (International Trade Administration, 2020). In line with this, the Philippine Government has been utilizing the use of both fossil fuels and renewable energy as solutions. However, the use of renewable energy which accounts for 24% of the energy mix has its own drawbacks.

The use of geothermal energy, hydropower, wind, and solar energy requires massive use of land space and

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leaves eventual residue specifically carbon footprints in production that can be harmful to people and the environment. They are also relatively expensive to set up which makes people reluctant to switch from what they were accustomed energy source to renewable energy. Therefore, the search for clean energy that has the least carbon footprints and is less expensive is one of the major goals of today's society.

Bioethanol is one of the most promising biofuels. Made from clean and renewable energy sources, it is an attractive energy source. Bioethanol can be produced from different kind of renewable feedstocks. The method of fermentation used by microorganism in producing bioethanol is determined by the raw materials. Fermentation of sugar-based raw materials such as sugarcane, corn, wheat and cassava belong to the first generation of bioethanol. Lignocellulosic materials typically contain lignin, cellulose and hemi-cellulose belongs to the second generation of bioethanol (Nielsen et al., 2020).

The first method of generating bioethanol is relatively easier in comparison with the second method but is not considered ideal. The raw materials used are all animal and human food. The consumption of such materials for biogas production will only add up to increasing reasons of food scarcity. The supply of these agricultural materials and their quality vary greatly depending on the geographical region as well as the season, year, and price. Relying on a single raw material alone can create supply barriers and can entail significant increase on raw material costs. Moreover, the consistency, composition, and quality of year-round supply of feedstock are unpredictable (Zabed et al., 2017).

The fermentation of lignocellulosic raw material is the second method for bioethanol generation. The materials are commonly acquired from agricultural waste and forestry products. This method is highly beneficial as it ferments agricultural residues which eliminates the competition with useful materials and helps with waste management. However, lignocellulosic materials require pretreatment to break down the structure of the lignin carbohydrate complex. This step is presently costly which makes it not commercially feasible (Rastogi and Shrivastava, 2017).

It would be ideal to find a source of bioethanol that is economically viable and would not compete for valuable resources. There are many species that can be a good source of bioethanol. Research has shown that duckweed is a potential starch source for ethanol production. Duckweeds is the fastest growing plants in the world and often doubles its biomass in a few days under optimal conditions (Cheng and Stomp, 2009). *Landoltia punctata*, commonly known as dotted duckweeds, is a strain of duckweeds that has a high starch content when converted into biomass (Jacono, 2002). Duckweeds, in general, has many advantages over other raw materials such as corn and sugarcane, making it an inexpensive and efficient source for bioethanol production. It is afloat and grows in highly concentrated wastewater and can be grown for high protein or high starch varieties. The high starch content is ideal for ethanol production and the high protein content has been proven to be equivalent to soy protein in animal feed (Christiansen et al., 2013).

The starch content of duckweed is reported to be between 4% and 10% by dry weight (Appenroth et al., 2017). It is known that starch accumulation in duckweed is affected by many factors such as temperature, nutrient deficiency (mainly nitrogen and phosphorus that can also be found in poultry wastewater) and duration of lighting, all of which can be altered to increase starch content in experimental or production systems (Cheng and Stomp, 2009). All sugars that can be absorbed and metabolized by microorganisms must be released as with minimal loss through the formation of the product and conversion to ethanol, hence the need for a proper culturing chamber. This research aims to create a growth chamber for *Landoltia punctata* that will specifically provide and

monitor the temperature, light duration and quality of nutrients uptake through manipulating the living environment for maximum starch production.

2 Methodology

2.1 Design criteria

The culturing chamber was designed and constructed based on the following criteria and considerations: 1) Modification and maintenance of the environmental conditions for dotted duckweeds to accumulate higher starch content; 2) Provision of light intensity of 110 $\mu mol\ m^{-2}\ s^{-1}$ throughout the conduct of the study and fulfilled through supplemental lighting; 3) Utilization of a photoperiod of 16:8, light: dark; 4) Maintenance of temperature range of 20°C to 30°C; 5) Capacity to culture duckweeds with three different dilution ratio of duckweeds all at once; 6) Capability to be operated by one operator; 7) Culturing efficiency of the chamber that should contain at least 10% starch content increase; 8) Materials used that should be from locally available materials; and 9) Ventilation through provision of ventilation hole.

2.2 The culturing chamber

Figure 1 presents the engineering drawing of the chamber outside and inside, respectively. It is comprised of frame, monitor, insulation wall, hot and cold side thermoelectric cooler Peltier, Peltier cooling system heatsink kit, temperature and humidity sensor, effluent container, light bulb, and Arduino.

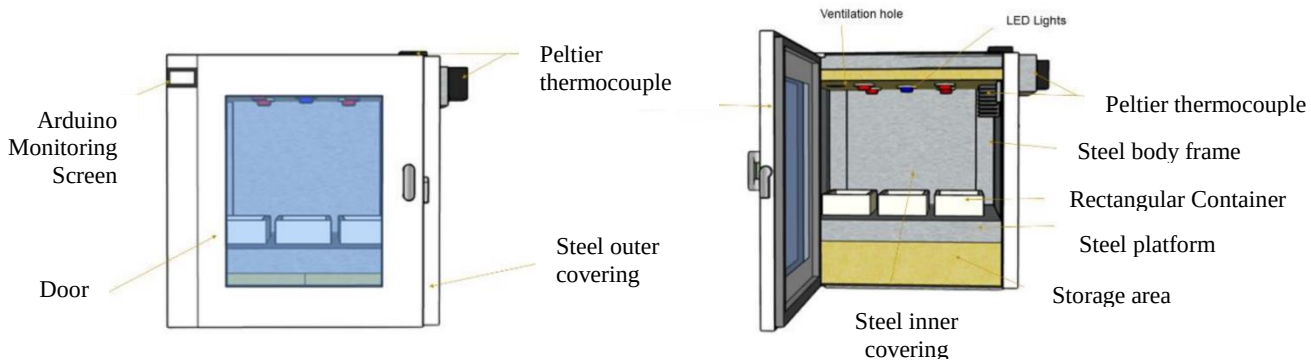

Figure 1 Outside and inside view of the arduino-based culturing chamber for duckweeds

Peltier Cooling System Heatsink Kit served as actuator whenever the sensor senses a temperature drop or increase from the programmed temperature range. It is composed of 2 fans, 2 heatsinks (cooling and heating block heatsink), and thermoelectric generator Peltier plate. The heating side was placed inside while the fan is on top, and the cooling side was attached on the outside wall but the heatsink was placed inside a pipe that connects inside the chamber. Meanwhile, SHT20 I2C Temperature and Humidity Sensor was the sensor used in the chamber to monitor and control the temperature inside the growth chamber. With length of 36.3 cm, width of 27.8 cm, and height of 7.5 cm, the food-grade aluminum tray that has a capacity of 3 Liters was used for culturing. It is a perfect fit in the chamber because of its characteristics. It was placed inside the chamber, on the center, 0.7 m from the light bulb. Moreover, 5 OMNI LED light bulbs, an energy saving light bulb with 300° beam angle and concealed with Aluminum heat sink enveloped in heat absorbing compound, were installed inside the chamber to meet the demand of light intensity of the duckweed's growth. Diurnal Variation Monitoring Device was assembled using the Weather Station Arduino Kit that are readily available in the market. This kit is used for monitoring and measuring the temperature, humidity, and atmospheric pressure as well as light sensor send data to the user. The Arduino-kit Fritzing diagram for the duckweed culturing chamber was shown in Figure 2.

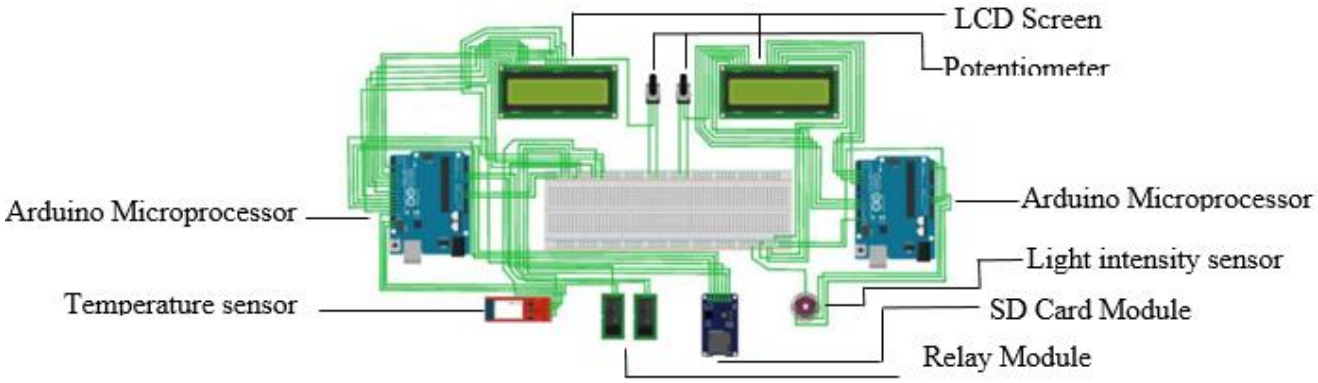

Figure 2 Arduino-kit fritzing diagram for the duckweed culturing chamber

Furthermore, body Frame was provided to support the overall weight of the machine. It has same dimensions as the machine and is made of steel. The insulation wall used has three layers excluding the inner and outer covering (steel). The calculations were considered using the heat transfer through walls. The outer layer was made of steel painted with white color, this also served as the covering of the chamber. The next layer from the outside is the polyethylene insulation foam with 5 mm standard thickness. Then in the middle layer will be the coconut fiber. In this study, 9 cm was the thickness of the fiber in order to achieve as low as 0.55 W of heat loss from the system. The thickness of the coconut fiber to be used in the growth chamber is 60 mm which is equivalent to 6 cm, derived from equation 1, considering 0.55 W heat loss in the wall.

$$Q = [K \cdot A \cdot (T2 - T1)] / d \quad (1)$$

where Q is the transfer of heat per unit time, K is the thermal conductivity of the body, A is the area of heat transfer, T_2 is the temperature of hot region, T_1 is the temperature of cold region, and d is the thickness of the body. Then, another PE insulation foam will be put before the inner body that is made of steel. This switches close and open circuits electromechanically/electronically using relays. It transformed the power from 220 V to 12 V for lights to 5 V for Arduino. It also took charge for the automatic turn on/off of lights in 16 h/8 h photoperiod.

The test material was prepared in such a way that the test sample for each treatment are conducted under same variety and condition, all were using *Landoltia punctata* strain and was cultured at the same time inside the chamber. The duckweeds were weighed and equally cultured in the culture medium with different dilution ratio. Before culturing, the effluents had already undergone pH level and NPK testing to determine the amount of Nitrogen, Phosphorus and Potassium present. The study was conducted at Mindoro State University Alcate Victoria. Figure 3 presents the Location of the study which is the Mindoro State University Main Campus, located at Alcate, Victoria Oriental Mindoro and is 9 km away from the nearest Automatic Weather Station. Since it is included in the 25 km radius from the coverage of the AWS, the data recorded can serve as a basis for the study especially in comparison of the conditions inside and outside the chamber.

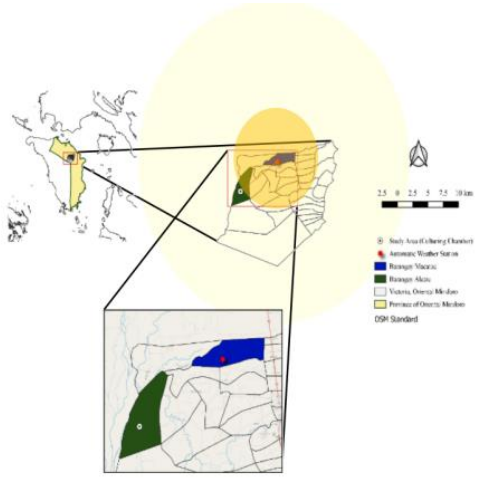

Figure 3 Location of the study

2.3 Design experiment

To test the performance of the chamber, the experimental set-up of culturing, starch accumulation and starch content testing was conducted as shown in Figure 4.

2.3.1 Culture medium composition analysis

The medium used for the treatment was swine effluent and was tested first using pH level and composition analysis where it determined the Nitrate, Phosphorus and Potassium Content (NPK) content. Once the pH level was tested lower or higher than the range of 5.0 to 7.0, buffer powder was provided to adjust the pH level. Then, the liquid was then again subjected to pH level test. As the pH level fell under the range, NPK analysis commenced. The swine effluent used was sent to Cavite Wastewater Laboratory for the analysis of nitrate, Phosphorus and Potassium content. Then, the swine effluent was mixed with water where three different dilution ratio of swine wastewater and water were used which are 1:2 (swine wastewater: water), 2:1 (swine wastewater: water), and 1:1 (swine wastewater: water). Each treatment was repeated three times.

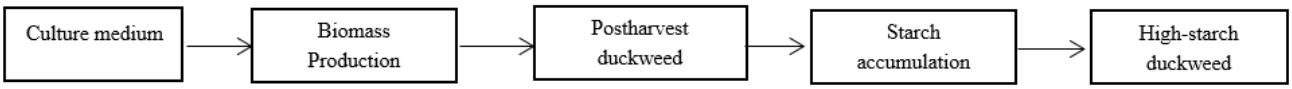

Figure 4 Flow chart of *Landoltia punctata* culturing chamber experimental set-up

2.3.2 Biomass production

Landoltia punctata was obtained in the river and pond in San Teodoro, Oriental Mindoro, Philippines. No specific permits or legal permission is required for the collection of the duckweed species since it does not involve endangered or protected species. Swine effluent was collected in pig farm in San Teodoro, Oriental Mindoro, Philippines.

Moreover, 0.51 g (501 mg) of fresh duckweed plants was put into the growth chamber at 20 to 30 ℃ under 16:8 light/dark with 110 μmol m^{-2} s^{-1} irradiance condition as suggested by past studies. The cultured duckweeds were harvested on third day to change its culture medium to water and then a sample was taken for starch testing. It was then fully harvested after 3 days after nutrient starvation. The same procedure was conducted with other treatment all under three trials.

While culturing the duckweeds inside the chamber, another 0.5 g of fresh duckweed was grown under normal setting at the same time. This was used to compare the biomass and starch accumulated from the samples inside the chamber and test the effectiveness of the chamber to increase its starch content. The fresh duckweeds cultured in normal setting was put in the container identical to the container used in the chamber. Tap water was used as culture medium and was harvested after 6 days.

2.3.3 Starch accumulation

The harvested duckweeds cultured on the different treatments proceeded with nutrient starvation to stimulate starch accumulation. The reason behind this is because the rapid proliferation of duckweed is supported by its great ability to absorb nutrients, especially nitrogen, from the medium to synthesize necessary proteins. At nutrient starvation, the protein synthesis was substantially reduced while the continual photosynthesis causes an increase in the relative proportion of starch in duckweed plants. Nutrient starvation, therefore, was applied to stimulate starch accumulation in temperature-treated duckweed (Cui, 2013). The same photoperiod, light intensity, pH level and temperature range were maintained all throughout the conduct of experiment.

After harvesting in 3 days, the duckweeds were put into normal water inside the chamber and left for another 3 days before it was fully harvested. Same procedure was applied to the duckweed grown in normal setting. Plants that have been harvested after six days were placed in a container and the sample from the duckweeds grown were subjected to iodine test for

starch content.

2.3.4 Starch content testing

Iodine Test was used to determine the starch content of *Landoltia punctata* cultured inside the chamber. The duckweeds were weighed first to get the biomass of the samples and then set aside to dry the water. Then the samples were measured, and the sample size was determined through the set-up with lowest biomass. To get the mass of the samples, Slovin's formula was used, as shown in Equation 2.

$$n = N / (1 + N \times e^2) \quad (2)$$

where *n* is sample size, N is the population and e is the margin of error. Each sample was boiled in a boiling water with temperature of 90 ℃ to100 ℃ for 2 minutes and was transferred in a test tube. The water inside the beaker was set aside for ethanol boiling. Consequently, the ethanol was added in the test tube. The test tube with ethanol and boiled duckweeds was placed in the water boiled in the beaker until the ethanol boils. The samples were transferred in the petri dish/small paper plate and 10 drops of Iodine was applied in each sample. The samples were set aside to dry, then oven dried for 5 minutes. The blackened area of the samples was separated to determine the potential starch content of the duckweeds cultured inside the chamber. The oven dried samples were weighed, and non-black color of the samples were removed. Then, the remaining black leaves were weighed again. To quantify the results, the mass of the total sample weighed after removing non-black leaves were rationed to the mass of the samples after oven drying.

3 Results and discussion

The average ambient temperature and solar radiation were recorded in Macatoc, Victoria, Oriental Mindoro, Philippines on 2020-2021, 9 km away from Mindoro State University where the study was conducted. It reflects the fluctuating temperature and solar radiation which ranges from 25 ℃ to 30 ℃ and 96 W m^{-2} to 232 W m^{-2} respectively. This indicates that there are days that the ambient temperature surpasses the required temperature (25 ℃ to 30 ℃) and solar radiation (110 μmol m^{-2} s^{-1}) as shown in Figure 5 for the optimal growth of duckweeds. This imposes the need of the culturing chamber to utilize the growth of *Landoltia punctata*.

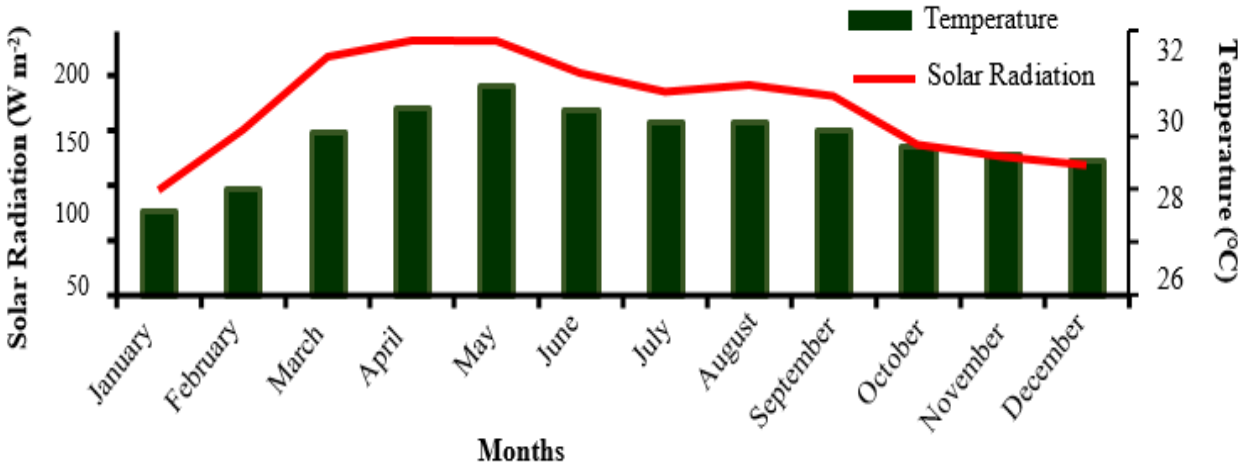

Figure 5 Average temperature and solar radiation in nearest AWS

Moreover, Figure 6 shows the room temperature recorded every 30 minutes as the culturing chamber starts growing *Landoltia punctata*. According to the graph, the room temperature upon the conduct of the study reached above 30 ℃ for Days 2 and 3. This indicates that the room temperature in the location still exceeds the required temperature which emphasizes the need for culturing chamber that will maintain the temperature for better growth of duckweeds.

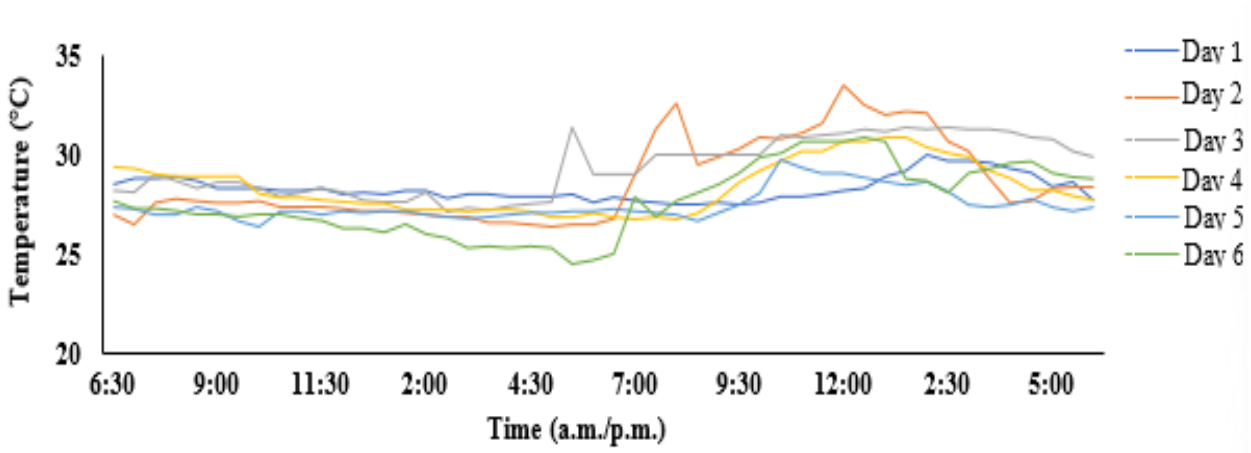

Figure 6 Room temperature from May 27 to June 2, 2022

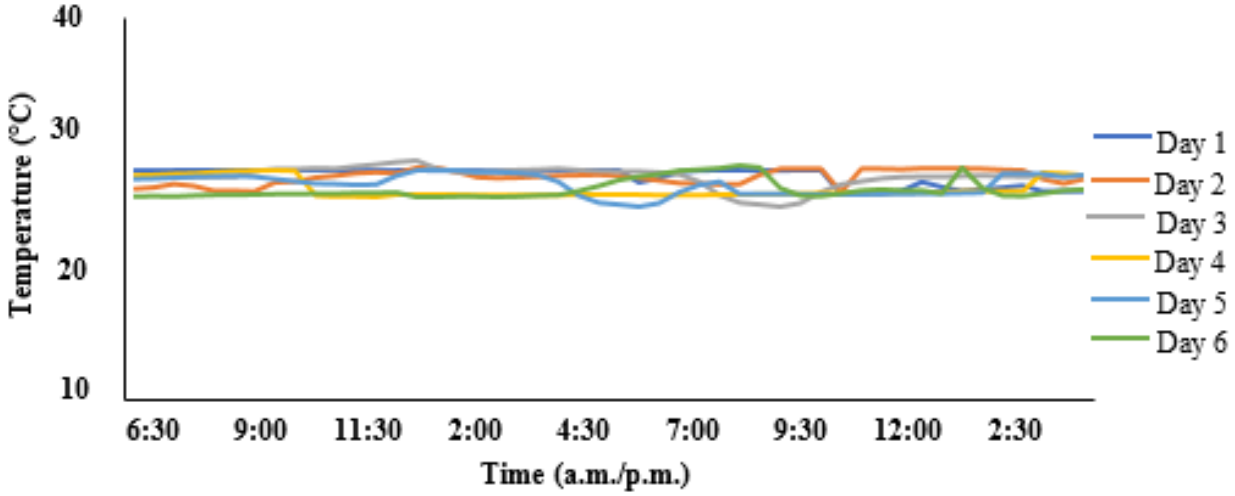

Figure 7 Temperature inside culturing chamber

The line graph of temperature inside the culturing chamber as it starts culturing *Landoltia punctata*, as presented in Figure 7, shows the values are in between 25 ℃ and 29 ℃. The temperatures recorded fit the required range which indicates that there is consistency in the temperature inside the chamber. The highest recorded temperature was 28.66 ℃ while the lowest is 24.97 ℃.

Furthermore, Table 1 and Figure 8 show the average room and chamber temperature per day. Standard deviation was also computed where the scatteredness of the data collected were determined. Based from Table 1, the average temperatures inside the chamber are still inside the required temperature range of duckweed's growth while the average room temperature in Day 4 exceeded the maximum temperature needed with 33.36 ℃. This indicates the effectiveness of the chamber in maintaining the temperature required for the plant's growth. Moreover, the standard deviation of the average temperatures was determined where the value in room temperature is greater than the average temperatures inside the chamber with a value of 2.18. It is further illustrated in Figure 8. This means that the data collected in room temperature is more scattered than temperature inside the chamber. This shows the consistency in the chamber where there is less fluctuation than room temperature. The heating system of the chamber comes from the light bulbs installed inside. Table 1 shows that the average temperature of is 26.96 ℃. Using Heat Transfer using Thermal Radiation, the heating system of the chamber is 0.0457 Watt-hour.

Table 1 Average temperature of room and chamber

	Average Temperature (degree Celsius)	
	Room Temperature (℃)	Temperature Inside Chamber (℃)
Day 1	28.30	27.41
Day 2	28.68	27.30
Day 3	29.37	27.37
Day 4	28.36	26.47
Day 5	27.51	26.77
Day 6	27.66	26.46
Average	28.31	26.96
SD	0.68	0.45

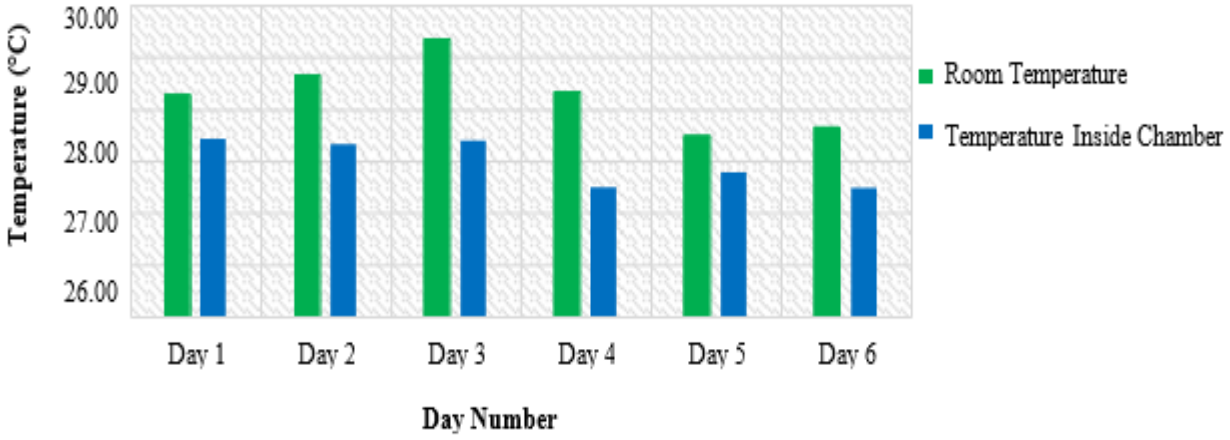

Figure 8 Average temperature of room and chamber

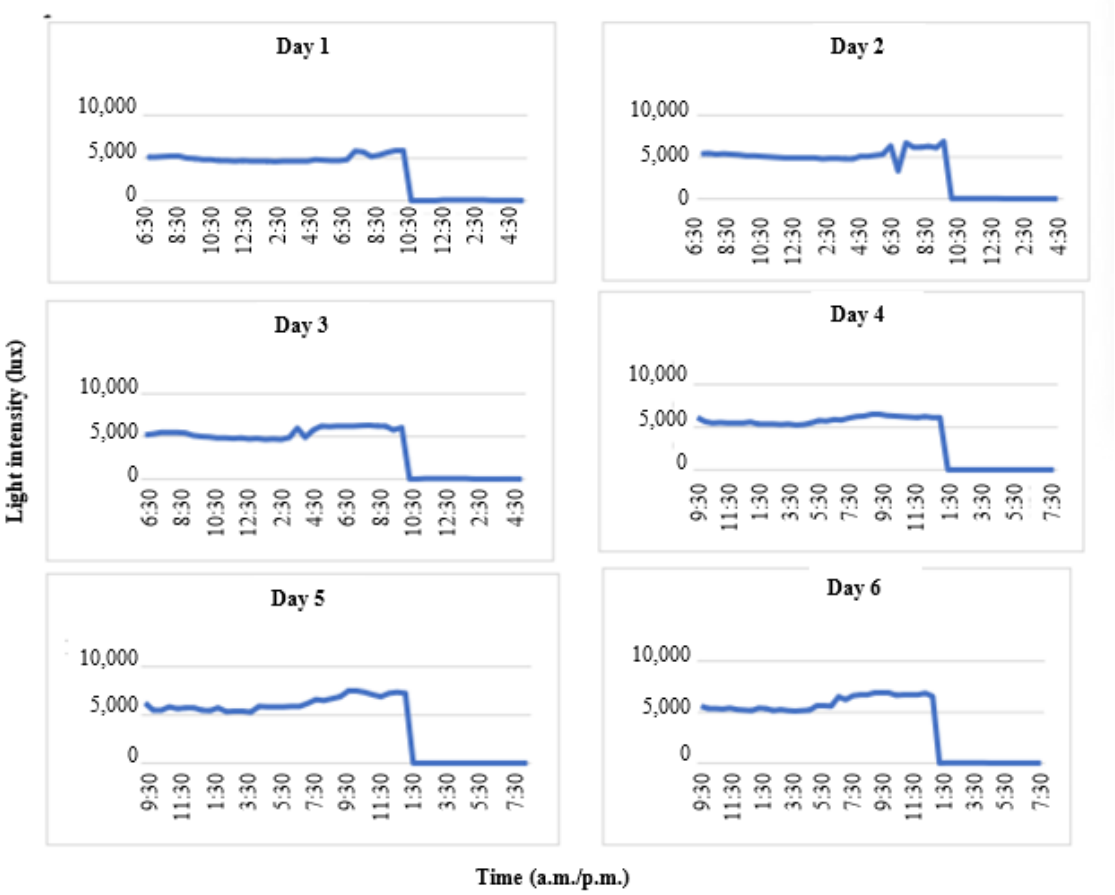

Figure 9 Light intensity inside culturing chamber

Figure 9 presents the light intensity recorded inside the chamber during the culturing period. The light intensity of 110 μmol m^{-2} s^{-1} was converted into watts and converted into lux and the Arduino measures and shows into the monitoring screen. The equivalent of 110 μmol m^{-2} s^{-1} ranges from 5000 to 6000 lux. The light intensity in all Figures lie at 5000 lux for 16 hours while the lights are turned on and became 0 for the remaining 8 hours of photoperiod. This indicates that the lights inside the chamber provide sufficient intensity needed for the growth of *Landoltia punctata*.

On the other hand, to illustrate the effect of the controlled environment, duckweed's nutrient recovery ability from wastewater and the effect of concentration of NPK from swine wastewater, *Landoltia punctata*, a strain of duckweeds commonly found on Southeast Asia was cultivated for six days inside a culturing chamber that provides the plant's optimal living condition: 20ºC to 30ºC temperature, 16:8 light-dark photoperiod ratio, 5.0 to 7.0 pH level, 110 μmol m^{-2} s^{-1} light intensity under three different dilution of swine effluents to water ratio: 1:2, 2:1 and 1:1.

As Figure 10 suggests, the biomass of duckweed plants grown in the culturing medium increased by nearly 4.7-fold from the initial 0.5059 grams to a maximum of 2.3562 grams during the 3 days of cultivation, with a maximum growth rate of about 0.42 mg cm^{-2} day^{-1}. However, the duckweeds cultured in the normal environment setting managed to grow for only about 0.0114 mg cm^{-2} day^{-1}. This indicates that the duckweeds grown on the chamber has a relatively higher biomass rate than that of the duckweeds cultured outside of the chamber. Duckweed often demonstrates near exponential growth rates and many species have doubling times of 2 to 3 days, depending on the environmental conditions. In this case, the duckweeds cultured with the complete environmental manipulation condition showed a favorable increase in biomass production in comparison to the samples grown in the normal condition. This is basically because the duckweeds cultured on a normal setting was heavily affected by the fluctuating temperature and light intensity. However, the starch content for the duckweeds cultured in the manipulated environment are on the decline while the starch content of the duckweeds cultured in the normal setting remains the same. This phase can be seen in Figure 11 during the first 3 days.

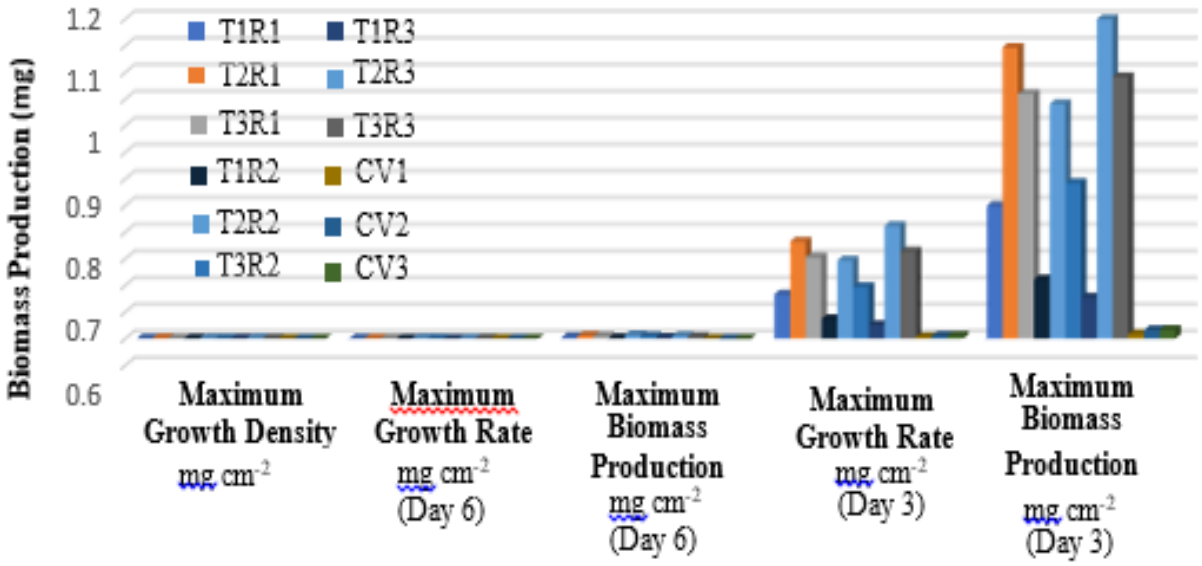

Figure 10 Biomass production, maximum growth intensity and maximum growth rate within six days cultivation period

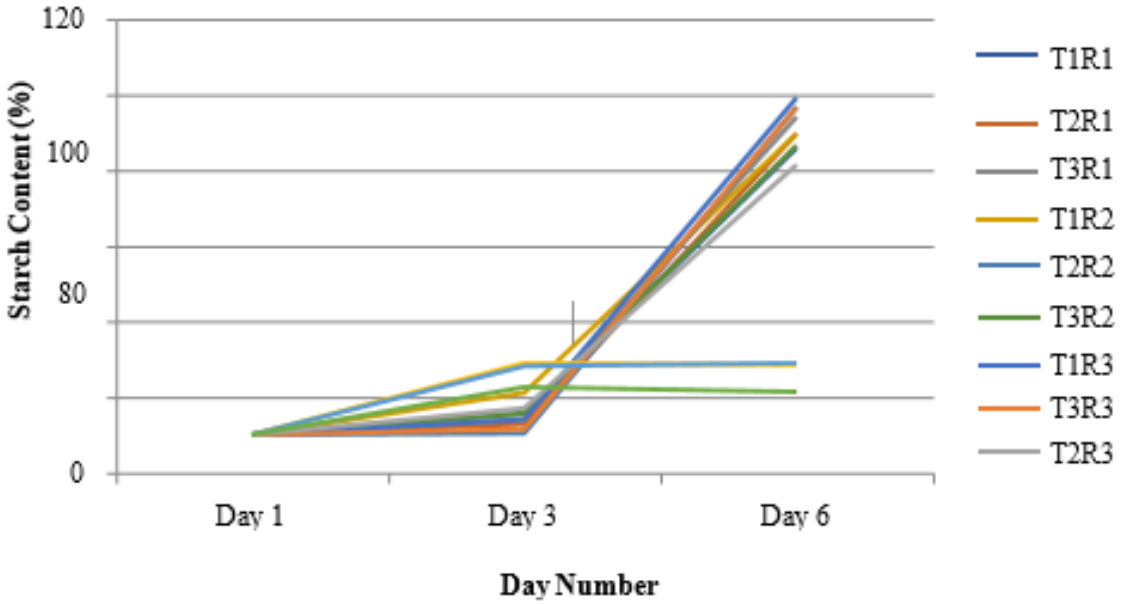

Figure 11 Starch content percentage

Starch content of duckweed was affected by many factors, especially nutrient deficiency. When the duckweeds were transferred to normal water as culturing medium, it stops from taking in nutrients. At nutrient starvation phase during the last three days of culturing, the protein synthesis was substantially reduced while the continual photosynthesis causes an increase in the relative proportion of starch in duckweed plants. This is due to the concentration of the culturing medium brought about by the nutritional component of swine wastewater. The maximum average starch content of the duckweeds cultured on the chamber was roughly 93.96% which was 67% higher than the duckweeds cultured on a normal environment.

There are 4 treatments identified in the study, treatment 1 with dilution ratio of 1:2 (1 L swine effluent, 2 L water), treatment 2 with 2:1 ratio (2 L swine effluent, 1 L water), treatment 3 with 1:1 ratio (1.5 L swine effluent, 1.5 L water) and treatment 4 which is the controlled variable (3 L pure water). Each of them has 3 replications. This was used for the Randomized Complete Block Design as statistical treatment in the study. Descriptive Statistics were used in order to get the data needed for ANOVA testing for statistical treatment which is presented in Table 2. As shown, treatment 1 garnered a mean of 1.23 and standard deviation of 0.3. Treatment 2 has an average of 2.49 and standard deviation of 0.32. Treatment 3 have an average of 1.86 and standard deviation 0.23. Treatment 4, the controlled variable, harvested a mean of 0.37 and standard deviation 0.18. Summing them up, the total mean of the treatments is 1.49 and standard deviation 0.85. The multiple comparisons were determined using Post Hoc Test in the treatments. It was found out that there is significant difference between the treatments and this table further analyzed which of the following treatments have significant difference. As shown in Table 2, there is significant difference between the biomass of treatment 1 and 2 having 0.003 value which is less than 0.05 (p-value). There is also significant difference between treatment 1 and 4 (controlled) having 0.021. Treatment 2 and 4 has also significant difference having 0.000 value. There is also significant difference between Treatment 3 and 4 (controlled) having 0.001 value.

Moreover, Table 3 describes the multiple comparisons in starch content of every set-up using

Post Hoc Test in the treatments. It was found out that there is significant difference between the treatments and this table further analyzed which of the following treatments have significant difference. As shown above, there is significant difference between the starch content of treatment 1 and 4, 2 and 4, and 3 and 4 since the values computed are less than 0.05 (p-value).

Table 2 Post Hoc test for biomass production

Multiple Comparisons - Post Hoc Test

Dependent Variable: Biomass Production

Tukey HSD

(I) Treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.2600*	0.20446	0.003	-1.9677	-0.5522
	3	-0.6242	0.20446	0.081	-1.3320	0.0835
	4	0.8668*	0.20446	0.021	0.1591	1.5746
2	1	1.2600*	0.20446	0.003	0.5522	1.9677
	3	0.6357	0.20446	0.076	-0.0720	1.3435
	4	2.1268*	0.20446	0.000	1.4190	2.8346
3	1	0.6242	0.20446	0.081	-0.0835	1.3320
	2	-0.6357	0.20446	0.076	-1.3435	0.0720
	4	1.4911*	0.20446	0.001	0.7833	2.1988
4	1	-0.8668*	0.20446	0.021	-1.5746	-0.1591
	2	-2.1268*	0.20446	0.000	-2.8346	-1.4190
	3	-1.4911*	0.20446	0.001	-2.1988	-0.7833

Table 3 Post Hoc test for starch content

Multiple Comparisons - Post Hoc Test

Dependent Variable: Maximum Starch Content

Tukey HSD

(I) Treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-2.7417	5.10370	0.947	20.4093	14.9258
	3	4.2815	5.10370	0.835	13.3860	21.9491
	4	65.1712*	5.10370	0.000	47.5037	82.8388
2	1	2.7417	5.10370	0.947	14.9258	20.4093
	3	7.0233	5.10370	0.555	10.6443	24.6908
	4	67.9129*	5.10370	0.000	50.2454	85.5805
3	1	4.2815	5.10370	0.835	21.9491	13.3860
	2	-7.0233	5.10370	0.555	24.6908	10.6443
	4	60.8897*	5.10370	0.000	43.2221	78.5572
4	1	-65.1712*	5.10370	0.000	82.8388	47.5037
	2	-67.9129*	5.10370	0.000	85.5805	50.2454
	3	-60.8897*	5.10370	0.000	78.5572	43.2221

The researchers have made general observations upon the conduct of the study. First, the temperature inside the chamber is always inside the temperature range required for duckweed's growth. Second, Peltier

cooling system automatically turns on as the temperature inside the chamber reaches 28℃ to 29℃. The pH level of the diluted effluent reaches the range of needed pH level for duckweed's growth, hence, there were no adjustments made. The scums inside hinders the duckweeds to afloat, hence, there is a need to stir the culture medium after 16 hours of light. While harvesting, there are duckweed fibers attached to the duckweeds in treatment 2, meaning the duckweeds continue to propagate while being starved with nutrients. The color of the leaves in controlled variable are yellow green while the color of the leaves in duckweeds cultured inside the chamber is dark green. Lastly, the rootlets in the fronds of duckweeds in treatment 2 are longer compared to other treatments.

4 Conclusion

Based on the findings, conclusions were drawn by the researchers. First, the duckweed (*Landoltia punctata*) culturing chamber is effective for growing duckweeds as it has successfully provided the needed living conditions. The temperature inside the chamber ranges from 25℃ and 29℃ which is inside the temperature range for culturing duckweeds. There is less fluctuation in the temperature inside the chamber compared to the temperature in the natural setting. Moreover, the light intensity of the chamber fits the required light intensity for growing *Landoltia punctata*. It is expected to yield 15,330 kg ha^{-1} of *Landoltia punctata* cultured inside the chamber in a year. The biomass production and starch content of the duckweeds cultured inside the chamber with swine effluent as culturing medium varies directly on the concentration of the culturing medium. The study provides optimized light conditions for future industrial large-scale duckweed cultivation. Lastly, the culturing chamber capacity is 3,852.09 mg with a culturing chamber efficiency of 67%.

5 Recommendations

Upon completing the study, the researchers recommend the following: first, to further quantify the starch content of *Landoltia punctata*, refer to a tested available Laboratory Center at Vietnam. Second, the duckweed culturing chamber is recommended for large scale production of duckweeds. The duckweed culturing chamber can also be modified for increasing the protein content of duckweeds. Fourth, try to culture another strain of duckweeds in the culturing chamber to compare which strain of duckweeds produce highest amount of starch inside the chamber. Use different culture medium like poultry wastewater, domestic wastewater or snap solution. Sixth, the yield starch contents of high-starch duckweeds as well as the starch-to-ethanol conversion rate could be utilized in a full-scale operation. Lastly, the researchers hope that this study will aid future large-scale industrial application for production of ethanol.

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