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Characteristics of Spirulina Platensis Cultivation with Comparison of Walne, Zarrouk, and Miquel Allen Methods

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ABSTRACT: *Spirulina cultivation requires specific treatment and conditioning since it commonly has unwanted microbes such as fungus, bacteria, and virus which will grow during the cultivation as a predator for the existence of spirulina. The easiest spirulina cultivation method can be conducted in various sizes of aquariums by adding various cultivating media. This research uses the composition of Walne, Zarrouk, and Miquel Allen fertilizers to discover which method is optimal to obtain the curve of growth for Spirulinaplatensis. From the variation of cultivating media method, analyses will be conducted about cell density through sunlight and another lighting method using TL 3000 Lux lamp. The result shows that the highest cell density is obtained through miquelallen method of 279,662.42 sinusoids using sunlight and the highest mean of cell density per day reaches 192,042.872 sinusoids.*

KEYWORDS: *Cell density, Cultivation, SpirulinaPlatensis ,Walne, Zarrouk, Miquel Allen*

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I. INTRODUCTION

Human dependence over energy needs has occurred since long time ago when early human civilization exists. The needs of energy increase over time due to human population hike. So far, the needs of energy are fulfilled by raw materials derived from fossil energy such as coal, natural oil, and gas which are not renewable. Their amounts get more limited and may be depleted due to their long recovery process.Moreover, the burning of fossil fuel has brought negative impacts to environment as it produces emission like carbon dioxide (CO_2) causing ozone layer depletion contributing to global warming. With such facts, the innovation and development of energy from renewable energy sources are necessary.

One of renewable energy sources frequently developed by researchers is a plant-based source including living plants in form of forest and agricultural products and their wastes. Moreover, lately, the potential of marine plant has started to be considered as they contain certain components which can be utilized as biofuel. Marine lives like algae often grow at the coasts of Java Island [12]. Marine microalgae like spirulina contain organic components such as protein, carbohydrate, fat, vitamin, and mineral [8, 1]. With relatively high carbohydrate content, spirulina has a potential to be an energy source which can be converted into bioethanol [8, 1, 3,18, 11]. Spirulina can be cultivated in artificial seawater whose nutrition resembles the nutrition in its original habitat (8, 16, 7, 9, 15, 4, 10], indoor or outdoor and in big or low scale.

Energy efficiency can be conducted by developing alternative energies besides fossil energy. Bioenergy raw material is one of biomass energy sources which can be developed and are observed by researchers lately. As biomass, spirulina microalgae play an important role to be studied as a new and renewable energy source. Not many researchers in alternative energy field study spirulina as an energy source. Most of them use spirulina as a food coloring and for pharmacy. Spirulina cultivation needs particular methods since during the cultivation, other microbes including fungus and virus will also grow as predators and attack spirulina[5].Spirulina cultivation can simply be conducted in aquariums or small vessels by adding cultivating media in form of the mixture of fresh water, sodium carbonate, sea salt, iron, and ash water [8]. Moreover, it can also be conducted by adding vinasse nutrition and AO media in the cultivation of Spirulina maxima [16], cheese whey [7], cow urine [7], Zarrouk media [7, 9, 15] and A_5 micronutrient [7]), Zarrouk media without $NaHCO_3$ (420) KT & MT Zarrouk Media [17], Walne media [17, 2]. Organic nutrition can be added with the extracts of bean sprout and urea fertilizer [2] and NPK fertilizer [9]. Amoniumsulfate and sodium nitrate are used by Ferreira as food for spirulina besides CO_2 gas feeds [4, 10, 6].Spirulina flourishes in the temperature of 25-35^oC [8, 16, 15, 10), pH levels of 10.5 [8], 8.5-10 [7] and 9.3 [16]; under LED lamp lighting for 6 hours to assist its photosynthesis process [8], the lamp is turned off at night to resemble its growth situation in its habitat, and other lighting needs to be turned on for 12 hours and turned off for 12 hours [16, 15] or 10-12 hours turned on [7]. Light intensity used by Zhang is the illumination of 100 mmol/m2/second [10] and 3000-4000 lux [17]. Agitation process with air circulation is also essential so the added

nutrition can be well-distributed and Spirulina will not clot. Spirulina's growth media is under 20% salt concentration [2]. The best harvest period is on 12th day [17], 15th day [7], 6th day [10] and 3rd week [16] by measuring daily absorbance using spectrophotometer on the wave lengths of 560 nm [7, 4] and 690 nm [9].

Different from the research conducted by Joshi which applied additional nutrition of cow urine [7], for the proposed research besides Zarrouk media nutrition, human urine will be added during cultivation process. It is conducted since cows only eat grass having more limited nutrition content than human consuming plant and animal-based foods so the nutrition contained in their urine is more complete to be used as spirulina's growth media. It is in line with the statement of Ranasinghe (2016) that human urine is secretion containing nutrition for plants such as nitrogen, phosphor, and potassium. In this research, we try to compare several fertilizer media with Walne, Zarrouk, and Miquel Allen methods under cultivation using sunlight and TL 3000 lux lamp to produce the most optimal spirulina by analyzingspirulina's cell density.

II. RESEARCH METHODOLOGY

The most economical and efficient method to produce spirulinaplatensis is by using aquariums with various sizes. To meet Ph and salinity, this method uses seawater mixed with RO water with the following methods:

1. To keep the media sterile, add natrium chloride in the aquarium filed with 80-liter water.
2. To prevent the water from releasing chlorine odor and to main the neutrality of the water's nature, add natrium thiosulfate with certain concentration.
3. Conduct initial analyses of pH, salinity, and temperature before seeds are put inside the media.
4. Conduct the initial analyses for 7 days to determine the value of salinity, pH, and temperature. The results of analyses are shown in Table 1.

- The results of analyses show that the temperature reaches 26 to 27^{o} C. It is suitable with optimal temperature for the growth of microalgae especially spirulinaplatensis which is 25 to 32^{o} C.
- Salinity ranges from 17 to 21 ppm. The value remains in the range of optimal salinity for the growth of spirulinaplatensis which is 17-25 ppm.
- Meanwhile, pH remains in the range of pH which is 7-8.

5. Since it has met the requirements for media of growth, spirulina seeds are then put inside the aquarium filled with 80-liter water.
6. Nutrition is added to the aquarium using walne formula of nutrition.
7. Observation is conducted every day by collecting samples to be observed using a sedgewick and their densities are observed using a microscope.
8. Observation is conducted until spirulina algae changes its color from green, bluish green, to yellow.
9. The green and bluish green colors show optimal growth while the yellow shows sign of death to the algae.
10. The observation to calculate cell density is resumed by changing walne nutrition with Miquelallen and zarrouk nutrition.

Cell Density Calculation with Sedgewick Rafter Counting Cell (SRCC)

Using SRCC, cell density calculation is conducted by collecting samples, putting them in a sedgewick and observing them using a microscope. First, adjust SRCC calculation scale by viewing them in low magnification. Next, change the view to high magnification and calculate the number of cell in 10 fields of view. SRCC has 20 lines of squares and 50 columns of squares so there are 1,000 squares in total. For spiral spirulinaplatensis, calculate the number of its sinusoids (one sinusoid is half of the top of circle).

Cell density (N) is calculated using the following formula:

$$N = \frac{\text{Amount of cell in 10 fields of view}}{\text{3.14 x number of field of view}} \text{ x } 1000$$

III. DISCUSSION

In the cultivation of this spirulinaplatensis, cell density factor is necessary to know the number of cell in the growth of spirulina. This research uses walne, Miquelallen, and zarrouk formulated fertilizers. Each has different and particular composition. The result of cell density analysis for each formula is shown in Table 1.

Table 1. Cell density of analysis

Day	Walne formula	Zarrouk formula	Miquelallen formula
1	79713.38	38057.32	54777.07
2	82777.07	98726.11	108282.25
3	253031.4	128025.47	140764.33
4	193949	143630.57	165286.62
5	155414	204324.84	245541.4
6	203343.9	218152.86	269745.22
7	411624.2	239490.44	279662.42
8	360987.4	223248.4	270063.69

9	35063.69	192356.68	196496.81
10	87643.31	152866.24	189808.91
Mean	186354.735	146575.793	192042.872

Referring to the mean of cell density per day, walne formula has 186.354,735, zarrouk formula has 146.575,793, and Miquelallen formula has 192.042,872. It means Miquelallen formula has the highest mean of cell density per day since it has the highest ratio of nitrogen and phosphor values namely 7.48 : 1. Meanwhile, the ratio for zarrouk formula is 4.68 : 1 and walne formula is 3.67 : 1. For the growth of microalgae, the most determining element is the content of nitrogen in fertilizer as Nitrogen is a primary component in cell protein which is the basic part of organism's life while phosphor is really needed in the process of protoplasma and cell nucleus, and it is a basic component to form nucleic acid, phospholipid, enzyme, and vitamin.

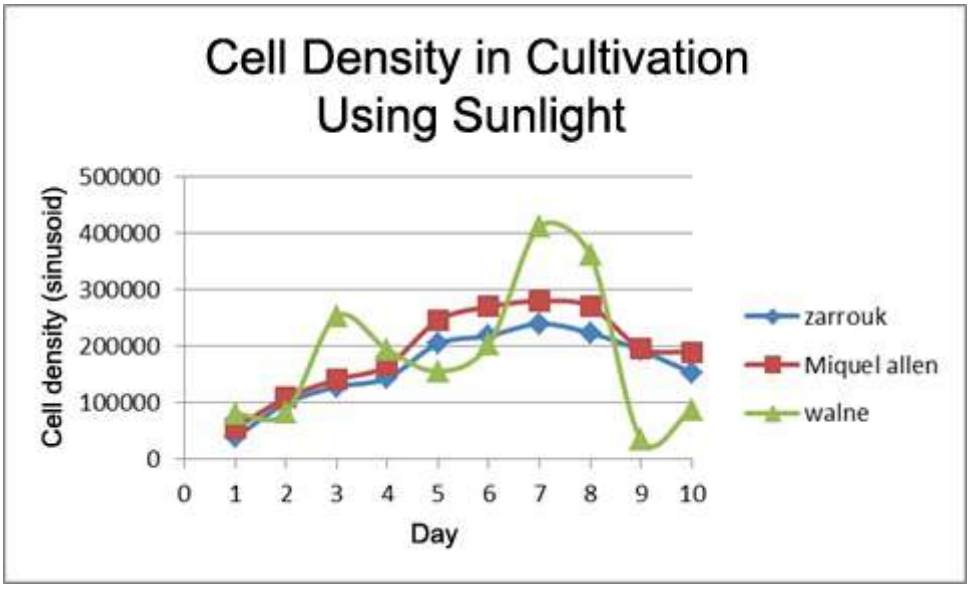

Image 1. Relationship between day and cell density in the growth phase of spirulinaplatensis using sunlight.

From image 1, the following observation data is obtained:

1. The growth of spirulina using walne formula shows irregular growth since after experiencing growth phase (exponential), the graphics drop and rise again and the highest number is achieved (stationary phase) in seventh day with cell density of 411,624.2. This is the highest value compared to Miquelallen and zarrouk formulas.
2. Meanwhile, the growth phase of spirulina seems to be stable in Miquelallen and zarrouk formulas, starting from the phases of adaptation, growth, stationary, and death.
3. Miquelallen formula generates higher cell density value than zarrouk formula for the growth of spirulinaplatensis.
4. Miquelallen and zarrouk formulas generate the highest cell density values in seventh day during stationary phase namely 279,662.42 for Miquelallen and 239,490.44 for zarrouk formulas.
5. Exponential phase shows that spirulinaplatensis' cell density starts to increase and will stop in stationery phase and cell density then starts to drop (death phase) after seventh day for walne, Miquelallen, and zarrouk formulas.
6. Growth phase starts with green color, then changes to bluish green, and to yellow showing death phase.

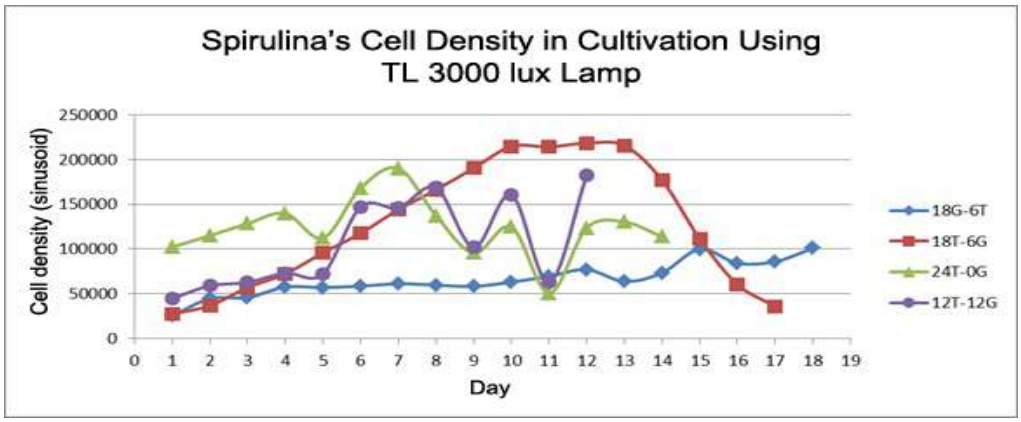

Image 2. Relationship between day and cell density in the growth phase of spirulinaplatensisusing TL 3000 luxlamp.

In image 2, spirulina cultivation uses another light besides sunlight which is TL lamp. Light is an energy source in photosynthesis process. Therefore, lighting intensity, quality, and time really affect the growth of spirulinaplatensis. Photosynthesis rate will increase upon high intensity of light and vice versa. Spirulina cultivation is conducted in a room with proper light intensity of 2500-4000 lux, 3000 lux is used in this research, with different lighting periods.

The observation of image 2 shows the following results:

1. The lighting periods of 12T – 12G (12 hours of lighting and 12 hours of darkness) and 24T – 0G (24 hours of lighting and 0 hour of darkness) generate irregular growth phases namely the phases of adaptation, growth, stationary, death, and growth again repeatedly.
2. The lighting period of 18G – 6T generates regular growth phases namely the phases of adaptation, growth, stationary, and finally death.
3. The lighting period of 18T – 6G generates the best growth phase among others. Besides regularity, the highest cell density value of 218,312.1 is achieved in 13th day.
4. Stationary phase is a phase in which spirulina has the most optimal growth marked with bluish green color in microalgae.

IV. CONCLUSION

This research generates the following conclusions:

1. The highest cell density is achieved in Miquelallen formula of 279,662.42 exposed with sunlight.
2. For the cultivation of spirulinaplatensis using the lighting of TL 3000 lux lamp, the highest density of 218,312.1 is achieved in the lighting period of 18T – 6G in 13th day.
3. Miquelallen formula has the highest mean of cell density per day of 192,042.872.

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