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EFFECT NUTRIENTS FOR GROWTH CHLORELLA VULGARIS AND SPIRULINA PLATENSIS

[1*]R Mohammad Alghaf Dienullah, [2]Harmin Sulistiyaning Titah
Faculty of Civil, Planning and Geo Engineering, Institut Tekonologi Sepuluh November
Email: alghaf31@gmail.com

ABSTRACT:
Microalgae are versatile organisms with the ability to adapt to various environments and exhibit rapid growth. They play a significant role in photosynthesis by converting carbon dioxide into oxygen and utilizing nutrients in their surroundings. This study aims to investigate the impact of nutrient availability on the growth of Chlorella vulgaris and Spirulina platensis. The experiment involves culturing the microalgae in mineral water and distilled water mediums supplemented with Walne medium nutrients. The growth of microalgae is monitored daily by calculating cell density using a counting chamber method. The pH and temperature of the culture are also measured. The results show that both microalgae species can grow in both mediums, but the usagee of mineral water medium promotes higher cell density and growth rates compared to distilled water medium. The pH values of the cultures vary between mediums, with mineral water medium generally exhibiting higher of pH. The temperature remains relatively stable throughout the experiment. These findings indicate that nutrient availability has a significant influence on microalgae growth, and the use of mineral water medium enhances their growth potential.

Keywords: Nutrients, Growth Rate, Microalgae, *Chlorella vulgaris*, *Spirulina platensis*

INTRODUCTION

In world nature, microalgae is one living things that have the potential to be studied due to their adaptability to anywhere environment (cosmopolite) and has fast growing ability. It can adapt until extreme environment such as pH, salt levels, and extreme climate. Besides that, microalgae is one of most significantly contributed photosynthetic activity. It can convert carbon dioxide to oxygen by metabolism process itself for cell growth (Thakur et al., 2020). While producing oxygen, microalgae using carbon dioxide by absorption process and assimilating some

nutrients in their life medium (Gani et al., 2016).

Some researchers using many species of microalgae in nature which commonly used for experiment, such as *Chlorella vulgaris*, and *Spirulina platensis*. *Chlorella vulgaris* is categorized as green microalgae from genus *Chlorella*, has 2-10 micrometer spherical microscopic cell and it growth to maturity range is range of 17-21 nanometer. It is also identified as eukaryotic and photosynthetic microorganism frrom family *chlorellaceae* (Daliry et al., 2017). *Chlorella vulgaris* reproduces asexually (Okoro et al., 2019). When reproduction, a mother cell of *Chlorella vulgaris* can reproduce 4 cell in range times about 19 hours (Daliry et al., 2017). While *Spirulina platensis* or also called *Arthospira platensis* is categorized as *Oscillatoria*, *Cyanophycean* and well known as blue-green microalgae (*Cyanophyta*). It mostly found in alkaline salt lake. It is also identified as ancient lower prokaryotic plant that composed of 5-10 micrometer wide, 200-500 micrometer long, a multicellular or single filamentous body like loose or tight regular spiral bending (Ai et al., 2023).

The best condition for grown microalgae is mostly depends on some factors, such as environment condition (pH, salinity, temperature, light intensity and its period), culturing technique, and availability of nutrients in their medium (Gani et al., 2016). Environment condition is categorized as physical factor, and availability of nutrients is categorized as chemical factor. Availability of nutrients is about amount and composition in culture medium. Nitrogen, carbon, phosphorus, silicon, vitamins, and some metals such as zinc, copper, iron can affect for growth microalgae. Among them, carbon is most important which has more effect on microalgae metabolism. It being energy source and the main structural component of microalgae cell. If microalgae categorized as autotropic culture, bicarbonate component or carbon dioxide is used as main carbon source. But if microalgae categorized as heterotrophic culture, glucose, sucrose, acetate, glycerol and other similar things are organic materials that used for source carbon (Daliry et al., 2017).

This study was focused to know the impact of the availability of nutrients for growth rate culture microalgae. By using *Chlorella vulgaris* and *Spirulina platensis* for object experiment, the results are expected to describe effect composition nutrient for *Chlorella vulgaris* and *Spirulina platensis* growth.

RESEARCH METHODS

Preparation Microalgae Culture

The microalgae species used for the experimental is the starter culture of *Chlorella vulgaris* and *Spirulina platensis*. The medium used is distilled water and mineral water. The nutrient in mineral water was contained: Calcium 9,14 mg/L; Magnesium 5,87 mg/L; Natrium 35 mg/L; Potassium 3 mg/L; Bicarbonate 118 mg/L; Sulfate 3,06 mg/L; Chloride <0,01 mg/L; Total Dissolved Solid (TDS) 177 mg/L; and pH 7,7. Both reactors containing distilled water and mineral water will be added with additional

nutrients in the form of Walne medium at a dose of 1 mL/L. It addition to culture only after inoculation. Walne medium are contained: NaNO3 100 g/L; H2BO3 33,6 g/L; Na2EDTA 45 g/L; NaH2PO4H2O 20 g/L; FeCl3.6H2O 1,3 g/L; MnCl2.4H2O 0,36 g/L; Vitamins solution 1 mL; and trace element 0,1 mL.

Experimental set-up

The experiment was carried out in a high growth rate microalgae reactor scale batch consisting of several components such as a 1.2 L tube, an aerator with an aeration rate of 2 liters O2/minute, and a 6000 lux cooldaylight LED lamp. The total volume of the reactor is 1 liter with 10% of which contains microalgae inoculum. aeration will be turned on for 24 hours, while the lighting time period is planned 12/12 (12 hours light, 12 hours dark), and the experiment will run for 7 days.

Determination of Microalgae Growth

Every day, the density of microalgae cells in each reactor will be calculated using the counting chamber method with the Haemocytometer Improved Neubauer on a microscope with a magnification of 100X (10X ocular lens and 10X objective lens). Besides that, every day the parameters of pH and temperature in all reactors are also observed.

RESULTS AND DISCUSSION

Effect Nutrients for Cell Growth

Chlorella vulgaris and *Spirulina platensis* studied able to grow in medium mineral water and distillated water during 7 days on batch reactor. The two microalgae were capable of developing in medium as long as they had a certain content that can promoted metabolic development (Sánchez-Bayo *et al*., 2020). The growth of *Chlorella vulgaris* are shown in Figure 1 and the growth of *Spirulina platensis* are shown in Figure 2.

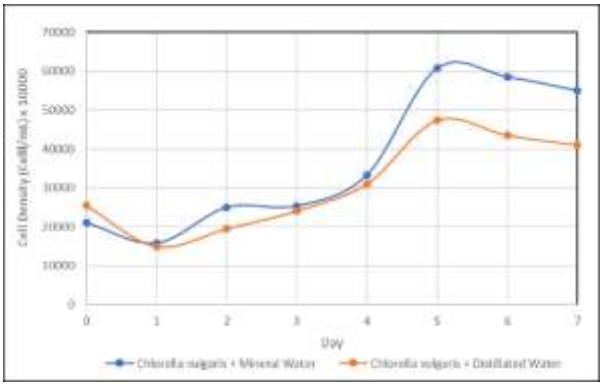

Figure 1. Cell Density *Chlorella vulgaris* at The Medium Mineral Water and Distillated Water

Cell density of *Chlorella vulgaris* showed increase from the first day to the fifth day. After that it began decrease to the seventh day. Both medium variations show the same trend of cell density changes. Until day fifth observation, increased cell density continuously caused by *Chlorella vulgaris* utilized the nutrients to reproduce and diffusion that can be affected on growth rates. It was also suspected that Walne had given more nutrients for cell proliferation (Hismayasari *et al*., 2021). Based on the growth curve on Figure 1, the largest cell density was shown on day 5, with a cell density of 6.08 x 10^8 cells/mL by using mineral water medium. (Wardani *et al*., 2022) refers that the increase in cell density day also showed that microalgae can utilize the nutrients in the medium for cell growth

and division. However, until the day there was a decrease in the number of cell densities indicating that the cells had reached their saturation point and the rate of cell death would increase which was not matched by the rate of cell growth.

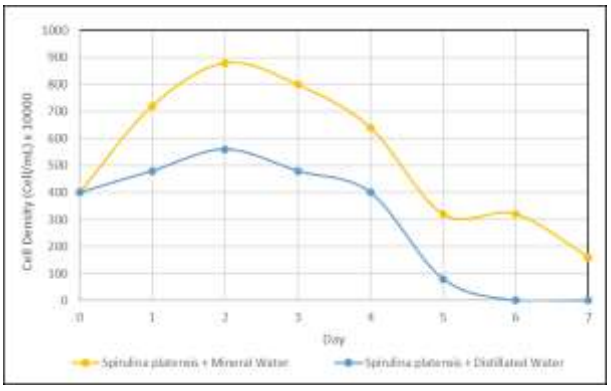

Figure 2. Cell Density *Spirulina platensis* at The Medium Mineral Water and Distillated Water

Cell density of *Spirulina platensis* showed increase from the first day to the third day. After that, it began decrease to the seventh day. Both medium variations showed the same trend of cell density changes. The increased density cell showed that under these conditions the microalgae cells have utilized the nutrients present in the media (Caturwati & Setyati, 2020; Hasim *et al*., 2022). Based on the growth curve on Figure 2, the largest cell density was shown on day 5, with a cell density of 8 x 10^6 cells/mL by using mineral water medium. But the cell density of *Spirulina platensis* when it is at its peak growth is not as much as *Chlorella vulgaris* when it is at its peak growth. The growth of microalgae is indeed influenced by the availability of nutrients in the growing media which are divided into macronutrients and micronutrients. However, giving excess nutrients will result in stress conditions on cultivated microalgae and lead to a decrease in biomass (Pratiwi *et al*., 2019).

Based on experiments, both species of microalgae showed that the use of mineral water containing nutrients as a medium is better than using distilled water. Besides from the growth curve on Figure 1 and Figure 2, the results of calculating the growth rate of microalgae also showed the same thing. Refers on (Andersen, 2005; Adriyanti *et al*., 2021), the growth rate is the rate of increase or increase in the number of microalgae cells in the culture at a certain time. *Chlorella vulgaris* which cultured in mineral water medium showed growth rate results 0,14 cell/mL/day. Whereas if it using the medium of distilled water, the growth rate decreased to 0.07 cell/mL/day. *Spirulina platensis* which cultured in mineral water medium showed growth rate results -0,13 cell/mL/day. Whereas if it using the medium of distilled water, the growth rate decreased to -0.32 cell/mL/day. These results bear out that the availability of nutrients has an influence on the growth rate of microalgae cells.

Effect Nutrients for pH

Abiotic factor can affect the microalgae growth. During the period of cultivation of microalgae, pH value indicated the photosynthetic assimilations of the carbon dioxide. It inferred another factor responsible microalgae for availability of carbon (Azov, 1982; Thakur *et al*., 2020). pH also has great significance in microalgal cultures, it determines the solubility of

minerals and CO2 in the medium (Chowdury *et al*., 2020).

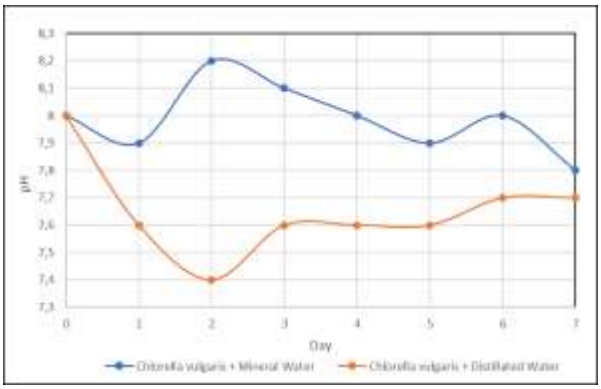

Figure 3. pH *Chlorella vulgaris* at The Medium Mineral Water and Distillated Water

Based on Figure 3, The pH of *Chlorella vulgaris* in distilled water and mineral water showed different results. Where the pH of microalgae in mineral water medium is higher than in distilled water. The use of distilled water will reduce the pH of the medium from 8 to 7,4 on the second day. But until the seventh day, the pH gradually increased to 7,7. Whereas if used mineral water medium, the pH of microalgae is in the range of 7.8 to 8.2. The microalgae pH was initially 8 after inoculation (at zero day), but increased to 8.2 on the second day, before gradually decreasing to 7.8 until the seventh day. In the last day, microalgae using mineral water medium had a higher pH value compared to using distilled water.

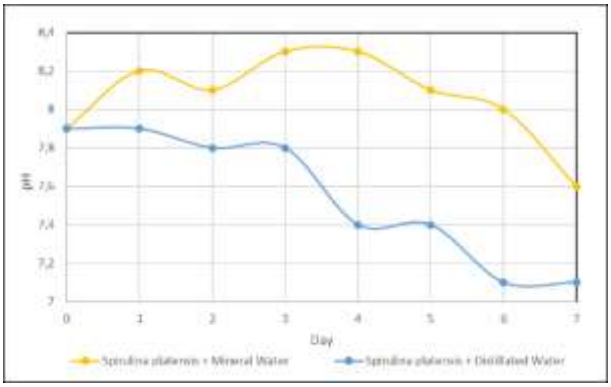

Figure 4. pH *Spirulina platensis* at The Medium Mineral Water and Distillated Water

Based on Figure 4, The pH of *Spirulina platensis* in distilled water and mineral water showed different results. Where the pH of microalgae in mineral water medium is higher than in distilled water. The use of distilled water will reduce the pH of the medium from 7,9 to 7,1 on the seventh day. The higher pH was 7,9 only until first day. After that, the pH values is decreased until day of 7. Whereas if used mineral water medium, the pH of microalgae was in the range of 7.6 to 8.4. The microalgae pH was initially 7,9 after inoculation (at zero day). It increased until 8,4 on fourth day until day of 5. After that, it decreased on until 7.6 at the day of 7. In the last day, microalgae using mineral water medium had a higher pH value compared to using distilled water.

Chlorella vulgaris reported can grow pH range 4 until 10, but most biomass productivity can be achieved if it lives in an environment with a pH 9 until 10 (Khalil *et al*, 2010; Daliry *et al*., 2017). Meanwhile, *spirulina platensis* can survive up to pH 11 and will grow well if it is at a pH in the range of 7,2 to 9,5 (Isnansetyo & Kurniastuty, 1995;

Ningsih *et al*., 2019). The changes in pH will cause produce changes in metabolism in microalgae cultures because they affect the balance of inorganic carbon. In a liquid medium, most of the carbon is in the form of bicarbonate in the pH range of 7 to 9. Microalgae cells can process bicarbonate into a carbon source due to the presence of the rubisco enzyme (Okoro *et al*., 2019; Meier *et al*., 2022). Then its converted to the enzyme carbonic anhydrase (Zhu *et al*., 2020). The decomposition of dissolved carbon causes a decrease in pH. However, the activity of the carbonic anhydrase enzyme will cause an increase in pH outside the cell due to the hydroxide ion transport which is closely related to the capture of H^+ ions to the inside of the thylakoid membrane (Kumar & Das, 2012). Referring to the experimental results, an increase in pH indicates that the microalgae cells carry out metabolic activities, while a decrease in pH may indicate a decrease in the metabolic activity of microalgae cells

Effect Nutrients for Temperature

Temperature is indicator that can significantly limit and affect growth of microalgae. It has varies value the optimum temperature for growh in the range 15^{o}C until 30^{o}C, but it depends on the microalgae species (Kaloudas *et al*., 2021).

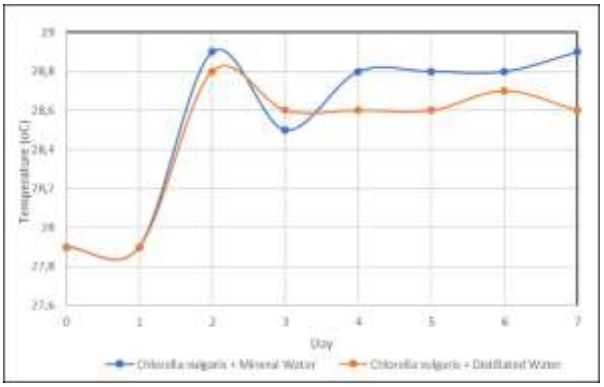

Figure 5. Temperature *Chlorella vulgaris* at The Medium Mineral Water and Distillated Water

Based on Figure 5, temperature of *Chlorella vulgaris* in distilled water and mineral water showed similar results. The use of mineral water medium produces a slightly higher final temperature than the use of distilled water medium. In day zero (after inoculation), the temperature in both mineral water and medium distilled water was 27.9^{o}C, then increased until the second day to a temperature of 28.9^{o}C for mineral water medium and 28.8^{o}C for distilled water medium. After the second day, the temperature in both mediums decreased slightly which lasted until the seventh day of the experiment.

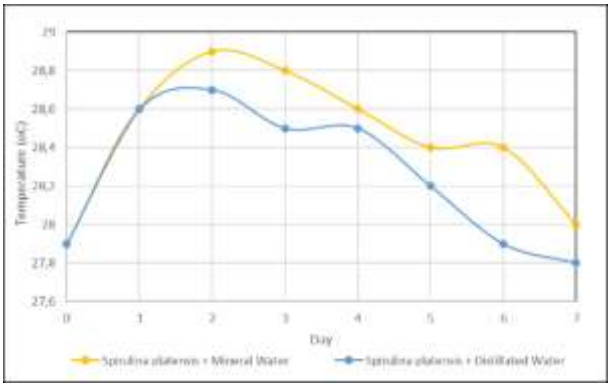

Figure 6. Temperature *Spirulina platensis* at The Medium Mineral Water and Distillated Water

Based on Figure 6, temperature of *Spirulina platensis* in distilled water and mineral water showed similar results. The use of mineral water medium produces a slightly higher final temperature than the use of distilled water medium. In day zero (after inoculation), the temperature in both mineral water and medium distilled water was 27.9^{o}C, then increased until the second day to a temperature of mineral water medium increased to 28,9^{o}C and temperature of distillated water medium increased to 28,7^{o}C. After the second day, the temperature in both mediums decreased until the seventh day of the experiment which mineral water medium reach temperature 28^{o}C and distillated water medium reach temperature 27,8 ^{o}C.

An increased temperature will have a positive effect on photosynthesis and cell division if it below on optimal growth temperatures. This trend is explained by the enhancement of enzymatic activities that related to the Calvin cycle. If growth temperature exceeding the optimal temperature, it can make growth rate of microalgae sharply decrease because the heat stress can make enzymes inactivated ,denaturation, and modify proteins which are involved in photosynthetic processes (Ras *et al*., 2013). *Spirulina platensis* has the optimum temperature for growth, biochemical, and antioxidants at 30^{o}C. At these temperatures, some antioxidant enzymes activity increased. Antioxidant affect to prevent the damage of cellular membranes by lipid preoxidation (Ismaiel & Piercey-Normore, 2020). Meanwhile, *Chlorella vulgaris* has the optimum temperatures to growth about 30^{o}C. But when it has an excessive temperatures up to 38^{o}C, it will make *Chlorella vulgaris* cell die and an abrupt halt in growth (Daliry *et al*., 2017). Referring to the experimental results, both types of microalgae showed that in seven days there are not microalgae in various media reached the optimum temperature for growth. It is known that the highest temperature reached by *Chlorella vulgaris* and *Spirulina platensis* is almost at the optimum temperature and does not reach the temperature that inhibits growth. In addition, experimental results prove that the highest temperature for both types of microalgae is obtained when using mineral water medium, so that nutrient availability can affect the temperature in the high growth rates reactor.

CONCLUSION

The effect of availability nutrients on the growth of *Chlorella vulgaris* and *Spirulina platensis* has evaluated. Nutrient availability was known to affect the growth rate of microalgae. *Chlorella vulgaris* and *Spirulina platensis* can grow well in a medium containing more nutrients. This was reinforced by several other parameters besides growth rate, namely pH and temperature parameters. The pH and temperature that were in the range close to the optimum indicate that microalgae carry out more metabolism, thus accelerating the growth of microalgae cells.

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