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The performance of *Chlorella vulgaris* growth on mass-scale cultivation

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ABSTRACT

Phytoplankton plays an important role, including as a primary producer, natural food, bioindicator, and water pollution treatment. For this reason, their availability needs to be managed, one of which is through cultivation. The purpose of this study was to analyze the mass scale cultivation of *Chlorella vulgaris*. The research was conducted at the Technical Implementation Unit of Freshwater and Brackish Water Aquaculture, Situbondo, using a descriptive method. The data were analyzed statistically using MS. Excel 2016 software, and a multiple linear regression test was carried out to determine the effect of water quality parameters on the growth of *C. vulgaris* using the SPSS 16.0 application. The cultivation process started from strain preparation, water and tank preparation, culture media preparation, inoculation, fertilization, and harvesting. The initial density of *C. vulgaris* used was 145 × 10^4 cell. mL^{-1} in tank 1 and 188 × 10^4 cell. mL^{-1} in tank 2. The results showed that the cell density value of *C. vulgaris* increased every day until it entered the exponential phase, that is on the 4^{th} day of the culture activity, which was 507 × 10^4 in tank 1 and 536 × 10^4 cell. mL^{-1} in tank 2. Furthermore, the value of water quality parameters that affected the growth of *C. vulgaris* in tank 1 and tank 2 was dissolved oxygen of 4.82–6.97 mg. L^{-1}, pH 8.2–9.1, transparency of 20–45 cm, temperature was 26.8–28.2 ºC, nitrate of 0.10–0.50 mg. L^{-1}, phosphate of 0.75–2 mg. L^{-1}, and salinity of 30–39 ppt.

INTRODUCTION

Phytoplankton is microorganisms that drift in the waters and their movements are influenced by currents. Phytoplankton act as a primary producer in the food chain in waters because they can carry out a photosynthetic process that is able to convert inorganic compounds into organic compounds by utilizing sunlight as an energy source, which is then utilized by organisms in the waters (Winder & Sommer, 2012; Salman et al., 2013; Persada et al., 2019). Phytoplankton plays an important role in aquatic ecosystems, such as a primary producer, natural food, bioindicators, and water pollution treatment. In addition, the high fat content of several microalgae species is being investigated for biodiesel production (Romdhane et al., 2013). The utilization of microalgae can also reduce greenhouse gas emissions because, in general, microalgae require a source of carbon dioxide to reproduce (Hadiyanto & Azim, 2012). Phytoplankton in the limited nature is the basis for the cultivation which serve to provide huge amounts of phytoplankton. Phytoplankton cultivation can be carried out at various scales according to the needs, including laboratory (Arsad et al, 2019), semi-mass, and mass-scales.

Mass-scale cultivation was carried out in ponds or concrete tanks with brackish water media. The cultivation of *C. vulgaris* was carried out in several stages, from preparation to harvesting. Moreover, water quality monitoring was done in the cultivation of *C. vulgaris* in this study. The understanding on what factors affect the growth of *C. vulgaris* is also very important, so that culture activities can run optimally (Chia et al., 2013).

In this study, *C. vulgaris* were used as natural food for Rotifers (*B. plicatilis*). This statement is in accordance to Novianti et al. (2017); Kandi (2018); Prayogo and Arifin (2015) stating that *C. vulgaris* can be used as natural food for zooplankton such as Rotifers (*B. plicatilis*). This species contains 51–58 % protein, 12–26 % carbohydrates, 2–22 % fat, and 4–6 % nucleic acids (Becker, 1994). This study aimed to analyze the cultivation management of *C. vulgaris* on a mass-scale and to analyze water quality parameters that affect the growth this species.

MATERIALS AND METHODS

Site and time. The research was conducted at the Technical Implementation Unit of Freshwater and Brackish Water Aquaculture, Situbondo, located on Pantai Pathek street, Gelung Selatan, Panarukan District, Situbondo Regency, East Java 68351 (7º 38'35.6 "S 113º 59'21.6" E) on July 13, 2020 – August 12, 2020. The geographic location of the research site can be seen in Figure 1.

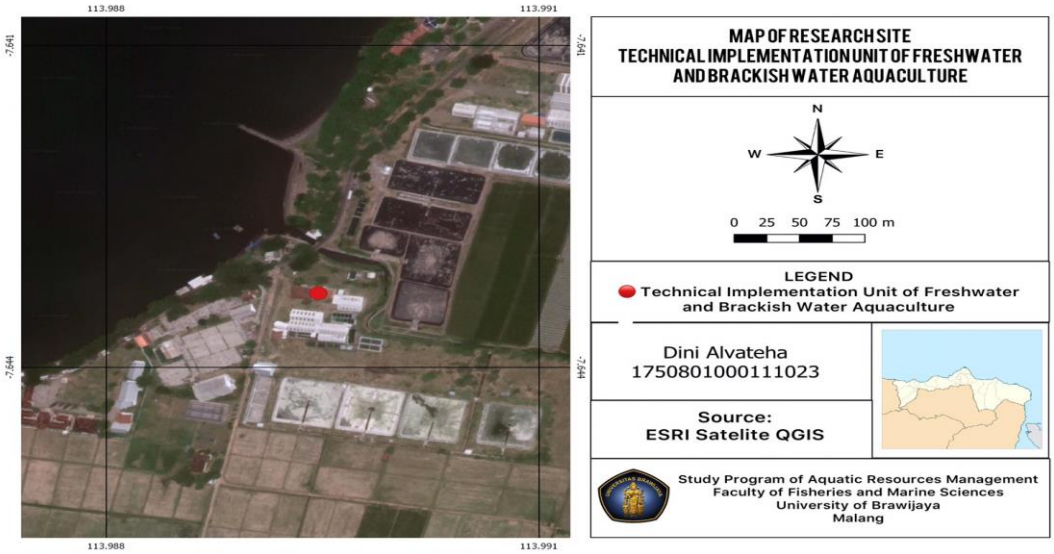

Figure 1. Map of Research Site.

Data collection technique. The parameters measured in this study included the main and supporting parameters. The main parameter was the density of *C*. *vulgaris* to see the dynamics of their growth. The supporting parameter was water quality, which included dissolved oxygen, pH, transparency, temperature, nitrate, phosphate, and salinity. The measuring instruments used included density (cell. mL^{-1}, Neubauer hemocytometer), dissolved oxygen (mg. L^{-1}, YSI 550A DO Meter), pH (pH Meter pHep by HANNA), transparency (cm, Secchi disk) (Maresi et al., 2015), temperature (^{0}C, YSI 550A DO Meter), nitrate (mg. L^{-1}, Merck KGaA nitrate test kit), phosphate (mg. L^{-1}, Merck KGaA phosphate test kit), and salinity (ppt, ATAGO refractometer).

Research method. The method used in this research was a descriptive method. The primary data were obtained directly through observation and documentation. The tanks used in the study were concrete tanks measuring 3 × 4 × 1.2 m^3 or having a volume of 12 tons (Figure 2), yet for culture activities, only 9 tons were filled in 2 tanks, namely tank 1 and tank 2, for the comparison purposes. Meanwhile, water quality parameters consisted of physical and chemical parameters. Physical parameters consisted of temperature and transparency, while chemical parameters consisted of dissolved oxygen, pH, nitrate, phosphate, and salinity. This is in accordance with the opinion of Boroh et al. (2019) and Daliry et al. (2017) that the growth of phytoplankton in culture media is strongly influenced by physical and chemical parameters of water quality, including the availability of nutrients in the form of nitrates and phosphates as well as temperature, salinity, dissolved oxygen, pH, transparency.

Figure 2. Cultivation tanks of *C. vulgaris*.

Cultivation stages. The cultivation stages of *C*. *vulgaris* consisted of strain preparation, water preparation, tank and culture media preparation, fertilizer production, inoculation and fertilization, and harvesting. The strain used for the culture of *C. vulgaris* came from the Technical Implementation Unit of Freshwater and Brackish Water Aquaculture, Situbondo, which entered the lag phase. The water used for the culture of *C*. *vulgaris* was seawater that had been treated or sterilized in a 4 × 5 × 2 m^3 tank, which was then given Hi chlon with a concentration of 10 ppm as much as 400 grams to kill bacteria and let stand for 5 hours. Moreover, it was added with Na-thiosulfate with a concentration of 5 ppm as much as 200 grams for neutralization and waited on for 2 hours before a chlorine test was carried out by dropping OTO (ortholidine) on the sample water to determine whether the water was neutral or not, and then the color change was observed. The sample water that changed color to yellow indicated that the water was not yet neutral, but if the water remained clear, it indicated that the water was neutral. The dry tank was then filled with seawater from the 70

cm seawater treatment reservoir. Aeration was used during the culture process so that *C. vulgaris* cells could obtain nutrition evenly because of the circulation of water in the culture tank. Moreover, the fertilizer used consisted of three types, namely 100 grams of urea, 50 grams of ZA fertilizer, and 100 grams of TSP fertilizer. The culture tank that had been filled with culture media were then filled with *C. vulgaris* strain as high as 20–30 cm so that the volume of water in the culture tank became 90–100 cm. The final step was harvesting, which was done by pumping them out using a submersible pump before flowing them through a hose connected to the Rotifera (*B. plicatilis*) culture tank. Harvesting was carried out partially, that is, when *C. vulgaris* entered an exponential phase, which was on the 4th day of culture activity. Meanwhile, the data collection was carried out for 7 days or until *C. vulgaris* entered the death phase.

Growth of C. vulgaris. Phytoplankton growth was observed every day by calculating cell density using a Neubauer hemocytometer (Salgueiro et al., 2016). The calculation and observation of the density of *C. vulgaris* were carried out using an Olympus binocular microscope with a magnification of 10 × or 40 × and with a hand tally counter counting tool. The calculation of the cell density of *C. vulgaris* was carried out during the culture activity for 7 days. The plankton calculations were carried out on four large hemocytometer boxes and the average cells per box were calculated (Gopal, 2004). The formula used according to the statement of Gopal (2004) is as follows:

$$\text{Density (cell. mL}^{-1}) = \frac{nA+nB+nC+nD}{4} \times 10^4$$

Information:
nA, nB, nC, nD : The number of phytoplankton cells in blocks A, B, C, D
4 : The number of boxes counted

Measurement of water quality parameters. Water quality was measured every day at 07.00 WIB (morning) for 7 days of culture activities, consisting of dissolved oxygen, pH, transparency, temperature, nitrate, phosphate, and salinity. These parameters were chosen because it could affect the growth of the *C. vulgaris*. Water quality control needed to be done because the mass-scale culture in this study was carried out in an open place (outdoor) so that the environmental factors would affect the water quality. The results of water quality measurements can be seen in Table 1.

Statistical analysis. The research data were analyzed using MS. Excel 2016 software. The multiple linear regression test was used to determine the effect of water quality parameters on the growth of *C. vulgaris* by data processing using the SPSS 16.0 application.

RESULTS AND DISCUSSION

Growth of C. vulgaris. The cell densities value from the 1st day to the 3rd day of culture activity shows a slow increase in tank 1 and tank 2 because there is still an adjustment or adaptation to the new culture media (Arsad et al., 2020a). Meanwhile, on the 4th day of culture activity the value increases rapidly in tank 1 and tank 2, and the 4th day itself is an exponential phase so that harvesting could be carried out. This statement is in accordance with the opinion of Creswell (2010) stating that after adjusting themselves, the algae cells divide rapidly so that the population will increase, which takes place on the 4th day or so of culture activity. The increased of cell population at the beginning of culture is due to the abundance of nutrients available in the culture media so that the *C. vulgaris* did cell division repeatedly. The cell density began to decline on the 5th day of culture activity, and will slowly decline again on the 6th and 7th day of culture activity. The cause of the decrease in cell density value is the decrease in nutrients and fed competition on *C. vulgaris*. The results of the calculation of cell density in tank 1 and tank 2 have different values, in which the value of cell density is higher in tank 2 because the hose used in tank 2 during the strain spreading is covered with cloth at the end of the hose to help filter the litter so that it does not get inside the culture tank, so

what escapes is only the desired phytoplankton. Meanwhile, the strain spreading that is carried out in tank 1 does not use a cloth at the end of the hose, so it allows a lot of litter or dirt to get into the culture tank, which makes the presence of phytoplankton lower or the density value smaller. The density value obtained in mass scale culture activities is higher than the density value for semi-mass and lab scale culture because the media used are different with different uses. The graph of the density and growth phase of *C. vulgaris* can be seen in Figure 3 and Figure 4.

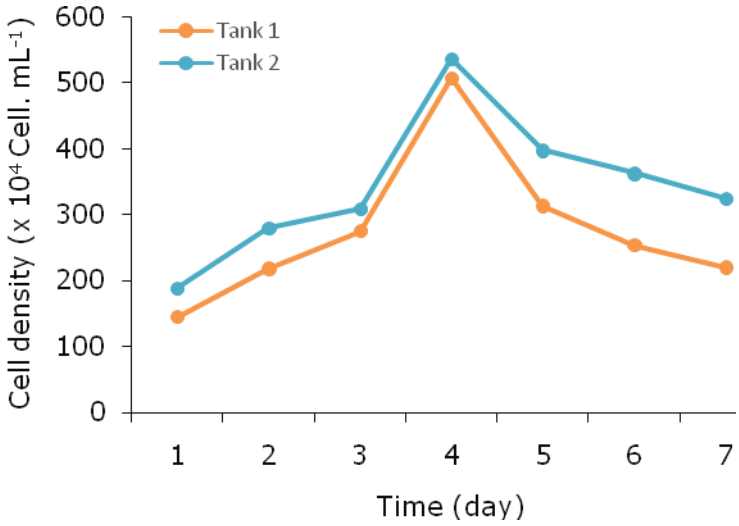

Figure 3. Graph of the calculation results of cell density of *C. vulgaris* in mass scale.

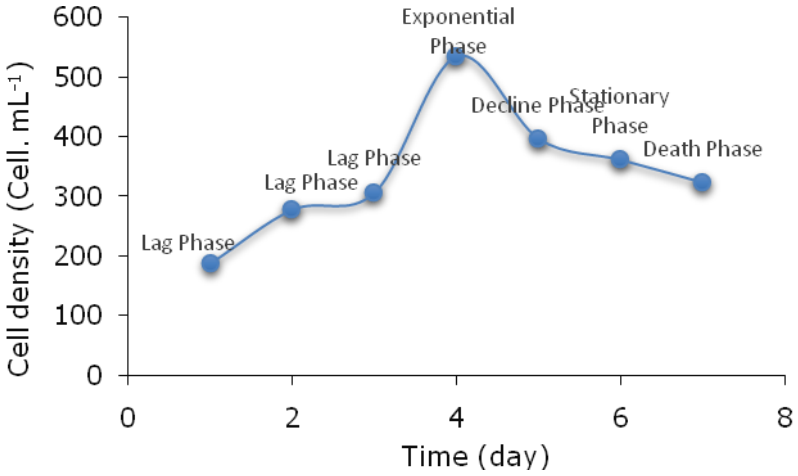

Figure 4. The growth curve of *C. vulgaris* during experiment.

Water Quality Parameters

Dissolved oxygen. Dissolved oxygen content is a reflection of the photosynthetic activity of phytoplankton biomass (Choudhury & Pal, 2010). The results of dissolved oxygen measurement in tank 1 and tank 2 for 7 days of culture range from 4.82–6.97 mg. L^{-1}. The dissolved oxygen value is ideal for meeting the needs of aquatic organisms for dissolved oxygen. This statement is in accordance with the opinion of Effendi (2003) stating that waters intended for fisheries purposes should have oxygen levels not < 5 mg. L^{-1}, and if the dissolved oxygen level is < 4 mg. L^{-1}, it will have an adverse effect on almost all aquatic organisms. The value of dissolved oxygen in tank 1 and tank 2 experiences an increase until the 4th day of culture activity due to the increasing number or density of *C. vulgaris*, which produce dissolved

oxygen through the photosynthesis process. The dissolved oxygen value will affect the metabolism of *C. vulgaris*. This statement is in accordance with the opinion of Prasetio et al. (2016) stating that dissolved oxygen can affect the physiology and metabolism of plankton. The dissolved oxygen value in the two tanks begins to decline from the 5th to the 7th day of culture activity due to the decrease in the density of *C. vulgaris*, so the dissolved oxygen produced through the photosynthesis process would also decrease. The average value of dissolved oxygen in tank 2 is higher than that in tank 1 because the density value of *C. vulgaris* is higher in tank 2.

Power of Hydrogen (pH). The results of pH measurements in tank 1 and tank 2 for 7 days of culture activity show a range of 8.2–9.1. This value is considered good or optimum to support the growth of *C. vulgaris*. This statement is in accordance with the opinion of Aprilliyanti et al. (2016) stating that the optimum pH limit value for growth of *Chlorella* sp. ranges from 8.25 to 9.82. The pH value in tank 1 and tank 2 increases every day until the 4th day, but then it tends to decrease on the 5th day to the 7th day of culture activity. The increase in pH value is due to the increase in the number or density of *C. vulgaris*, which utilize carbon dioxide for the photosynthesis process so that there is less carbon dioxide in the water. This causes the pH value to rise or become alkaline because the pH value is inversely proportional with the presence of carbon dioxide in the water. One of the reasons for the decrease in pH value is the decrease in the density of *C. vulgaris*. The average pH value obtained in tank 2 is greater than that in tank 1. This difference is due to the higher density of *C. vulgaris* in tank 2 so that carbon dioxide in the water decreases because it is used more, and the dissolved oxygen content is higher, which makes the pH of the water become alkaline.

Transparency. The results of transparency measurements obtained in tank 1 and tank 2 for 7 days of culture activity range from 20–45 cm. This value is considered good for supporting the growth of *C. vulgaris*. This statement is in accordance with the opinion of Junda (2018) and Boyd (1990) stating that the optimum transparency value for plankton growth is 20–50 cm. The transparency value in both tanks decreases from the 1st day to the 4th day of culture activity due to the increasing density value of *C. vulgaris* which could inhibit sunlight into the water. The brightness value experiences another decrease on the 5th day to the 7th day of culture activity because the culture tank is contaminated by Rotifers (*B. plicatilis*), which increases the density of the culture tank and causes lower intensity of sunlight entering the water. The average transparency value obtained in tank 2 is smaller than that in tank 1. This difference is due to the higher density value of *C. vulgaris* which causes the darker color of the water in the culture tank so that the intensity of sunlight that could penetrate the water occurs at shallower depths, which then causes the transparency value in tank 2 to be smaller.

Temperature. The results of temperature measurements in tank 1 and tank 2 obtained for 7 days of culture activity range from 26.8–28.2 ^{o}C. This value is considered good for supporting the growth of *C. vulgaris*. This statement is in accordance with the opinion of Nurlaili et al. (2015) and Arsad et al. (2020b) stating that the optimal temperature in microalgae culture is generally between 25–35 ^{o}C. The temperature value increases on the 1st day to the 4th day of culture activity due to the increase in the density value, so the heat generated from the metabolic process of the organism can increase the water temperature. The temperature value decreases on the 5th to the 7th day of culture activity due to the decreasing density value. The average temperature value obtained in tank 2 is greater than that in tank 1. This difference is due to the fact that tank 2 has a higher density of *C. vulgaris*, so the heat generated from the metabolic processes of the organism is also higher, which leads to an increase in water temperature.

Nitrate. The results of nitrate measurements in tank 1 and tank 2 for 7 days of culture activity show a range of 0.10–0.50 mg. $L^{-1.}$ This value is considered good or optimum to support the growth of *C. vulgaris*. This statement is in accordance with the opinion of Wardoyo (1982) stating that the optimal nitrate level for phytoplankton growth is 0.1–3.5 mg. L^{-1}, and nitrate will be a limiting factor for the growth of *Chlorella* sp. when the nitrate level reaches 1.8 mg. L^{-1} or above and is below 0.02 mg. L^{-1}. The nitrate value in the culture tanks only

decreases until the 2nd day and did not change until the 7th day. This value only shows the range value, and will be more accurate if measured using modern measuring instruments such as a spectrophotometer. Nitrate content in the culture tanks decreases because it is used by *C. vulgaris* as a nitrogen source for growth, but the presence of nitrate will never run out.

Phosphate. The results of phosphate measurements in tank 1 and tank 2 for 7 days of culture activity show a range of 0.75–2 mg. L^{-1}. This value is considered good or optimum to support the growth of *C. vulgaris*. This statement is in accordance with the research of Rumanti, et al. (2014) stating that the optimal phosphate content for phytoplankton growth is in the range of 0.27–5.51 mg. L^{-1}, while the phosphate content of less than 0.02 mg. L^{-1} will be a limiting factor. The phosphate measurement results in the culture tanks decrease every day. This decrease is due to the utilization of phosphate by the *C. vulgaris* as a nutrient for their growth. The phosphate values in the two culture tanks measured using a phosphate test kit show the same value, and may have different values when measured using a measuring instrument that has higher accuracy such as a spectrophotometer because the density value of *C. vulgaris* in both tanks shows a different value.

Salinity. The results of salinity measurements in tank 1 and tank 2 for 7 days of culture activity show a range of 30–39 ppt. This value is considered good for supporting the growth of *C. vulgaris*. This statement is in accordance with the opinion of Rachmawati (2019) stating that salinity with a value of 30 ppt is a very good level for the growth of *C. vulgaris* and has the ability to withstand high salinity in the case that it can tolerate salinity between 33–40 ppt. The salinity value in the two tanks tends to increase from the 1st day to the 4th day of culture activity due to the increasing density value of *C. vulgaris* which is able to excrete salts from its cell metabolism. The energy produced by phytoplankton will have an impact on the evaporation of the culture media, so the salinity value of the media will increase (Widayat et al., 2018). The salinity value in both tanks increases on the 6th and the 7th day of culture activity due to the absence of additional water in the culture tanks and the evaporation process. The average salinity value in tank 2 is higher than that in tank 1 because tank 2 has a higher density value of *C. vulgaris* than does tank 1.

Table 1. The Water Quality Value in the Cultivation Tanks

Water Quality Parameter	Value				Quality standard value (Reference)
	Tank 1 (Seawater/ Cultivation Media)	Tank 2 (Seawater/ Cultivation Media)	Tank 1 (7 days of cultivation activity)	Tank 2 (7 days of cultivation activity)	
Dissolved Oxygen (mg. L^{-1})	5.29	5.28	4.82–6.23	5.11–6.97	> 4 m (Effendi, 2003)
pH	7.9	7.9	8.2–9	8.3–9.1	8.25 – 9.82 (Aprilliyanti et al., 2016)
Transparency (cm)	100 %	100 %	25–45	20–43	20–50 (Junda, 2018 & Boyd, 1990)
Temperature (ºC)	28.3	28.2	26.8–28	27.1–28.2	25–35 (Nurlaili et al., 2015)
Nitrate (mg. L^{-1})	0.10	0.10	0.10–0.50	0.10–0.50	0.1–3.5 (Wardoyo, 1982)
Phosphate (mg. L^{-1})	0.25	0.25	0.75–2	0.75–2	0.27–5.51 (Rumanti et al., 014)
Salinity (ppt)	35	35	30–37	33–39	33–40 (Rachmawati, 2019)

Effect of water quality parameters on the growth of C. vulgaris. The effect of water quality on the growth of *C. vulgaris* is analyzed using multiple linear regression analysis. From the results of the analysis, it is obtained the correlation coefficient (r) of 0.978 (α = 0.05). This value indicates that the relationship of dissolved oxygen, pH, transparency, temperature, nitrate, phosphate, and salinity is simultaneously very strong towards the growth of *C. vulgaris*, which means that overall water quality greatly affects the growth of *C. vulgaris*. Based on the multiple linear regression analysis (Table 2), water quality parameters simultaneously have a very strong effect on the growth of *C. vulgaris* with a percentage of dissolved oxygen of 46.32 %, pH of 25.08 %, transparency of 11.48 %, temperature of 9.92 %, nitrate of 3.43 %, phosphate of 2.09 %, and salinity of 1.68 %.

Table 2. Score of SE and SR

Parameter	Score of SE	Score of SR
Dissolved oxygen (X1)	44.30	46.32
pH (X2)	23.99	25.08
Transparency (X3)	10.98	11.48
Temperature (X4)	9.49	9.92
Nitrate (X5)	3.28	3.43
Phosphate (X6)	2.00	2.09
Salinity (X7)	1.61	1.68
R Square/Total	95.63	100

Effective Contribution (SE) and Relative Contribution (SR)

CONCLUSION

The conclusion obtained from the present research on the management of mass-scale cultivation of *C. vulgaris* is that water quality parameters simultaneously have a very strong influence on the growth of *C. vulgaris* with a percentage of dissolved oxygen of 46.32 %, pH of 25.08 %, transparency of 11.48 %, temperature of 9.92 %, nitrate of 3.43 %, phosphate of 2.09 %, and salinity of 1.68 %. The main differences between mass, semi-mass, and laboratory scale microalgae cultivation are the culture media used, the amount of fertilizer required, the number of microalgae produced, and the utilization.

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