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DENSITY, DIAMETER, GROWTH RATE, CHOROPHIL A AND LIPIDS OF CHLORELLA VULGARIS ON FERMENTATION OF GREEN BEAN SPROUTS (*Phaseolus radiatus*)

Kepadatan, Diameter, Laju Pertumbuhan, Klorofil A dan Lipid Chlorella Vulgaris Pada Fermentasi Tauge Kacang Hijau (*Phaseolus radiatus*)

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ABSTRACT

Chlorella culture requires large quantities of nutrients, such as nitrogen and phosphorus, for growth and development. One way to increase nutrients in microalgae culture is by fermentation with Green Bean Sprouts (*Phaseolus radiatus*). Green bean sprout fermentation is a natural medium that meets the criteria for proper microalgae growth. This investigation aimed to ascertain the impact of the concentration of Green Bean Sprouts fermentation on the density, diameter, growth rate, chlorophyll a, and lipid formation of the microalgae *C. vulgaris*. The experiment will employ a variety of fermentation procedures for green bean sprouts, including concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 mL. The Duncan test was implemented after the analysis of variance (ANOVA) data analysis. The density, chlorophyll a, and lipid of the results of this study were the highest of *C. vulgaris* in the 1 mL concentration treatment, which was 1,249 x 10^4 cells/mL, 0.220 mg/mL, and 5.98%. The highest cell diameter of *C. vulgaris* was found in the 1.5 mL concentration treatment, which was 7.91 µm, and the growth rate ranged from 12.20-14.56. Environmental factors in the form of water quality, such as temperature obtained during culture, ranged from 26.3-29.3ºC, pH 7.19-7.80, salinity 35-39 ppt, dissolved oxygen (DO) 3.21-4.89 mg/L, and light intensity of 3000 lux. The results of this study could be significantly different because this treatment has a complete nutrient composition, such as phytohormones and phosphorus, under the needs of *C. vulgaris*.

Key words: Phytoplankton, Marine Resources, Aquaculture, Sustainable Fisheries, Life Feed

ABSTRAK

Kultur *Chlorella* membutuhkan nutrien seperti nitrogen dan fosfor dalam jumlah besar untuk pertumbuhan dan perkembangannya. Salah satu cara untuk meningkatkan nutrien dalam kultur mikroalga yaitu dengan fermentasi dengan bahan tauge kacang hijau *(Phaseolus radiatus)*.

Fermentasi tauge kacang hijau dapat digunakan sebagai media alami dan memenuhi kriteria media yang tepat untuk pertumbuhan mikroalga. Tujuan penelitian ini adalah untuk mengetahui bagaimana konsentrasi fermentasi tauge kacang hijau berpengaruh terhadap tingkat fermentasi terhadap kepadatan, diameter, laju pertumbuhan, pembentukan klorofil a dan lipid mikroalga *C. vulgaris*. Penelitian akan dilakukan dengan beberapa perlakuan fermentasi tauge kacang hijau yaitu konsentrasi 0; 0,5; 1; 1,5; 2 dan 2,5 mL. Setelah itu, analisis data dengan *Analysis of Variance* (ANOVA) dilanjutkan uji Duncan. Hasil penelitian ini tertinggi pada kepadatan, klorofil a dan lipid *C. vulgaris* terdapat pada perlakuan konsentrasi 1 mL yaitu sebesar 1.249 x 10^4 sel/mL, 0,220 mg/mL dan 5,98%. *C. vulgaris* diameter sel tertinggi terdapat pada perlakuan konsentrasi 1,5 mL yaitu sebesar 7,91 µm dan laju pertumbuhan berkisar antara 12,20-14,56. Faktor lingkungan berupa kualitas air seperti suhu yang didapat pada selama kultur berkisar antara 26,3-29,3^{o}C, pH 7,19-7,80, salinitas 35-39 ppt, oksigen terlarut (DO) 3,21-4,89 mg/L, dan intensitas cahaya yaitu 3000 lux. Hasil penelitian ini dapat berbeda nyata terjadi karena pada perlakuan ini memiliki komposisi nutrient yang lengkap seperti fitohormon dan fosfor yang sesuai dengan kebutuhan *C. vulgaris*.

Kata Kunci: Fitoplankton, Sumber Daya Kelautan, Budidaya, Perikanan Berkelanjutan, Pakan Alami

INTRODUCTION

Microalgae consist of cell chemicals such as proteins, carbohydrates, lipids, and nucleic acids, as well as organic materials such as hormones, vitamins, minerals, and also secondary metabolite compounds (Kotasthane, 2017). This microalgae is widely used as fish feed, food supplements and to strengthen the human immune system (Phukan et al., 2011). The high role of *C. vulgaris* needs to be developed, one way to increase the yield of *C. vulgaris* is that it needs to be cultured. The main factors are nutrients, namely nitrogen and phosphorus, which are food for phytoplankton and limiting factors for phytoplankton life. Chlorella culture requires large amounts of nitrogen and phosphorus for growth and development. The nitrogen and phosphorus requirements of *C. vulgaris* are 14-70% and phosphorus 1.5-61% (Triastuti et al., 2011).

Generally, *C. vulgaris* cultures use Walne fertilizer as a nutrient. One alternative to replace Walne fertilizer in microalgae culture is the use of green bean sprouts, because bean sprouts are easy to obtain, economical, environmentally friendly and do not cause dangerous residues (Hamad & Kristiono, 2013). The nutrients in bean sprouts can be maximized into simple compounds so they are easily absorbed by microalgae is by how to ferment. The nitrogen and phosphorus content in fermented bean sprouts is 13.7% nitrogen and 3.7% phosphorus (Nurhasanah, 2017). Posphor (P), sodium (Na), copper (Cu), zinc (Zn), selenium (Se), and manganese (Mn) are minerals found in bean sprouts (Maulana, 2010). leucine, isoleucine, and valine are essential amino acids found in bean sprouts. Mung bean sprouts contain many vitamins. These include vitamin C, riboflavin, thiamin, niacin, panthothenic acid, folate, beta-carotene, vitamin A, vitamin E (alpha tocopherol), and vitamin K (Maulana, 2010).

Fermented fertilizer has the benefit of encouraging and increasing the photosynthetic ability of microalgae and nitrogen absorption (Marpaung et al., 2014). Previous research conducted by Prihantini (2005) showed that the highest cell density in bean sprouts media was 5,677,625 cells/mL. Research conducted by Prihantini et al. (2007), showed that the highest cell density for Chlorella used a 2% concentration of green bean sprout extract, namely 30,700,000 cells/mL. Imelda et al. (2018) conducted research on the cultivation of local isolates of microalgae in bean sprout extract (MET) medium, showing that the highest cell growth for Chlorella was 7.4 x 10^8 cells/mL with the addition of 10% bean sprout extract. The highlight of this research is that fermented mung bean sprout fertilizer has the ability to increase Chlorella culture yields, so it is necessary to carry out research on the effect of fermented mung bean

sprouts concentration on density, diameter, growth rate, chlorophyll a and lipids of *C. vulgaris*.

METHODS

Research location, Anatomy and Cultivation Laboratory, Faculty of Fisheries and Maritime Affairs and Nutrition Laboratory, Faculty of Public Health, Airlangga University in 2023. The method that will be used in research preparation is 24 3L jars, hemocytometer, dropper pipette, pH meter (ATC-SKU pH 2 Pro), thermometer, aeration stone, aeration hose, LP20 aerator, Nikon E-100 Binocular Microscope, object glass, cover glass, ATC refractormeter, cotton wool, stationery, gauze, 3mL sample bottle, and lamp. The materials that will be used for research preparation are *C. vulgaris* inoculant, green bean sprouts, Effective Microorganism 4 (EM4), granulated sugar, sea water, Aquades Waterone, 60 ppm chlorine, Na-Thiosulfate SAP Chemicals, 70% alcohol, label paper and Lugol. Completely Randomized Design (CRD), six treatments and four repetitions for research. The following is the research that will be carried out: A fermented green beans 0 mL/L, B fermented green beans 0.5 mL/L, C fermented green beans 1 mL/L, D fermented green beans 1.5 mL/L, E fermented green beans 2 mL/L, and F fermented green beans 2.5 mL/L.

Making and Fermenting Green Bean Sprouts

The modified research method of Arsad et al. (2024) is that green bean sprouts are first washed to remove dirt. Next, two kilograms of bean sprouts are dried and crushed with a blender. Put the bean sprouts in a container containing 10 liters of distilled water, 500 milliliters of EM4 and 500 grams of granulated sugar. After that, the bottle is closed tightly and left in another place for eight days. First, the bean sprouts are fermented in the autoclave. 50 milliliters of Erlenmeyer was added to ferment the bean sprouts, then autoclaved, covered with aluminum foil and cotton, and then put into the autoclave at a temperature of 121 degrees Celsius and a pressure of 1 atm for thirty minutes. Put 2,400 mL of fermented bean sprouts into a container.

Environment and Culture Media of *C. vulgaris*

Preparation of culture media using sea water and fresh water. The sea water used comes from Dukuh Kupang Surabaya and fresh water comes from the Faculty of Fisheries and Maritime Affairs, Airlangga University. The culture medium used in this research was fermented green bean sprouts and according to the prescribed dosage. The salinity of the seawater used is between 25 ppt, the dilution process is carried out if the seawater salinity level is too high. The degree of acidity (pH) for microalgae culture is between 6.6-7.0 (Amaliandini et al., 2023) and the temperature is between 23 - 30°C (Pratama, 2020).

C. vulgaris Stock Preparation (density)

The *C. vulgaris* inoculant was obtained from the Situbondo Brackish Water Aquaculture Fisheries Center (BPBAP). To prepare stock, prepare an aquarium or jar as a culture container. First, samples are taken from the stock media and counted under a microscope to calculate the stock density. To make it easier to calculate the sample population, you can use a hand counter (Suyoso et al., 2022). After the seeds are counted, place them in sterile culture media. During maintenance, the culture media is aerated to avoid contamination. The desired initial density during the study is 1,500 $x10^4$ cells/mL. The dilution density of microalgae can be calculated using the following formula (Trikuti et al., 2016).

$$V_1 \times N_1 = V_2 \times N_2$$

Note:

V1 shows the initial seed volume (mL), N1 shows the initial number of seeds (cells/mL), and V2 shows the desired media volume (mL), and N2 shows the desired number of seeds (cells/mL).

C. vulgaris culture process

Before carrying out the culture process, the culture equipment is sterilized by soaking it in chlorine for 24 hours, then rinsing with running water (Musa et al., 2021). Then, sea water is sterilized using Na-thiosulfate. Next, the culture process begins by adding fermented green bean sprouts and microalgae inoculant, then placing them in a glass jar. Then given aeration to maintain the stability of dissolved oxygen. The culture rack is covered with black plastic to keep the room temperature stable and prevent contamination. After that, *C. vulgaris* was maintained for several days using 24 hours of light (Azmi et al., 2020). According to the Chlorella growth curve, the right time to harvest is in the middle of the exponential phase (Prasetya et al., 2023).

Calculation of *C. vulgaris* Density

C. vulgaris growth was quantified by measuring cell density. *C. vulgaris* density calculations were carried out every day from the start of culture to harvest. Cell density was determined using a hemacytometer and observations were carried out under a microscope (Apriliyanti et al., 2016). The density formula is based on Kwangdinata et al. (2013).

$$\text{Phytoplankton density (cells/mL)} = \frac{nA+nB+nC+nD}{4}\ x\ 10^4$$

Note :
nA, nB, nC, nD = Number of phytoplankton cells in blocks A, B, C, D

Calculation of Growth Rate of *C. vulgaris*

The growth rate of *C. vulgaris* was calculated using the formula from Krichnavaruk et al. (2004).

$$\mu = \frac{\ln Nt - \ln No}{Tt - To}$$

Note:
Nt is the population number at time t, No is the number of cell populations at time 0, To is the initial time, and Tt is the observation time.

C. vulgaris Cell Diameter Measurement

Cell diameter measurements were carried out by dropping 1 milliliter of sample on an objective micrometer with an accuracy of 0.01 millimeters. This measurement was carried out three times with the help of a microscope at 40× magnification. The results are then averaged (Mahardani et al., 2017).

Measurement of chlorophyll-a content of *C. vulgaris*

Chlorophyll-a content in *C. vulgaris* was measured by spectrophotometry. 10 milliliters of microalgae were taken to be centrifuged for 10 minutes at 6,000 rpm until supernatant and sediment were formed. The supernatant was discarded and 10 milliliters of absolute methanol was added to the bottom sediment of the tub. Then, using a vortex for 15 seconds, the mixture of precipitate and solvent was placed on a hot plate for 10 minutes at 70°C to make the sample homogeneous. Using a spectrophotometer with a wavelength of 665 nm, the chlorophyll-a content was measured by vortexing the sample and centrifuging it again for ten minutes at a

speed of 6,000 rpm. The chlorophyll-a content was calculated using the equation according to Prasetya et al. (2023), namely:

$$\text{Cha-a} = 12{,}9774 \text{ x A665}$$

Note:
Cha-a: chlorophyll-a content (µg/L) 13.9 = absorbance coefficient, A665: absorbance at a wavelength of 665 nm

Lipid Extraction

A modified method studied by Wijoseno (2011) was used for lipid extraction. Putting 10 mL of culture sample into a centrifuge tube was used to extract *C. vulgaris* lipids. The sample was then centrifuged for ten minutes at a speed of 6000 rpm. After that, the pellets are taken and dried for twenty-four hours in an oven at 80 degrees Celsius. Using 1 mL of aquabides, the dried *C. vulgaris* biome was suspended. Then 0.5 mL of ethanol solution and 0.25 mL of chloroform solution were added. After vortexing for 2 minutes, add 0.25 mL of aquabides and 0.25 mL of chloroform. Then centrifuged for 10 minutes at a speed of 6000 rpm. After that, the precipitated lipids were taken and put into a petridish. Heating was carried out for fifty minutes at a temperature of 80 degrees Celsius to remove the mixture of chemical solutions that had been used previously. Lipid content is calculated using the following formula:

$$\text{L}(\%) = \frac{\text{LW}}{\text{BW}} \times 100$$

Note:
L: Lipid content (%), Lw: Sample lipid weight (g), Bw: Sample biomass weight (g) (Ningsih, 2017)

RESULT

Density of *C. vulgaris*

The density of *C. vulgaris* using fermented mung bean sprouts with different concentrations was cultured for 10 days. Based on Figure 1, for all treatments the highest average density of *C. vulgaris* occurred on day 5. Treatment C showed the highest density of 1,249 x 104 cells/mL.

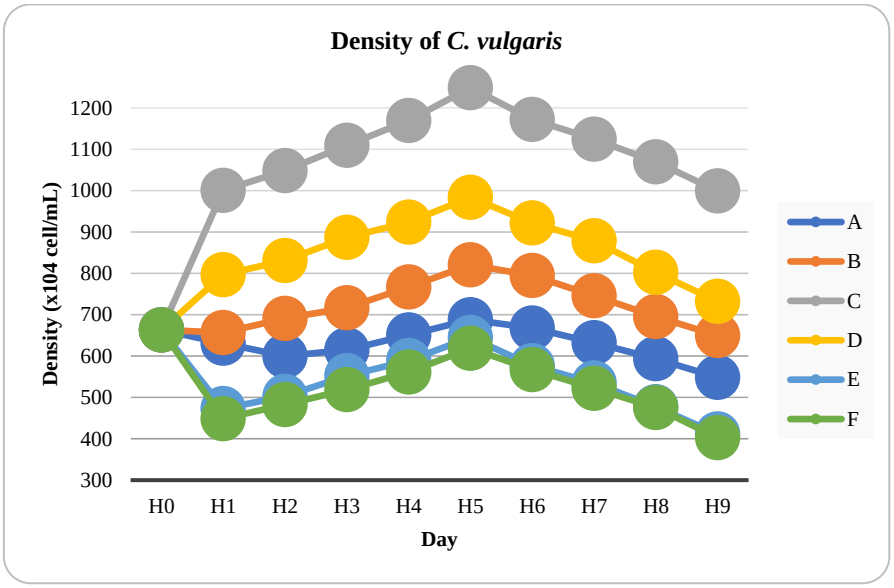

Figure 1. *C. vulgaris* Density Graph

C. vulgaris Cell Diameter

The diameter of *C. vulgaris* cells using fermented green bean sprouts with different concentrations was cultured for 10 days. Data on *C. vulgaris* cell diameter during the study can be seen in Table 1.

Table 1. Average cell diameter during the culture period (µm)

Day	Average cell diameter (µm)					
	Treatment					
	A	B	C	D	E	F
H0	$2.29^{a} \pm 0.06$	$2.36^{ab} \pm 0.04$	$2.88^{d} \pm 0.05$	$3.34^{e} \pm 0.09$	$2.54^{c} \pm 0.09$	$2.44^{bc} \pm 0.06$
H1	$3.29^{a} \pm 0.23$	$3.33^{a} \pm 0.45$	$3.75^{a} \pm 0.16$	$4.30^{b} \pm 0.39$	$3.36^{a} \pm 0.22$	$3.29^{a} \pm 0.38$
H2	$3.91^{a} \pm 0.14$	$4.08^{a} \pm 0.40$	$4.69^{b} \pm 0.18$	$5.32^{c} \pm 0.49$	$4.29^{ab} \pm 0.37$	$4.09^{a} \pm 0.24$
H3	$4.80^{a} \pm 0.22$	$4.96^{ab} \pm 0.41$	$5.51^{b} \pm 0.14$	$6.23^{c} \pm 0.47$	$5.09^{ab} \pm 0.45$	$4.93^{a} \pm 0.23$
H4	$5.60^{a} \pm 0.27$	$5.68^{a} \pm 0.42$	$6.50^{b} \pm 0.06$	$7.22^{c} \pm 0.48$	$5.85^{a} \pm 0.52$	$5.50^{a} \pm 0.22$
H5	$6.21^{a} \pm 0.35$	$6.51^{a} \pm 5.22$	$7.49^{b} \pm 0.32$	$7.91^{b} \pm 0.21$	$6.67^{a} \pm 0.50$	$6.59^{a} \pm 0.37$
H6	$5.57^{a} \pm 0.14$	$6.07^{bc} \pm 0.17$	$6.78^{d} \pm 0.06$	$7.46^{e} \pm 0.23$	$6.29^{c} \pm 0.07$	$5.84^{ab} \pm 0.04$
H7	$4.48^{a} \pm 0.17$	$5.32^{bc} \pm 0.54$	$5.95^{c} \pm 0.32$	$6.74^{d} \pm 0.76$	$5.56^{bc} \pm 0.42$	$5.15^{ab} \pm 0.35$
H8	$3.55^{a} \pm 0.40$	$4.51^{ab} \pm 0.68$	$5.08^{bc} \pm 0.69$	$5.79^{c} \pm 1.03$	$4.81^{bc} \pm 0.52$	$4.29^{ab} \pm 0.57$
H9	$2.67^{a} \pm 0.71$	$3.74^{abc} \pm 0.87$	$4.26^{bc} \pm 0.90$	$4.98^{bc} \pm 1.29$	$4.01^{abc} \pm 0.64$	$3.46^{ab} \pm 0.71$

Note: Treatment A (Control / 0 mL), Treatment B (Concentration of fermented green bean sprouts 0.5 mL), Treatment C (Concentration of fermented green bean sprouts 1 mL), Treatment D (Concentration of fermented green bean sprouts 1.5 mL), Treatment E (2 mL fermented concentration of green bean sprouts), and Treatment F (2.5 mL fermented concentration of green bean sprouts). Different notations indicate significant differences ($P<0.05$)

Based on the average *C. vulgaris* cell diameter data obtained, the highest cell diameter data was shown on day 5. The results showed that treatments A and B were not significantly different from treatments E and F, but were significantly different from treatments C and D. Cell diameter The measurements obtained ranged from 2.29 – 7.91 µm for 10 days.

C. vulgaris Cell Growth Rate

The growth rate of *C. vulgaris* cells using fermented green bean sprouts with different concentrations was cultured for 10 days. Data on *C. vulgaris* cell diameter during the study can be seen in Table 2.

Table 2. Average Specific Growth Rate of *C. vulgaris* during the culture period (days)

Growth Rate (cell/day)	Treatment					
	A	B	C	D	E	F
H0	12.49	11.25	15.02	14.09	14.45	14.56
H1	12.56	12.57	13.06	12.67	12.53	12.63
H2	12.46	12.43	13.30	13.19	13.11	12.74
H3	12.69	13.1	13.28	12.70	12.94	12.86
H4	12.79	13.18	13.57	13.29	13.21	13.21
H5	12.20	12.37	13.48	13.26	13.33	13.10

H6	12.78	13.06	13	12.94	12.93	12.98
H7	12.77	13.02	13.17	13.53	13.16	12.99
H8	12.99	12.99	13.42	13.39	13.39	13.48

Data on the average cell diameter of *C. vulgaris* were obtained, the highest cell diameter data was shown on day 5. The results showed that treatments A and B were not significantly different from treatments E and F, but were significantly different from treatments C and D. The cell diameters were The size obtained ranged from 2.29 – 7.91 µm for 10 days.

Chlorophyll-a content in *C. vulgaris*

Table 3 Results The highest average *C. vulgaris* occurred on day 5 for all treatments. Treatment C showed the highest chlorophyll-a at 0.220 mg/mL, followed by treatment D at 0.206 mg/mL. The lower average chlorophyll-a achieved by treatment B was 0.179 mg/mL, treatment E was 0.171 mg/mL and treatment A was 0.165 mg/mL. The lowest average chlorophyll-a was with treatment F at 0.159 mg/mL. The average chlorophyll-a of *C. vulgaris* during the study can be seen in Table 3.

Table 3. Average Chlorophyll-a of *C. vulgaris* during the culture period (mg/mL)

Day	Average Chlorophyll-a Content of *C. vulgaris* (mg/mL)					
	Treatment					
	A	B	C	D	E	F
H0	0.083 ±	0.083 ±	0.083 ±	0.083 ±	0.083 ±	0.083 ±
H1	0.080^{a} ± 0.014	0.112^{c} ± 0.003	0.126^{d} ± 0.004	0.111^{bc} ± 0003	0.101^{b} ± 0.003	0.108^{bc} ± 0.004
H2	0.114^{ab} ± 0.008	0.123^{c} ± 0.003	0.137^{d} ± 0.006	0.119^{bc} ± 0.002	0.109^{a} ± 0.002	0.126^{c} ± 0.003
H3	0.134^{b} ± 0.006	0.158^{c} ± 0.006	0.190^{d} ± 0.007	0.131^{b} ± 0.007	0.115^{a} ± 0.005	0.139^{b} ± 0.005
H4	0.149^{b} ± 0.006	0.167^{c} ± 0.004	0.198^{d} ± 0.005	0.161^{c} ± 0.006	0.135^{a} ± 0.004	0.145^{b} ± 0.008
H5	0.165^{ab} ± 0.006	0.179^{c} ± 0.007	0.220^{e} ± 0.008	0.206^{d} ± 0.008	0.171^{bc} ± 0.004	0.159^{a} ± 0.006
H6	0.155^{b} ± 0.003	0.167^{c} ± 0.004	0.210^{d} ± 0.006	0.141^{a} ± 0.005	0.161^{b} ± 0.006	0.144^{a} ± 0.006
H7	0.134^{ab} ± 0.006	0.158^{c} ± 0.005	0.177^{d} ± 0.005	0.127^{a} ± 0.007	0.146^{b} ± 0.006	0.140^{b} ± 0.004
H8	0.120^{ab} ± 0.005	0.129^{bc} ± 0.008	0.141^{d} ± 0.008	0.114^{a} ± 0.006	0.138^{cd} ± 0.005	0.121^{ab} ± 0.003
H9	0.109^{b} ± 0.003	0.108^{b} ± 0.004	0.124^{c} ± 0.006	0.113^{b} ± 0.005	0.099^{a} ± 0.004	0.096^{a} ± 0.006

Based on the results of the Anova test which was carried out in the form of data on lipid content in *C. vulgaris* as the main parameter. During culture *C. vulgaris* increased on day 6. The highest lipid content in *C. vulgaris* was found on day 6 with treatment C (1 mL) with a lipid content of 5.98%, followed by treatment D (1.5 mL) of 5.65%. Lower lipid content levels occurred on day 0 in treatment E (2 mL) by 2.1%%. Data on the average lipid content of *C. vulgaris* for the 10 day culture period with different concentrations of fermented mung bean sprouts can be seen in Figure 2.

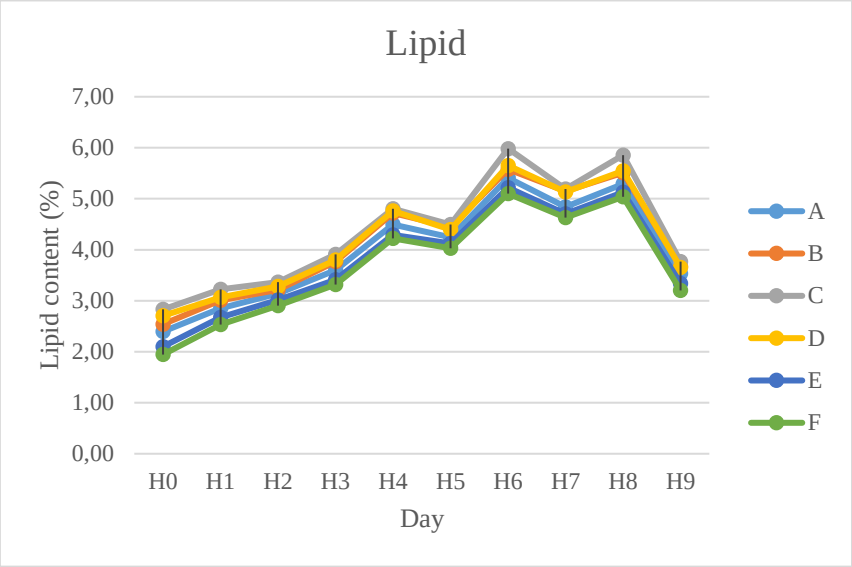

Figure 2. Chlorophyll-a graph of *C. vulgaris*

Water Quality

One of the factors that influence the growth of *C. vulgaris* is environmental factors. Environmental factors in *C. vulgaris* culture include temperature, pH, salinity and DO. Water quality measurements are carried out every day during maintenance. The results of water quality measurements during the research can be seen in Table 5.

Table 5. Data on water quality parameters during the culture period

Treatment	Water Quality Parameters			
	pH	Temperature (ºC)	DO (mg/L)	Salinity (ppt)
A	7.51-7.91	27.8-29.3	5.23–6.61	25–28
B	7.52-7.74	27.9-29.4	5.18–6.76	25–27
C	7.46-7.72	27.3-28.6	5.47–6.96	25–28
D	7.54-7.72	28.1-29.5	5.34–6.87	25–29
E	7.57-7.79	28.8-29.7	5.71-7.06	26–30
F	7.59-7.73	28.6-29.6	5.66-7.11	27–30
Optimum range	7-9 (Jusadi, 2003).	23-30 (Pratama, 2011).	5-7 (Facta et al., 2015).	25-28 (Isnanstyo & Kurniastuty,1995).

RESULT

Fermentation of mung bean sprouts in *C. vulgaris* culture increased cell density, according to the results of the ANOVA statistical test ($P<0.05$). The use of nutrients in the media used for microalgae growth caused the results of these six treatments to be significantly different. The nutrients found in fermented green bean sprouts are the elements N, P, K, Ca, Mg, S, Cu, Zn, and Mn. Many important nutrients for Chlorella are N and P. In this study, mung bean sprouts contained an N content of 45.39 mg/L and a P content of 27.58 mg/L. Nitrogen and phosphorus requirements of Chlorella sp. are 0.7–0.14 g/l and 0.62-0.015 g/l, while Walne fertilizer only contains 0.016 g/l and 0.004 g/l (Triastuti, 2011).

This increased nitrogen value is thought to be caused by the large amount of EM4 added, thereby increasing the ongoing organic material decomposition processes, such as

aminization, ammonification and nitrification. The breakdown of proteins by microorganisms into amino acids is known as the aminization process. This process occurs in organic materials, where microorganisms break down proteins into amino acids. Next, microorganisms destroy these amine and amino acid compounds to produce ammonia (NH_3) and ammonium (NH_4) compounds. This process is known as ammonification. Furthermore, the nitrogen element in fermentation increases because fermentation bacteria convert ammonia into nitrate (Wulandari et al., 2015). Fermentation time influences low phosphorus levels. The longer the fermentation process takes, the fertilizer will lose some of its nutrients, namely phosphorus. This happens because most phosphorus is chemically bound by other elements, making it a compound that is difficult to dissolve in water. This results in the rapid transformation of orthophosphate ions ($H_2PO_4^+$) and these secondary orthophosphate ions into phosphorus compounds. Orthophosphate (PO_4) is the simplest form of phosphorus that can be absorbed by microalgae. P-solubilizing bacteria are another bacteria found in EM_4; These bacteria convert ATP produced during the initial fermentation process into P elements which can be used directly by microalgae (Riniati et al., 2021).

C treatment with a concentration of 1 mL affected the density of *C. vulgaris* at the highest level, reaching 1,249 x 10^4 cells/mL on the fifth day. This can happen because C treatment produces a complete nutrient composition such as nitrogen and phosphorus that suits the needs of *C. vulgaris*, which allows microalgae to grow well. Since nitrogen and phosphorus play an important role in the photosynthesis process, these two nutrients are essential for the growth of microalgae. Crishmadha (2006) stated that in *C. vulgaris* photosynthesis, nitrogen plays a role in the formation of chlorophyll and protein. Cells produce ATP from phosphorus, which is responsible for energy transfer (Amanatin., 2013). In the process of photosynthesis, light plays an important role. At this stage, light energy is converted into chemical energy which is stored in molecules originating from CO_2 and H_2O. Photosystem I works on red light and Photosystem II works on green light to carry out photosynthesis reactions. Photosynthesis occurs in both of them in the same way, but the types of microalgae pigments in the chloroplasts are different, so the wavelengths of light absorbed are more different. Photosystem II has a wavelength of 680 nanometers, and Photosystem I has chlorophyll molecules with a wavelength of 700 nanometers. The two types of photosynthesis reactions are light (requires light) and dark (requires carbon dioxide). The working principle of dark reactions is that light energy is converted into chemical energy ATP and NADPH. On the other hand, light reaction energy comes from ATP and NADPH which are used to convert carbon dioxide into sugar. Photosynthesis will produce glucose and energy which is used in cell metabolism. As a result, the growth of *C. vulgaris* will increase (Robi, 2014). During the study, the lowest *C. vulgaris* culture solids were found at a concentration of 2.5 mL. The presence of excessive amounts of nutrients to meet the needs of Chlorella cells causes this treatment to have the lowest levels of *C. vulgaris*. Excess nutrients will cause ammonia levels to increase. Large levels of ammonia will be toxic, which can inhibit growth. This is due to the fact that the nature of the poison will directly interfere with the effectiveness of cell metabolism.

In the exponential phase, Chlorella cell density reaches its peak. On the third and fifth days, the exponential phase occurs. On the fifth day, the results are completely different because cells divide rapidly due to the availability of nutrients that support their growth (Boroh et al., 2019). Calculating the diameter of *C. vulgaris* cells, we found that there were significant differences between each treatment. This is supported by research conducted by Trikuti et al. (2016), which found that the availability of constituent nutrients in each medium determines the size of cells produced by *C. vulgaris* cultured in various types of treatment. Nutrients such as phytohormones and phosphate are responsible for the metabolism and physiology of algae. In addition, microalgae cell size is also influenced by environmental variables such as CO_2 concentration, light intensity and temperature. There is a possibility of increasing nutrient

levels and changes in microalgae cell size due to increasing CO_2 concentrations in the culture media (Khairuddin & Sihabuddin, 2013). As long as the culture is not too high or low, the temperature must be adjusted because high temperatures will also reduce cell size. Microalgae at low light intensity have larger cell sizes due to their ability to absorb more nutrients than microalgae at high light intensity. During ontogenesis, chlorella cells differ in size. The development of an organism from a fertilized egg to the adult form is known as ontogenesis. Because microalgae carry out photosynthesis in the early phase of growth (adaptation), the size of *C. vulgaris* will greatly increase. During the stationary phase, cells continue to divide even though nutrients have been used up. Because cells in the stationary phase lack nutrients and thus become more resistant to extreme conditions such as heat, cold, radiation, and chemicals, the composition of cells may differ from cells growing in the logarithmic phase. However, microalgae have the largest cell size which is spread within 3-8 µm with an average diameter of 4.34 ± 0.90 µm and there are no cells below 2 µm (Hu, 2014).

The largest cell size was produced by treatment D with a concentration of 1.5 mL, amounting to 7.91 µm. This could be due to the fact that this treatment provides complete nutrients, such as phytohormones and phosphorus, which meet the needs of *C. vulgaris*. Phytohormones can increase the size of microalgae cells created during cell division. Auxin is a phytohormone that influences the growth and metabolism of microalgae. Although auxin has the ability to stimulate growth and increase biomass, higher auxin concentrations also have the ability to inhibit cell growth (Wang et al, 2022). In the process of microalgae cell growth, the mechanism of action of auxin (IAA) begins with loosening of the cell wall through the proton (H^+) pump. This process plays an important role in the cell growth reaction to auxin. Acidification of cell walls activates the enzyme expansin, which loosens cell wall material by breaking hydrogen bonds between cellulose microfibrils and other cell wall constituents. As the membrane potential increases, more ions will be taken into the cell. This will cause an increase in water osmosis and turgor. Cells can grow if the level of turgor and cell wall plasticity increases.

The lowest cell size produced by treatment A (0 mL concentration) was 2.29 µm. Due to the lack of nutrients needed by Chlorella cells, *C. vulgaris* cell size was lowest during this treatment. The formation of chlorophyll-a ($C_{55}H_{72}O_5N_4Mg$) and chlorophyll-b ($C_{55}H_{70}O_6N_4Mg$) is influenced by a deficiency of the macronutrients N and Mg. Because Mn is an enzyme activator in the light reaction of photosynthesis, a lack of micronutrients such as Mn can affect the photosynthesis process. This will affect the rate of photosynthesis. The quantity of products (carbohydrates) produced is determined by the rate of photosynthesis. Carbohydrates produced by microalgae photosynthesis are used for cell growth and respiration. Carbohydrates remaining after some are used in the respiration process are insufficient for cell growth if photosynthesis yields are reduced. Cell size will be influenced by growth inhibition. An increase in cell size indicates microalgae growth.

According to the results of the ANOVA statistical test ($P<0.05$), fermentation of mung bean sprouts in *C. vulgaris* culture changed the level of chlorophyll-a in cells. Due to the use of nutrients in the media used to contain chlorophyll-a in microalgae, the six treatments are different. Nutrients and water quality are important components in the formation of chlorophyll-a. Among the nutrients found in fermented green bean sprouts are the elements N, P, K, Ca, Mg, S, Cu, Zn, and Mn. Many important nutrients for Chlorella are N and P. In this study, mung bean sprouts contained an N content of 45.39 mg/L and a P content of 27.58 mg/L. Chlorophyll-a is formed mostly in the stationary phase, namely H-4 to H-5. The metabolic pattern and biochemical composition of cells in a microalgae culture will change greatly after passing through the exponential phase and then entering the stationary phase. One of the characteristics of the stationary phase of microalgae is a low or stable chlorophyll content. Treatment C with a concentration of 1 mL produced high chlorophyll levels of 0.220 mg/mL.

This is due to the fact that the elements that form chlorophyll are available in larger quantities compared to other fermented concentrations of mung bean sprouts. Nitrogen, the main nutrient that helps form chlorophyll-a, is brought in by this treatment. Nitrogen, a macronutrient, has the ability to influence microalgae chlorophyll development in various cellular metabolic processes, including transport, catabolism, assimilation, and especially protein biosynthesis.

Nitrogen also has an effect on enzymes that control all metabolic processes and chlorophyll synthesis. There are three reaction pathways in the formation of chlorophyll which are controlled by core genes. This includes the reaction pathway between protoporphyrin and protochlorophyll to chlorophyllide controlled by the CHLD, CHLI, CHLH, and CDR genes; the change in protochlorophyll to chlorophyllide is controlled by the VDR, POR genes, and the chlorophyll-b synthesis pathway is controlled by the CAO gene. The amount of chlorophyll in microalgae can increase due to the presence of nitrogen. Higher protein content will have higher nitrogen levels. Chlorophyll content increases in media with lots of nitrogen (Amaliandini et al, 2023). The relationship between growth and chlorophyll content increases at the time of highest growth. This is because chloroplasts have to convert a lot of light energy into chemical energy during maximum growth. The more chlorophyll needed to absorb light energy, the more parts of the chloroplast work. Thus, the amount of chlorophyll formed will increase. Connected with the amount of protein formed, an increase in the amount of chlorophyll is followed by a decrease in the amount of protein. In other words, microalgae cells only have the ability to increase protein or chlorophyll.

Chloroplasts contain protein and are the site of the chlorophyll formation process, so that chlorophyll formation is reduced when chloroplasts form protein and vice versa. In contrast to other treatments, treatment F had the lowest chlorophyll-a content of 0.159 mg/mL with a concentration of 2.5 mL. This can occur because this treatment lacks nutrient composition such as Mg which is needed for the formation of chlorophyll. The precursors of the chlorophyll molecule are the amino acid glycine and its derivative, d-amino levulinic acid (ALA). One of the components of chlorophyll is Mg with 130 ppm Mg in fermented green bean sprouts (Nurhasanah, 2013). This may be related to the absorption of metallic nutrients, such as Mg, which form chlorophyll molecules. As shown by the ANOVA test results, fermentation of mung bean sprouts in *C. vulgaris* culture had a significant impact on the level of lipid content of *C. vulgaris*. Nitrogen (N) and phosphorus (P) are important nutrients for *C. vulgaris*. In this study, fermented mung bean sprouts contained 45.39% nitrogen (N) and 27.58% phosphorus (P). This is in line with research by Triastuti et al. (2011), who found that *C. vulgaris* requires nitrogen (N) between 14 and 70 percent to increase its lipid content. According to Hu & Gao (2006), nitrogen (nitrate) loss is a condition where sufficient nitrogen content can increase lipid content levels by around 2.8%. This condition is known as high carbon dioxide (CO_2) levels.

Fermentation time affects phosphorus (P) levels because phosphorus compounds are easily bound by other chemicals before the fermentation process begins. Phosphorus compounds are easily absorbed by microalgae because the fermentation process makes it difficult for them to bind with other chemicals. This rapidly converts secondary orthophosphate and orthophosphate ions into phosphoric acid compounds. One form of phosphorus that can be absorbed by microalgae is orthophosphoric acid (H_3PO_4). On day 6, with treatment C (1 mL), the lipid content of *C. vulgaris* was highest at 5.98%. This is caused by environmental stress, the ratio of nitrogen (N) and phosphorus (P), which is limiting for microalgae, causing an increase in lipid content levels. This is in accordance with research conducted by Ciu et al. (2008), where the limited ratio of nitrogen and phosphorus is considered as an environmental pressure that causes stress in microalgae, thus helping to increase the accumulation of lipid content levels. If there is a low ratio of nitrogen to phosphorus (N), proteins can be broken down into amino acids. Acetyl-CoA is then formed, which results in increased lipid levels Hartati et al. (2013). The production of glycerol and fatty acids is needed to convert

carbohydrates into fat. The alcohol reaction begins with triglycerides (TGA) turning into diglycerides (DGA), then diglycerides (DGA) turning into monoglycerides (MGA), and finally glycerol is formed. Formation of fatty acids through acetyl Co-A acetate units. Chloroplasts produce fatty acids from the enzyme pyruvate dehydrogenase which is made during glycolysis in the cytosol. Additional acetyl Co-A comes from free acetate from mitochondria. Plastids will absorb free acetate and convert it into acetyl Co-A for use in lipid formation (Hartati et al., 2013).

Fermentation increases levels of the nutrient nitrogen (N) and phosphorus (P). The ammonization process, in which microorganisms break down proteins into amino acids, is the initial stage of the fermentation process. Production through protein breakdown and amino acid synthesis is known as the aminosation process. Microorganisms then destroy amino acid and amine compounds, producing ammonia NH_3^+ and ammonium NH_4 compounds. This process is also referred to as ammonization. Furthermore, ammonia is converted into nitrate by fermentation bacteria, which causes an increase in nitrogen (N) content during the fermentation process (Christi, 2007). With the help of the lipase enzyme catalyst, the condensation of glycerol and three fatty acid molecules initiates lipid biosynthesis in microalgae cells. Some Acetyl-CoA, two pairs of electrons (2NADPH), and ATP energy are required to form fatty acids. ATP, NADH, and pyruvic acid are produced from glycolysis during the process. ATP derived from glycolysis of pyruvate and NADPH can be obtained through the pentose phosphate respiration pathway. Acetyl CoA carboxylase increases lipid production (Hartati et al., 2013).

The first day of the study showed the lowest *C. vulgaris* lipid content, with treatment F (2.5 mL) at 1.95%. This is caused by excess nutrition compared to the needs of *C. vulgaris* cells. High ammonia levels are caused by excessive nutrition. It is possible that high levels of ammonia, known as toxic ammonia, will inhibit growth. Due to its dangerous nature, cell metabolism will be disrupted. The increase in lipid content is influenced by the growth process of *C. vulgaris*. The growth process of *C. vulgaris* consists of five stages: adaptation, exponential phase, growth decline phase, stationary phase, and death phase. After the inoculum is introduced into the culture medium, the adaptation phase begins. This phase shows a large increase in cell size and protein synthesis. Although metabolism continues, cell division does not occur intensively. As a result, due to the process of adaptation to the new environment, cell density does not immediately increase. Because cell activity reaches its peak in the exponential phase, cell division increases rapidly. For harvesting *C. vulgaris* as natural feed or fish seeds, the exponential phase is the best. Next is the phase of decreasing growth rate, which is characterized by weak cell division and a tendency to decrease growth rate. The growth process of *C. vulgaris* reaches its peak in the stationary phase. The balance of cell growth and cell death rates in microalgae shows this. Nutrient deficiency will cause stress to microalgae during the stationary phase. Microalgae on roads tend to produce lipids as food reserves. The end of the microalgae growth phase is the death phase, because the rate of cell death is higher than the rate of cell growth (Chilmawati et al., 2008).

CONCLUSION

Providing fermented mung bean sprouts (*Phaseolus radiatus*) with different concentrations during the culture period showed a significant effect on the concentration, density, diameter, growth rate, formation of chlorophyll a and lipids of *C. vulgaris*. The highest results regarding density, chlorophyll a, and lipids of *C. vulgaris* were found in the 1 mL concentration treatment, which reached 1,249 x 10^4 cells/mL, 0.220 mg/mL, and 5.98%. The highest *C. vulgaris* cell diameter was found in the 1.5 mL concentration treatment, which reached 7.91 µm, with a growth rate of 12.20–14.56. Water quality included culture temperature 26.3–29.3°C, pH 7.19–7.80, salinity 35–39 ppt, dissolved oxygen (DO) 3.21–4.89

mg/L, and light intensity 3000 lux. Fermented mung bean sprouts have complete amounts of nutrients, such as nitrogen and phosphorus, which meet the needs of *C. vulgaris*.

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