Mass Production and Growth Performance of Spirulina on Salinity Reduction

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Mass Production and Growth Performance of Spirulina on Salinity Reduction

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Abstract

Spirulina, a multicellular and filamentous cyanobacterium, has already known for its bioactivities as a functional food and fishes diets. In this study, mass production of Spirulina was demonstrated in modified salinity for 25 days. This study aimed to determine the best salinity to obtain the optimal growth rate of-Spirulina. The cultivation method was small to large-scale and evaluated the water quality. Results showed that salinity reduction influences algal biomass. Spirulina's density was $41.8x10^3$ cells ml^{-1} in 15 ppt; $81x10^3$ cells ml^{-1} in 20 ppt; $145x10^3$ cells ml^{-1} in 23 ppt; $160x10^3$ cells ml^{-1} in 25 ppt; and $270x10^3$ cells ml^{-1}. The most efficient treatment was 20 ppt with the cells density $510x10^3$ cells ml^{-1} in the 23rd day in salinity 20 ppt after cultivated in large-scale production. The results suggest that mass-produced Spirulina can be characterized using a modified salinity technique.

Keywords: Cyanobacteria, Cells Density, Large-Scale Culture, Spirulina, Water Quality.

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Introduction

The genus *Arthrospira* which is currently known as *Spirulina*, has already learned of being used as a food source for every organism, including fishes (Nowicka-Krawzyk et al., 2019). This superfood has demonstrated its bioactivity as fish pellet and had proven to increase fish health and body coloration, especially on ornamental fishes. Skin pigmentation is responsible for ornamental fish's coloration; therefore, the carotenoid pigment in spirulina acts as a vital nutrient for color and growth, reproduction, and metabolism (Bakshi etal., 2018). For this reason, it is essential to mass-produce Spirulina products due to their value as fish food.

Several cultural methods have already been demonstrated to enhance their mass production. The controllable factors to promote Spirulina's growth are pH, temperature, light intensity, and carbon dioxide concentration (Jin et al., 2020). Although *Spirulina platensis* species in freshwater microalgae, nevertheless it still needs a high concentration of salts to improve its growth. Thus, the modification of Spirulina medium components was further studied to gain a cost-effective outcome for its mass production (Choi et al., 2016).

In this study, we report the growth performance of Spirulina's culture in a small scale laboratory to mass-production

with gradual salinity reduction. Several controllable parameters tested were pH, temperature, salinity, dissolved oxygen, and density.

Materials and Methods

Spirulina Production

Spirulina seeds were prepared withthe addition of salts 30 ppt in 1000 mL. Wayne fertilizer as Spirulina's nutrition was added in 2% concentration (v/v) of the mixture. Lamp with 3200 lux of white light(around 20 watts) in room temperature and aeration was added to the culture system.

After seven days, Spirulina seeds were moved to more significant cultural conditions. These seeds are added to an aquarium in 20 liters of saline water (30 ppt). This culture then was added urea (nitrogen) fertilizer 60 ppm and TSP 30 ppm. Lamp and aeration were prepared as medium control at room temperature. In this scheme, Spirulina's salinity was degraded by water addition to 25 ppt – 20 ppt every three days in 14 days. Its density was also checked, and other parameters, such as temperature, dissolved oxygen, carbon dioxide, and pH to find out Spirulina's quality.

Spirulina's mass production has been conducted in fiberglass tanks in salinity concentration 20 ppt, started on day 15. A mixture of $Na_2SiO_{35}H_2O$ 0.3% (w/v), 2mL of TSA, and Urea was used as fertilizer and silicate enrichment. Sunlight was maximized during the daytime, and several 3200 lux of white light were installed in this system in the evening. The Spirulina was mixed manually every day with the addition of several aeration systems. This systemwas installed for nine days.

After culturing Spirulina, on the 24th day, they were harvested using a 200 mesh sieve and dried under sunlight for 4 hours to minimize protein reduction. This Spirulina was dried in an oven (50°C) for 120 min and stored at room temperature.

Density Calculation

Spirulina's sample was initially checked under a trinocular microscope (Olympus BX43; Shinjuku; Japan) to analyze its condition. Seeds were diluted in 1:100 (v/v) to reduce its density and observed.

To analyze its density, a little sample of Spirulina was taken out in a hemocytometer and analyzed under a microscope with five fields of view. The formula was calculated as follows:

Cell Density $: \frac{(A1+A2+A3+A4+A5)}{5} \times 25 \times 10.000$

A = Cell count in 1 chamber
5 = Total experiments
25 = Total of the chamber (5 fields of view)
10.000=Chamber's density volume

Water Quality Measurement

To ensure the optimal growth of Spirulina, the water quality was evaluated every day. Several parameters, such as dissolved oxygen, pH, and temperature, were analyzed using DO meters (Pro20; YSI; USA), and water salinity was analyzed by salinometer (CT3080; Kedida; Shenzen; China).

Results and Discussion

Spirulina is blue-green cyanobacteria with filamentous morphological structure, helical and unbranched. Cells form a series with a thin cell wall. This genus was

autotroph and converted sunlight energy to chemical energy in carbohydrates form (Soni et al., 2017). This research was investigated Spirulina with blue-green helical twisting filamentous structure, as seen in Figure 1, suggesting microalgae characteristics. The Spirulina showed spherical shapes on the surface. Our results were similar to other Spirulina powder products for animal feed or functional food (Singh, 2011).

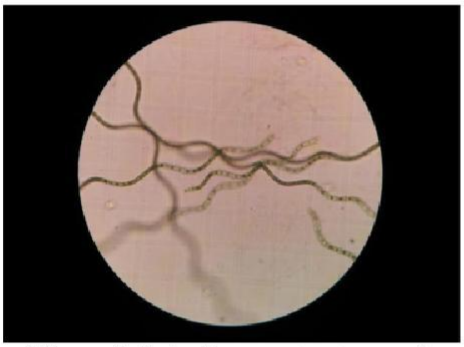


Figure 1. *Spirulina* sp. structure under microscope

Like plants, Spirulina is capable of synthesizing chlorophyll. Therefore they need considerable illumination to increase their production. There is an interaction between the lighting and the temperature, stated that higher temperature and enough light intensity would increase the final cell concentration and higher cell productivities of Spirulina (Danesi et al., 2011).

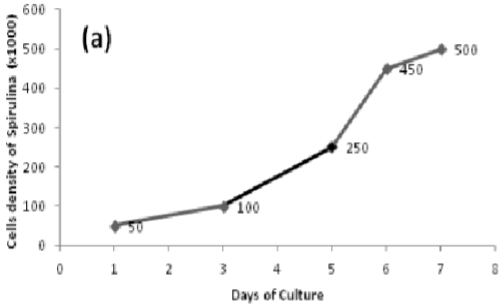
In this study, Spirulina was cultured in small and medium scale in the laboratory. The light intensity and temperature were maintained at 25°C, and ± 3200 lux of white light, with the aeration, were always maximized to bloom the algae, as seen in Figure 2. This parameter condition was good enough to enhance Spirulina production.

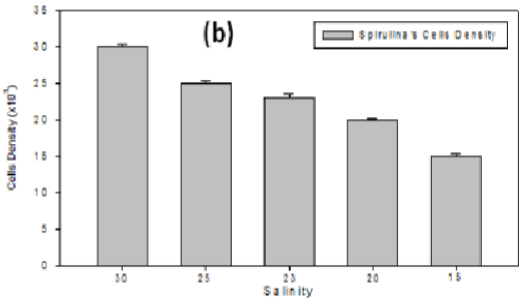
Figure 2. Spirulina culture in medium-scale

The salinity of Spirulina was maintained at 30 ppt in small-scale culture for seven days. In this cultural type, the cell density of Spirulina was significantly increased due to its favorable condition (Mahrouqi et al., 2015). The density of Spirulina was increased from $50x10^3$ cells ml^{-1} to $500x10^3$ cells ml^{-1} in seven days, as seen in figure 3a. After that, the salinity was gradually diminished every three days in medium-scale culture. Nevertheless, Spirulina's density was slowly decreased from $270x10^3$ cells ml^{-1} to $160x10^3$ cells ml^{-1} in 25 ppt; $145x10^3$ cells ml^{-1} in 23 ppt; $81x10^3$ cells ml^{-1} in 20 ppt; and $41.8x10^3$ cells ml^{-1} in 15 ppt (figure 3b). The cell density of Spirulina in 15 ppt was quite unstable. Therefore mass-production was optimized in 20 ppt. In this study, although each treatment had different salinity, nevertheless the water parameter was the least different (figure 3c). For this reason, we conclude that the degradation of Spirulina's cell density was majorly due to its reduced salinity.

In a large scall production, we also optimized the water quality of Spirulina to increase its productivity. As stated before, the mass culture of Spirulina has conducted at salinity concentration 20 ppt and cultured from day 15 to 23. The pH condition was varied, though it still ranges between 8-11 (Figure 4a). Similarly, the dissolvedoxygen concentration of Spirulina's mass culture was also stable from 4.8 to 7 gr/l, depending on Spirulina's density (Figure 4b). The highest dissolved oxygen was

might be another parameter Spirulina's production was not quite optimal, as well as its salinity. Several factors influence Spirulina's productivity, such as stirring speed, luminosity, pH of 8.5-10.5, temperature ±30°C, and water quality (Ciferri, 1983). These factors eventually influence the biomass of Spirulina to increase significantly (Figure 4d), and it showed the growth curve of Spirulina in large-scale cultivation was always supplement with a lower growth





(c)

Salinity	pH	DO	Temperature
30	10.678±0.1	5.28±0.9	30±0.6
25	10.256±0.7	5.3±0.6	28.84±0.5
23	10.298±0.9	5.58±0.4	28.28±0.5
20	10.08±0.5	5.72±0.7	28.4±0.9
15	10.004±0.6	5.12±0.6	29.26±0.8

Figure 3. The cultivation of Spirulina in small and medium-scale production. (a) Cells density of Spirulina within seven days in small-scale culture; (b) The Average cells density of Spirulina with different salinity in medium-scale culture; (c) Water quality parameters in different salinity of Spirulina in medium-scale culture, results were expressed in mean±SD.

7.1±0.3 when Spirulina's denseness was lowest. These variable conditions were still optimal for Spirulina's production.

On the other hand, the water temperature of Spirulina's large scale culture tends to be unstable, though the range was still between 28-30°C, respectively (Figure 4c). This condition

performance was 22nd to 23rd day, due to alower temperature and higher pH. This result, similar to Soni (2019) study, which cultivated Spirulina with manual aeration inan open pond, gained an increased dry biomass weight within the first seven days

and steadily started from the 8th day.

The pH influences the carbon dioxide and mineral solubility in the medium and interfering with Spirulina metabolism. The existence of bicarbonate and inorganic carbon in the autotrophic growth medium usually depended on pH. When the pH is optimal, the proportions of HCO_{3^-} and CO_{3^-} and CO_{2^-} increase, but when the cultivation medium's pH is low, carbon CO_{2^-} was the predominantly carbon source used by algae (Pereira et al, 2019).

contain 50% (w/w) of the element. Moreover, carbon was also used for autotrophic growth, which is very suitable for mass-production cultivation (Borges et al., 2013). Nevertheless, nitrogen and phosphorus were also essential for microalgae cultivation. When nitrogen is limited, cell division rate significantly declined and decreased dramatically (Wong et al., 2017). In cultures supplemented with nitrogen source from reduced cost media, it showed that the concentration of Spirulina's

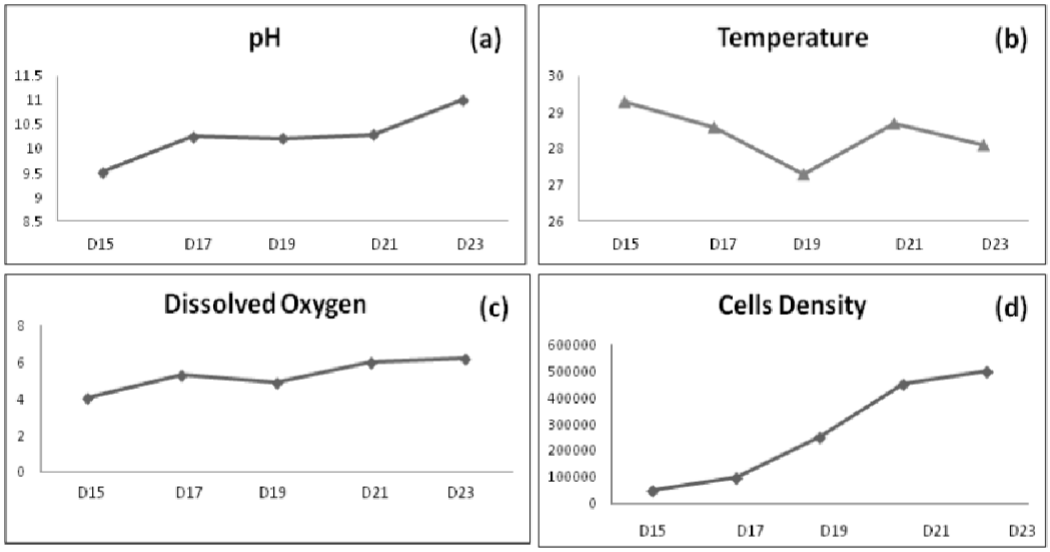


Figure 4. Water quality parameters and cell density of Spirulina in large-scale production. (a) the pH level of water in day 15th to 23rd cultivation; (b) temperature of water in day 15th to 23rd cultivation; (c) dissolved oxygen level of water in day 15th to 23rd cultivation; (d) Cells density of Spirulina in day 15th to 23rd cultivation.

Several essential nutrients such as carbon, nitrogen, and phosphorus were added to the experimental cultivation to enhance microalgal biomass. In this study, $Na_2SiO_35H_2O$ fertilizer was used to qualify Spirulina's nutrition, containing the significant nutriment above. Between these nutrients, carbon was the primary nutrient required for Spirulina, because the cells

biomass increased as long as the increase ofnitrogen concentration (Madkour et al., 2012).

For the boosting or enrichment of Spirulina, several approaches can be followed, including increasing the speed ofstirring and maintain the temperature. Another important parameter was also including the development of a culture

system with water quality stability to maximized Spirulina's mass production(Harel and Clayton, 2004).

In aquaculture, Spirulina has a significant role in feeding fish or enriching the zooplankton of larvae. This microalga could be quite popular due to its essential nutrients, including protein, vitamins, and minerals. As a feed additive or supplement,its extract also has an interesting point: antioxidant and anti-inflammatory due to itspigment, such as carotenoids (El-Kassas et al., 2015). This pigment was also used as ornamental fishes diets to maintain theircolors. Therefore, it is a considerableopportunity enriching the mass production of Spirulina to encounter these needs, as food or drugs, especially in Aquaculture major.

Conclusion

Spirulina is known as nutritious food due to its richness in protein, minerals, andnecessary fatty acids, particularly useful as fish diets in aquaculture. Spirulina growth in lab-scale to mass production in reduced salinity evidently gained a high production of algal biomass, potentially reduceabundant salts requirement and its expense.Further research is needed to cultivate Spirulina in different mediums or treatments to boost biomass production.

References

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