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Effect of Fermented Mother Liquor (FML) Substitution Enriched with $NaNO_3$ as a *Chlorella vulgaris* Culture Media

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ABSTRACT

Chlorella vulgaris is a natural feed that plays an important role in cultivation due to its ease of cultivation and high nutritional value. However, its production is hindered by the high cost of the culture media, which makes the overall production expensive. One potential solution is to use a medium made from fermented mother liquor (FML) enriched with $NaNO_3$, as its nitrogen-phosphorus (N/P) content is beneficial for the growth of *C. vulgaris*. This study aimed to assess the impact of FML and $NaNO_3$ substitution as a culture medium for *C. vulgaris* and to determine the optimal dose. The treatments used in this study were P1 (Walne, serving as the control with an N/P ratio of 17:1), P2 (10:1), P3 (15:1), P4 (17:1), and P5 (20:1). The results demonstrated that media with FML substitution enriched with $NaNO_3$ can not only replace Walne fertilizer but also outperform it. The optimal dose of FML substitution enriched with $NaNO_3$ for maximizing chlorophyll-a, chlorophyll-b, and carotenoid content in *C. vulgaris* on a laboratory scale was found to be the N/P ratio of 15:1, with a population density of 10.92 x 10^6 cells/ml (day 7), a growth rate of 25 x 10^6 cells/ml (day 3), chlorophyll-a content of 0.49µg/ ml, chlorophyll-b of 0.46µg/ ml (day 7) and carotenoids of 0.16µg/ ml (day 7).

INTRODUCTION

Chlorella vulgaris plays an important role in fish and shrimp cultivation due to its function as live feed **(Ahmad *et al.*, 2020)**. *C. vulgaris* has advantages over other phytoplankton, such as being easy to cultivate and reproducing quickly **(Thirugnanasambantham *et al.*, 2020)**. It also contains various essential nutrients required by cultivated species, including protein, carbohydrates, unsaturated fatty acids, vitamins, chlorophyll, enzymes, and high fiber **(Mtaki *et al.*, 2021)**. Nutrients like nitrogen (N) and phosphorus (P) are crucial factors in the culture of *C. vulgaris*, enabling its large-scale production and use in aquaculture **(Kumari *et al.*, 2021)**.

C. vulgaris culture on a laboratory scale generally uses a medium with a practical fertilizer named Walne **(Trinh, 2023)**. The use of Walne culture media promotes the growth of green microalgae more effectively than other media, such as Guillard, due to its more complete nutritional content **(Putri & Alaa, 2019)**. However, Walne culture media is relatively expensive, making it challenging to achieve high production quantities of *C. vulgaris* **(Putri & Alaa, 2019)**. This is because Walne fertilizers contain a mix of macro and micronutrients derived from various sources such as animal manure, compost, bone meal **(Hidayati *et al.*, 2022)**. Walne fertilizer can also leach into water bodies, causing eutrophication and harming aquatic ecosystems **(Jwaideh *et al.*, 2022)**. Therefore, alternative media or substitutes with nutrient content equivalent to Walne are needed.

One approach is to substitute alternative materials like FML (Fermented Mother Liquor) in the *C. vulgaris* culture medium. FML is a liquid byproduct from the production of monosodium glutamate (MSG), obtained through the fermentation of molasses or sugarcane. It contains high levels of nutrients, such as nitrogen (0.49g/ L), phosphorus (13.69g/ L), protein (22.98%), and amino acids (3-5%) **(Urry *et al.*, 2017)**. FML is also relatively inexpensive, making it a viable culture medium for *C. vulgaris* **(Yulanda *et al.*, 2021)**. However, FML-based media must be enriched with $NaNO_3$ to enhance the productivity of *C. vulgaris*. FML typically does not contain significant levels of nitrates, as it is primarily composed of crude protein, amino acids, and minerals **(Kananurak, 1987)**. The addition of $NaNO_3$ increases the N/P ratio, making the nutrient content comparable to that of Walne's medium, as $NaNO_3$ is rich in nitrates and nitrogen **(Kilroy *et al.*, 2020)**. Various studies on FML and $NaNO_3$ have been conducted on other phytoplankton, such as *Nannochloropsis* sp. and *Galdieria* sp. **(Kurniawan *et al.*, 2023; Wiryadi & Witono, 2018)**. However, research on the potential of FML as a substitute medium for *C. vulgaris* remains limited, and the combination of FML with $NaNO_3$ has not been widely explored. This study aimed to assess the impact of substituting FML and $NaNO_3$ as a culture medium for *C. vulgaris*, focusing on its productivity and nutritional content.

MATERIALS AND METHODS

1. Experimental design

The *Chlorella vulgaris* inoculant used in this study was obtained from BBPBAP Situbondo (Indonesia) while fermented mother liquor (FML) was sourced from PT Ajinomoto Indonesia in Surabaya. $NaNO_3$ was obtained from the Faculty of Fisheries and Marine (Airlangga University). This study employed an experimental design, substituting FML enriched with $NaNO_3$ to evaluate its effect on the productivity of *C. vulgaris*. The results were compared with Walne medium to assess performance. The study followed a completely randomized design (CRD), with the treatment involving the addition of

nitrogen (N) to the FML, creating different N/P ratios. The experiment included 5 treatments with 4 replications, as shown in Table (1).

Table 1. Formulation of N/P used as treatment

Treatment		N/P ratio
P1 (control)	Walne	17:1
P2	FML + $NaNO_3$	10:1
P3	FML + $NaNO_3$	15:1
P4	FML + $NaNO_3$	17:1
P5	FML + $NaNO_3$	20:1

2. Ethics statement

The necessary ethical approvals were obtained from the Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, and the guidelines outlined in the Laboratory Code of Animal Care were strictly followed. No animals or fish were harmed or improperly treated during this study.

3. FML preparation

FML was tested for nitrogen and phosphorus content. Furthermore, FML was filtered using filter paper, then put into an erlenmeyer flask and sterilized in an autoclave. FML was put into an erlenmeyer flask covered with cotton and gauze, then wrapped in aluminum foil. Furthermore, it was sterilized by autoclaving at a temperature of 121^{o}C and a pressure of 1 atm for 15 minutes to kill any remaining microorganisms **(Nikhilesh *et al*., 2013)**. After sterilization, FML was continued with the addition of $NaNO_3$ with different concentrations so that FML had N/P that was in accordance with the treatment and was confirmed by testing its content. The addition of $NaNO_3$ was in accordance with the procedure detailed in a previous study **(Hidayati *et al*., 2020)**.

4. Algal materials and culture condition

The medium used was seawater which was then added with a nutrient source of Walne fertilizer, $NaNO_3$, and the addition of FML. The inoculant was spread after the medium was given fertilizer with a dose of 1ml/ L of culture media water **(Aulia *et al*., 2021)**. After determining the desired initial density, the initial step taken was to calculate the density of the pure *C. vulgaris* seed or starter stock. The initial density used in this study refers to previous study of **Brautovic (2000)**, which was 1.25 x 10^6 cells/ml of inoculant volume. Inoculant seeds were taken for treatment culture using the percentage method of 20% inoculant and 80% media. The inoculant was spread after the media was given FML enriched with $NaNO_3$ for the growth of phytoplankton to be cultured. The container for microalgae culture is a glass jar with a volume of 2.5 liters. In this study, the environmental factors were as follows: salinity ranged from 25 to 35ppt; pH levels varied between 4.5 and 9.3; temperatures ranged from 25 to 35°C, and light intensity was

between 500 and 5000 lux, with a light period of 16 hours and a dark period of 8 hours **(Selvika *et al.*, 2016; Deniz, 2020; Febrieni *et al.*, 2020; Lamadi *et al.*, 2022)**.

The algae culture process was carried out for 11 days in a closed room to determine the density and growth rate of *C. vulgaris* from the adaptation phase to the decline **(Wang *et al*., 2023)**.

5. Analysis parameter

The biological indices (density and growth rate) of *C. vulgaris* were evaluated by the following formula **(Hidayati *et al*., 2020)**:

Phytoplankton cell density (cells/ml) = $\frac{\mathrm{nA+nB+}nC\mathrm{+nD+nE}}{5\ x\ 4}$ x 10^6

Note :

nA = number of phytoplankton cells in blocks A (cells/mL)
nB = number of phytoplankton cells in blocks B (cells/mL)
nC = number of phytoplankton cells in blocks C (cells/mL)
nD = number of phytoplankton cells in blocks D (cells/mL)
nE = number of phytoplankton cells in blocks E (cells/mL)

Growth rate is a parameter that describes the speed of cells in dividing themselves per unit of time. The calculation formula used refers to **Paes *et al*. (2016)**:

Growth rate (cells/day) = $\frac{\mathrm{Nt-(Nt-1)}}{\Delta \mathrm{t}}$

Note :

Nt = number of cells at a specified time (cells/day)
Nt-1 = number of cells in the 24 hours before the specified time (cells/day)
Δt = cell calculation time difference (days)

Other parameters measured in this study included the chlorophyll and carotenoid content, assessed daily until *C. vulgaris* reached the death phase. The analysis of pigment levels was conducted following the method described by **Pisal and Lele (2005)**. A UV-Vis spectrophotometer was employed to obtain absorbance at wavelengths of 470, 646.8, and 663.2nm. Pigment levels were calculated using the formula provided by **Lichtenthaler and Buchmann (2001)**:

Ca = 12.25 A663.2 – 2.79 A646.8
Cb = 21.50 A646.8 – 5.1 A663.2
Cx+c = (1000 *A*470 − 1.82 Ca − 85.02 Cb) /198

Note :

Ca = chlorophyll a content (µg/mL)
Cb = chlorophyll b content (µg/mL)
Cx+c = total carotenoid content (µg/mL)

Temperature was measured using a thermometer; pH was measured using a pH meter; salinity was measured using a refractometer, and light intensity was measured using a lux meter as described by previous studies **(Hidayati *et al*., 2020; Santanumurti *et al*., 2023)**. Measurement of supporting parameters was carried out once a day during maintenance.

6. Statistical analysis

The data obtained are quantitative and show the density parameters and growth rate of *C. vulgaris* with FML substitution. The effect of treatment on parameters was analyzed using the analysis of variance (ANOVA) test. If the analysis results show a significant difference, the calculation is continued with Duncan's multiple range test **(Bahar *et al*., 2022)**. As a tool for implementing statistical tests, laptop software was used with the SPSS program.

RESULTS

1. Population density of *C. vulgaris*

Table 2. Average results of *C. vulgaris* density with different N/P ratios (x10^6 cells/ml)

Day(s)	P1	P2	P3	P4	P5
0	1.25±0.00	1.25±0.00	1.25±0.00	1.25±0.00	1.25±0.00
1	1.33±0.14^{a}	1.75±0.25^{b}	2.00±0.25^{b}	1.25±0.00^{a}	1.25±0.00^{a}
2	1.58±0.14^{a}	2.75±0.25^{b}	4.00±0.50^{c}	2.08±0.14^{a}	1.83±0.14^{a}
3	2.58±0.38^{a}	3.33±0.14^{a}	6.25±0.90^{b}	2.75±0.25^{a}	3.00±0.50^{a}
4	4.17±1.04^{a}	3.91±0.14^{a}	7.08±0.38^{b}	3.42±0.29^{a}	4.33±0.38^{a}
5	4.25±1.15^{a}	4.41±0.14^{a}	7.83±0.28^{b}	3.92±0.38^{a}	3.83±0.14^{a}
6	4.17±0.95^{a}	5.33±0.52^{b}	8.67±0.29^{c}	3.50±0.43^{a}	3.83±0.14^{a}
7	6.42±0.94^{a}	6.75±0.90^{a}	10.92±1.13^{b}	6.75±1.25^{a}	6.17±0.52^{a}
8	8.42±1.18^{a}	8.42±0.63^{a}	9.50±0.75^{a}	8.08±1.01^{a}	8.41±0.38^{a}
9	7.33±0.58^{a}	8.00±0.50^{a}	8.33±0.80^{a}	7.42±0.52^{a}	7.17±0.52^{a}
10	3.67±0.52^{bc}	2.75±0.25^{ab}	4.42±0.95^{c}	2.58±0.80^{ab}	1.67±0.72^{a}

Note: Different letters indicate the significant differences between treatments.

The results of Table (2) demonstrated the population density of *Chlorella vulgaris* in this study. This study showed that the substitution of FML enriched with $NaNO_3$ gave a significantly different effect on days 1 to 7 and day 10. The ANOVA results of this study showed that the use of FML and $NaNO_3$ with different N/P ratios had a significantly different effect on the density of *C. vulgaris*. P3 with N/P 15:1 showed a significantly different value, even with Walne fertilizer as control. This indicated that P3 was the treatment that gives the highest population density value. The highest density

peak occurred on the seventh day, namely in P3 with a density of 10.92 x 10^6 cells/ml, and was significantly different from all treatments. The differences between each treatment are presented in Fig. (1).

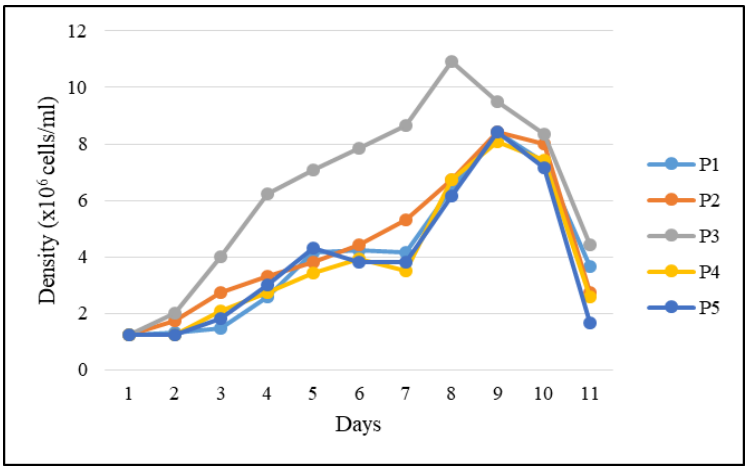

Fig. 1. Average results of *C. vulgaris* density with different N/P ratios (x10^6 cells/ml)

2. Growth rate of *C. vulgaris*

Table 3. Average results of *C. vulgaris* growth rate with different N/P ratios (x10^6 cells/ml)

Day(s)	P1	P2	P3	P4	P5
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	-0.08±0.14^{a}	0.33±0.29^{bc}	0.50±0.25^{c}	0.00±0.00^{ab}	0.00±0.00^{ab}
2	0.50±0.00^{a}	1.17±0.52^{bc}	1.58±0.14^{c}	0.92±0.14^{ab}	0.50±0.00^{a}
3	0.92±0.38^{a}	0.50±0.00^{a}	3.25±0.50^{b}	0.67±0.29^{a}	0.83±0.14^{a}
4	2.08±0.52^{b}	0.83±0.38^{a}	1.42±0.52^{ab}	1.17±0.38^{a}	1.58±0.29^{ab}
5	0.00±1.75^{a}	0.50±0.00^{a}	0.67±0.29^{a}	-0.33±0.14^{a}	-0.42±0.29^{a}
6	-0.08±0.29^{a}	0.58±0.78^{a}	1.33±0.38^{b}	-0.08±0.29^{a}	0.00±0.43^{a}
7	2.83±0.8^{a}	1.92±0.14^{a}	2.00±0.90^{a}	3.08±0.72^{a}	2.42±0.63^{a}
8	1.25±0,43^{b}	2.00±0.43^{c}	-1.42±0.38^{a}	0.92±0.29^{b}	2.75±0.25^{d}
9	-0.42±2.02^{a}	-0.50±1.00^{a}	-0.75±0.25^{a}	-0.75±0.00^{a}	-1.13±0.38^{a}
10	-3.92±0.14^{ab}	-4.92±0.14^{a}	-3.50±1.00^{b}	-4.25±0.90^{ab}	-4.75±0.43^{a}

Note: Different letters indicate the significant differences between treatments.

The results of the growth rate values in this study are shown in Table (3). Statistically, the results of the research analysis except on days 5, 7, and 9 showed that the substitution of FML enriched with $NaNO_3$ had a significantly different effect on the growth rate of *C. vulgaris* on days 1-4, 6, 8, and 10. P3 with N/P 15:1 showed significantly different values, even with Walne fertilizer as the control. This indicated that P3 was the treatment that provides the highest growth rate value. On day 3, P3 had

the highest growth rate value of 3.25 x 10^6 cells/ml which was significantly different (P<0.05) from all treatments. The differences between each treatment are presented in Fig. (2).

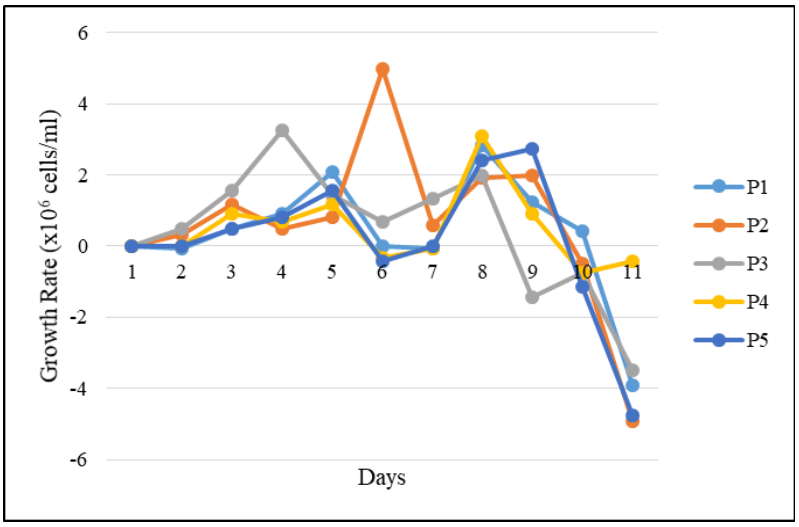

Fig. 2. Average results of *C. vulgaris* growth rate with different N/P ratios (x10^6 cells/ml)

3. Chlorophyll-a of *C. vulgaris*

Table 4. Average results of chlorophyll-a of *C. vulgaris* with different N/P ratios (µg/ml)

Day(s)	P1	P2	P3	P4	P5
0	0.08±0.07^{a}	0.14±0.01^{a}	0.06±0.05^{a}	0.10±0.02^{a}	0.08±0.03^{a}
1	0.10±0.08^{ab}	0.16±0.03^{b}	0.07±0.05^{ab}	0.14±0.03^{ab}	0.07±0.02^{a}
2	0.22±0.06^{b}	0.21±0.04^{b}	0.10±0.04^{a}	0.20±0.03^{b}	0.13±0.03^{a}
3	0.27±0.08^{b}	0.24±0.03^{b}	0.12±0.06^{a}	0.20±0.04^{ab}	0.20±0.07^{ab}
4	0.01±0.00^{a}	0.05±0.00^{c}	0.01±0.00^{a}	0.01±0.00^{ab}	0.03±0.00^{b}
5	0.09±0.02^{b}	0.07±0.01^{ab}	0.06±0.00^{a}	0.06±0.00^{a}	0.05±0.00^{a}
6	0.24±0.13^{a}	0.27±0.01^{a}	0.15±0.06^{a}	0.24±0.04^{a}	0.14±0.02^{a}
7	0.27±0.10^{a}	0.27±0.04^{a}	0.49±0.10^{b}	0.26±0.04^{a}	0.24±0.08^{a}
8	0.39±0.08^{a}	0.39±0.07^{a}	0.24±0.14^{a}	0.38±0.03^{a}	0.32±0.05^{a}
9	0.53±0.07^{b}	0.26±0.06^{a}	0.21±0.11^{a}	0.41±0.03^{b}	0.19±0.04^{a}
10	0.35±0.16^{b}	0.19±0.15^{ab}	0.09±0.05^{a}	0.27±0.12^{ab}	0.12±0.03^{a}

Note: Different letters indicate the significant differences between treatments.

The chlorophyll-a content values in this study are displayed in Table (4). Statistically, FML substitution enriched with $NaNO_3$ had a significantly different effect on the chlorophyll-a content of *C. vulgaris*, except on days 0, 6, and 8. P1 using Walne fertilizer had a significantly different chlorophyll-a content value compared to other treatments. The highest chlorophyll-a content value was found in P1 on day 9 with a

value of 0.53 ± 0.07µg/ ml. However, this value was not significantly different from P3 on day 7 (0.49 ± 0.10µg/ ml). This indicated that P3 containing N/P 15:1 could produce the same chlorophyll-a as Walne. The differences between each treatment are depicted in Fig. (3).

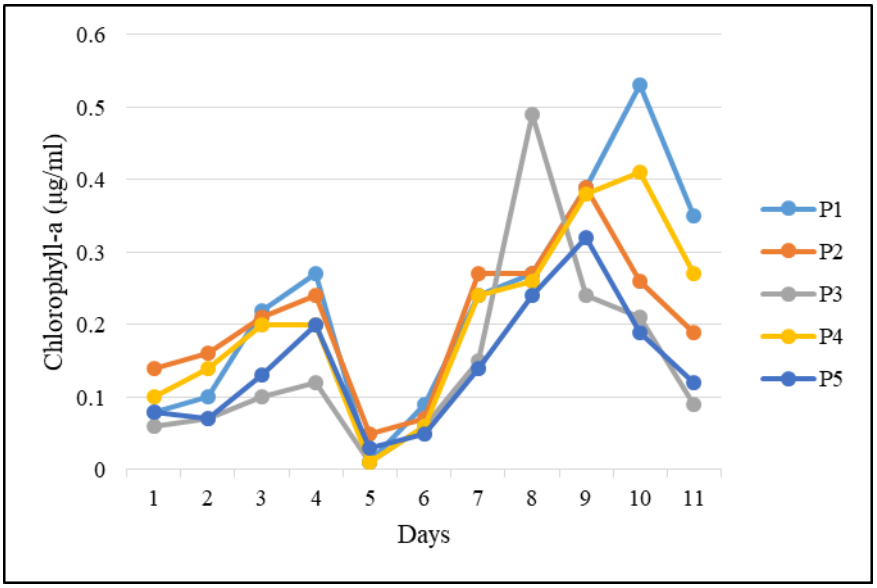

Fig. 3. Average results of chlorophyll-a of *C. vulgaris* with different N/P ratios (µg/ml)

4. Chlorophyll-b of *C. vulgaris*

Table 5. Average results of chlorophyll-b of *C. vulgaris* with different N/P ratios (µg/ml)

Day(s)	P1	P2	P3	P4	P5
0	0.03±0.01^{ab}	0.11±0.01^{c}	0.01±0.00^{a}	0.04±0.01^{b}	0.03±0.00^{ab}
1	0.05±0.04^{a}	0.10±0.05^{ab}	0.09±0.04^{ab}	0.13±0.02^{b}	0.06±0.03^{ab}
2	0.10±0.01^{a}	0.20±0.03^{b}	0.10±0.05^{a}	0.15±0.09^{ab}	0.08±0.03^{a}
3	0.12±0.03^{a}	0.23±0.03^{b}	0.15±0.10^{ab}	0.16±0.05^{ab}	0.17±0.03^{ab}
4	0.01±0.00^{a}	0.05±0.01^{b}	0.02±0.00^{a}	0.05±0.01^{b}	0.02±0.01^{a}
5	0.04±0.01^{ab}	0.07±0.01^{c}	0.04±0.00^{ab}	0.05±0.01^{bc}	0.02±0.01^{a}
6	0.21±0.02^{a}	0.25±0.03^{a}	0.19±0.09^{a}	0.26±0.01^{a}	0.26±0.05^{a}
7	0.31±0.07^{a}	0.27±0.01^{a}	0.46±0.08^{b}	0.29±0.04^{a}	0.28±0.03^{a}
8	0.31±0.07^{a}	0.44±0.07^{a}	0.32±0.12^{a}	0.40±0.04^{a}	0.31±0.06^{a}
9	0.33±0.02^{a}	0.24±0.08^{a}	0.30±0.03^{a}	0.30±0.04^{a}	0.29±0.07^{a}
10	0.24±0.12^{c}	0.04±0.03^{a}	0.17±0.03^{bc}	0.11±0.06^{ab}	0.16±0.04^{abc}

Note: Different letters indicate the significant differences between treatments

The chlorophyll b value in this study is presented in Table (5). The results of the analysis during the study showed that the substitution of FML enriched with $NaNO_3$ had a significantly different effect on the chlorophyll-b content of *C. vulgaris*, except on days 6, 8, and 9. P3 produced the highest chlorophyll value on day 7 (0.46 ± 0.08µg/ ml)

compared to all treatments. The differences between each treatment was presented in Fig. (4).

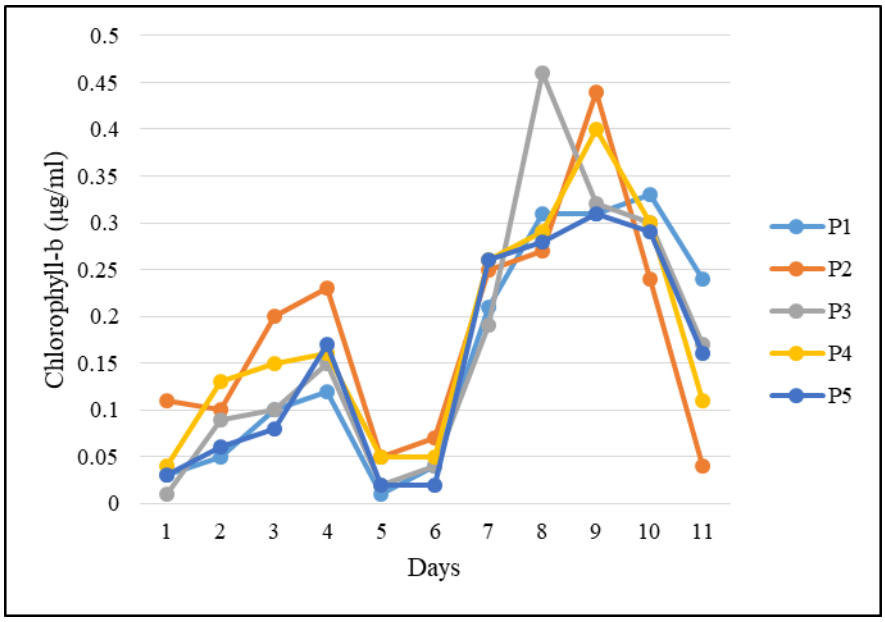

Fig. 4. Average results of chlorophyll-b of *C. vulgaris* with different N/P ratios (µg/ml)

5. Carotenoids of *C. vulgaris*

Table 6. Average results of *C. vulgaris* carotenoids with different N/P atios (µg/ml)

Day(s)	P1	P2	P3	P4	P5
0	0.06±0.02^{b}	0.03±0.00^{ab}	0.01±0.00^{a}	0.05±0.03^{b}	0.03±0.02^{ab}
1	0.05±0.03ab	0.03±0.00^{ab}	0.02±0.00^{a}	0.06±0.02^{b}	0.03±0.02^{ab}
2	0.05±0.03^{ab}	0.04±0.00^{ab}	0.02±0.00^{a}	0.07±0.02^{b}	0.03±0.02^{ab}
3	0.07±0.02^{a}	0.04±0.01^{a}	0.15±0.01^{b}	0.07±0.02^{a}	0.06±0.01^{a}
4	0.01±0.00^{abc}	0.01±0.00^{bc}	0.01±0.00^{ab}	0.01±0.00^{a}	0.02±0.00^{c}
5	0.04±0.01^{b}	0.02±0.00^{b}	0.01±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{ab}
6	0.07±0.03^{b}	0.08±0.02^{b}	0.15±0.010^{c}	0.10±0.02^{b}	0.04±0.00^{a}
7	0.09±0.02^{a}	0.09±0.02^{a}	0.16±0.02^{b}	0.09±0.03^{a}	0.06±0.02^{a}
8	0.17±0.06^{c}	0.10±0.02^{ab}	0.05±0.010^{a}	0.12±0.01^{bc}	0.08±0.02^{ab}
9	0.19±0.03^{b}	0.05±0.03^{a}	0.05±0.00^{a}	0.04±0.01^{a}	0.05±0.01^{a}
10	0.11±0.07^{b}	0.08±0.06^{ab}	0.02±0.00^{a}	0.04±0.00^{ab}	0.01±0.00^{a}

Note: Different letters indicated the significant differences between treatments.

Carotenoid values in this study are demonstrated in Table (6). The results of statistical analysis during the study showed that FML substitution enriched with $NaNO_3$ had a significantly different effect on the carotenoid content of *C. vulgaris*. The highest carotenoid value was found in treatment P1 on day 9 with a value of 0.19 ± 0.03µg/ ml.

However, this value was not different from P3 on day 7, with a value of 0.16 ± 0.02µg/ml. This showed that FML substitution enriched with NaNO3 could produce the same carotenoid value. The differences between each treatment are exhibited in Fig. (5).

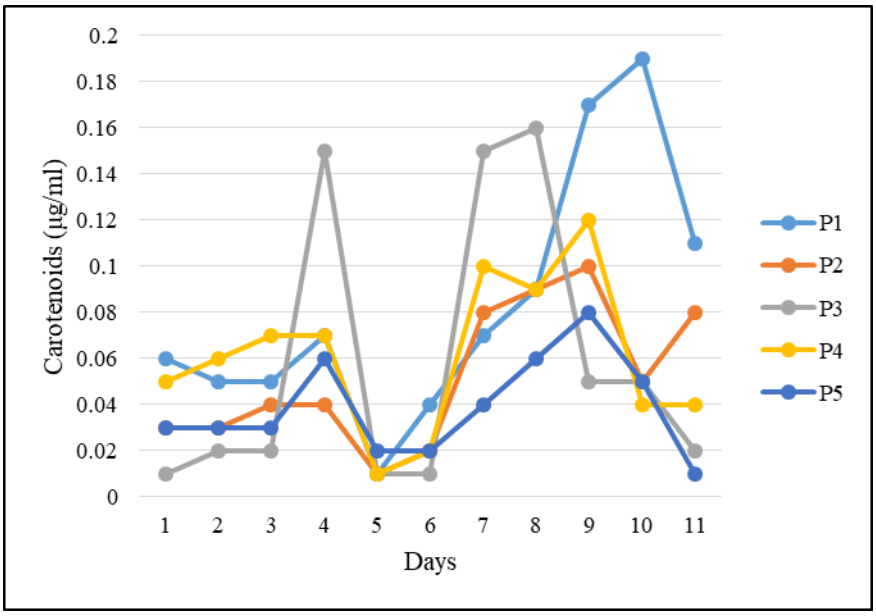

Fig. 5. Average results of *C. vulgaris* carotenoids with different N/P atios (µg/ml)

6. Water quality parameters

The results of water quality observations of *C. vulgaris* culture are illustrated in Table (7). Based on the results of observations during the study, the data obtained were water temperature of 26.6-28.7ºC; salinity ranging from 25-35ppt, pH 8.5-9, and light intensity ranging from 1,922-1,997 lux. The water quality values are within the optimal range of *C. vulgaris* culture expected in the study.

Table 7. Water quality parameters in this study

No	Parameter	Value	Standard
1	Temperature (ºC)	26.6-28.7	25-35 **(Lamadi *et al.*, 2022)**
2	Salinity (ppt)	25-35	25-35 **(Selvika *et al.*, 2016)**
3	Light intensity (Lux)	1,922-1,997	500-5,000 **(Febrieni *et al.*, 2020)**
4	pH	8.5-9	4.5-9.3 **(Deniz, 2020)**

DISCUSSION

The results of the study showed that FML enriched with $NaNO_3$ can produce better density and growth rates of *Chlorella vulgaris* than Walne fertilizer. This is influenced by the provision of optimum nitrogen and phosphorus nutrients for the growth

of *C. vulgaris*. The elements N and P are the main nutrients because they are limiting factors that are needed in large quantities for phytoplankton growth **(Prihardianto *et al*., 2023)**. An increase in P and N in the growth medium can increase photosynthetic pigments in microalgae **(Martins *et al*., 2011)**. Based on research by **Waluyo *et al*. (2016)**, nitrate is one of the important elements in the formation of chlorophyll and amino acids. The N element is absorbed by microalgae in the form of nitrate, then the nitrate is reduced to amino acids (NH_2) by the nitrate reductase enzyme, which requires energy for cellular metabolic activities. These nitrate (NO_3^-) and nitrite (NO_2^-) compounds can be absorbed directly by microalgae to meet their nutritional needs. Nitrate (NO_3^-) and nitrite (NO_2^-) can be converted into ammonia (NH_4^+) through the nitrogen assimilation process in microalgae cells. Microalgae take a form of nitrogen that is easier to use as a source of nutrition, namely in ammonia (NH_4^+) **(Prihardianto, 2023)**. Under normal conditions, ammonium nitrogen is catalyzed by glutamate dehydrogenase to produce glutamine. Glutamine will be formed by α-ketoglutarate to form amino acids **(Xiao *et al*., 2016)**. Then, these amino acids can be combined into proteins that are used for the growth and metabolism of microalgae cells **(Pozzobon *et al*., 2021)**. These amino acids are also used to duplicate themselves so that their density increases **(Liu *et al*., 2021)**. P3 with an N/P value of 15:1 showed higher density and growth rates than other treatments. The optimal N/P concentration can affect the density of *C. vulgaris* with the substitution of FML enriched with NaNO3 menu. If the concentration is too low, the absorbed amino acids cannot increase metabolism optimally **(Pozzobon *et al*., 2021)**. Conversely, too high N content can inhibit the growth of *C. vulgaris* **(Pozzobon *et al*., 2021)**. The culture media used can also affect the growth of microalgae. FML has a high nutrient content such as nitrogen (0.49g/ L), phosphorus (13.69g/ L), protein (22.98%), and amino acids (3-5%) so that it can be consumed by *C. vulgaris* for its metabolism **(Urry *et al*., 2017)**.

The results showed that FML enriched with $NaNO_3$ could produce chlorophyll-a and chlorophyll-b values of *C. vulgaris* that were comparable to those produced by Walne fertilizer. The chlorophyll-a and chlorophyll-b content of P3 (N/P 15:1) showed the same values as P1 (Walne fertilizer) on the 7th day (0.49 and 0.46µg/ ml). This is because the FML substitution enriched with $NaNO_3$ has the main nutrient element N which can meet the basic needs of *C. vulgaris* pigment biosynthesis. The main component of chlorophyll is nitrogen (N) and chlorophyll synthesis begins with the entry of basic nitrogen materials into the cells **(Fathi, 2022)**. This is because nitrogen plays a role in cell formation and as a basic material for chlorophyll production, specifically the amino acid glutamate **(Sabadel *et al*., 2022)**.

The results showed that FML enriched with $NaNO_3$ could produce carotenoid content of *C. vulgaris* that was not different from Walne fertilizer. The carotenoid content of P3 (N/P 15:1) showed the same value as P1 (Walne fertilizer) on the 7th day (0.16µg/ ml). This is thought to be because N from the media was absorbed and used for chlorophyll. The reduction in N in the media and *C. vulgaris* itself is indicated by the

onset of the death phase on the 8th day. This is in accordance with the opinion of **Kusumaningrum and Zainuri (2013)**, who stated that the production of *C. vulgaris* pigments increases in line with the increasing age of the cells and will continue to decline until reaching the cell death phase. Cell death is caused by reduced N content (N deficiency). Based on research by **Borowitzka *et al*. (1991)**, the increase in carotenoid content occurs under certain conditions due to N deficiency conditions. This shows that the N deficiency factor stimulates a rapid physiological response, which is then directed into the secondary metabolite biosynthesis pathway including carotenoids. In microalgae culture conditions with low N content, microalgae will use carbon (C) contained in cells and media for carotenoid synthesis, while cultures rich in nitrogen use carbon as a material for nitrogen assimilation. In general, N deficiency has a better effect than excess N on carotenoid content **(Moussa *et al*., 2017)**.

FML has advantages over Walne fertilizer as a medium culture for *C. vulgaris*. FML has a cheaper price because the basic material of FML is the liquid that remains after the fermentation process in MSG production **(Aprilliza *et al*., 2023)**. In addition, FML is a by-product of MSG production, and thus its use will help minimize waste in fermentation processes, as it repurposes leftover liquid that would otherwise be discarded **(Yoshimura, 2001)**. With the same performance, or even better than Walne fertilizer, FML can be a substitute option as a medium for *C. vulgaris*.

CONCLUSION

The results indicated that media substitution using FML enriched with NaNO3 could effectively replace Walne fertilizer for *C. vulgaris* culture, as it demonstrated superior values. The optimal N/P ratio for the FML-enriched NaNO3 culture medium, in terms of chlorophyll-a, chlorophyll-b, and carotenoid content of *C. vulgaris* on a laboratory scale, was found to be 15:1. This condition yielded a population density of 10.92 x 10^6 cells/ml on day 7, a growth rate of 25 x 10^6 cells/ml on day 3, chlorophyll-a content of 0.49µg/ ml, chlorophyll-b content of 0.46µg/ ml (both on day 7), and carotenoids at 0.16µg/ ml (day 7).

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REFERENCES

Ahmad, M. T.; Shariff, M.; Yusoff, F. M.; Goh, Y. M. and Banerjee, S. (2020). Applications of microalga *Chlorella vulgaris* in aquaculture. Rev. Aquac., 12(1): 328–346.

Aprilliza, M.N.; Krishna, N.H. and Pamungkas, D. (2023). Feed Durability and feed quality of concentrate feed with addition of monosodium glutamate manufacturing by-products. IOP Conf. Ser. Earth Environ. Sci., 1177(1): 012053.

Aulia, A.E.; Maimunah, Y. and Suprastyani, H. (2021). Penggunaan ekstrak daun lamtoro (*Leucaena leucocephala*) sebagai pupuk dengan salinitas yang berbeda terhadap laju pertumbuhan, biomassa dan klorofil-a pada mikroalga *Chlorella vulgaris* [The use of lamtoro leaf extract (*Leucaena leucocephala*) as fertilizer with different salinity on growth rate, biomass and chlorophyll-a in microalgae *Chlorella vulgaris*]. J. Fish. Mar. Res., 5(1): 47–55.

Bahar, H.S.; Djunaedi, A. and Widianingsih. (2022). Perbedaan lama fotoperiode terhadap total lipid kultur mikroalga *Chlorella vulgaris* [Differences in photoperiod length on total lipids of *Chlorella vulgaris* microalgae culture]. J. Mar. Res., 11(1): 92–97.

Borowitzka, M.A.; Huisman, J.M. and Osborn, A. (1991). Culture of the astaxanthin-producing green alga *Haematococcus pluvialis* 1. Effects of nutrients on growth and cell type. J. Appl. Phycol., 3(4): 295–304.

Brautovic, I. (2000). The influence of light intensity on growth of the marine planktonic alga *Chlorella* sp. under laboratory conditions. Institute of Oceanography and Fisheries Dubrovnik Croatia, Dubrovnik, pp. 322.

Deniz, I. (2020). Determination of growth conditions for *Chlorella vulgaris*. Mar. Sci. Technol. Bull., 9(2): 114–117.

Fathi, A. (2022). Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: a. Agrisost, 28: 1–8.

Febrieni, V.N.; Sedjati, S. and Yudiati, E. (2020). Optimization of light intensity on growth rate and total lipid content of *Chlorella vulgaris*. IOP Conf. Ser. Earth Environ. Sci., 584(1): 012040.

Jwaideh, M.A.; Sutanudjaja, E.H. and Dalin, C. (2022). Global impacts of nitrogen and phosphorus fertiliser use for major crops on aquatic biodiversity. Int. J. Life Cycle Assess., 27(8): 1058–1080.

Hidayati, N.A.; Anggraini, I.D.; Marno, S.; Fikri, R.A.; Lathifah, W.; Putri, N.; Fitriani, I.N.; Prabowo, B. and Fitriani, R. (2022). Development of fertilizer-based medium for the growth rate improvement of *Chlorella* sp. in the laboratory scale. IOP Conf. Ser. Earth Environ. Sci., 1034(1): 012021.

Hidayati, P.A.; Mubarak, A.S. and Sudarno (2020). The optimal n/p ratio of shrimp culture waste liquid fertilizer on growth of *Chlorella vulgaris*. IOP Conf. Ser. Earth Environ. Sci., 441(1): 012090.

Kananurak, P. (1987). Effects of fermented Mother Liquid (FML) Supplementation to Growing and Finishing Swine Ration. Agric. Nat. Resour., 21(3): 263–270.

Kilroy, C.; Brown, L.; Carlin, L.; Lambert, P.; Sinton, A., Wech, J.A., and Howard-Williams, C. (2020). Nitrogen stimulation of periphyton biomass in rivers: Differential effects of ammonium-N and nitrate-N. Freshw. Sci., 39(3): 485–496.

Kumari, K.; Samantaray, S.; Sahoo, D. and Tripathy, B.C. (2021). Nitrogen, phosphorus and high CO 2 modulate photosynthesis, biomass and lipid production in the green alga *Chlorella vulgaris*. Photosynth. Res., 148, 17–32.

Kurniawan, S.A.; Budiati, T. and Rahman, D. Y. (2023). Kultivasi mikroalga *Galdieria* sp. dalam *limbah gula (molases)* [Microalgae cultivation of *Galdieria* sp. in sugar waste (molasses)]. J. Food Eng., 2(1): 12–18.

Kusumaningrum, H.P. and Zainuri, M. (2013). Aplikasi pakan alami kaya karotenoid untuk post larva *Penaeus Monodon* Fab [Application of natural feed rich in carotenoids for *Penaeus monodon* Fab. post larvae]. Ilmu Kelaut., 18(3): 143–149.

Martins, A.P.; Junior, O.N.; Colepicolo, P., and Yokoya, N.S. (2011). Effects of nitrate and phosphate availabilities on growth, photosynthesis, and pigment and protein contents in colour strains of *Hypnea musciformis* (Wulfen in Jacqu.) J.V. Lamour. (Gigartinales, Rhodophyta). Braz. J. Pharmacogn., 21(2): 340–348.

Lamadi, A.; Mulis and Kristanto, A. E. (2022). The growth of *Chlorella* sp. cultivated in walne media with different intensities of light. Tomini J. Aquat. Sci., 3(1): 1–7.

Lichtenthaler, H.K. and Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr. Protoc. Food Anal. Chem., 1(1): F4.3.1–F4.3.8.

Liu, X.; Wang, M.; Zhang, J.; Wei, L. and Cheng, H. (2021). Immobilization altering the growth behavior, ammonium uptake and amino acid synthesis of *Chlorella vulgaris* at different concentrations of carbon and nitrogen. Bioresour. Technol., 320: 124438.

Moussa, I.D.B.; Chtourou, H.; Karray, F.; Sayadi, S. and Dhouib, A. (2017). Nitrogen or phosphorus repletion strategies for enhancing lipid or carotenoid production from *Tetraselmis marina*. Bioresour. Technol., 238: 325–332.

Mtaki, K.; Kyewalyanga, M.S. and Mtolera, M.S. (2021). Supplementing wastewater with NPK fertilizer as a cheap source of nutrients in cultivating live food (*Chlorella vulgaris*). Ann. Microbiol., 71: 1–13.

Nikhilesh, B.; Sachin, Z.A.; Vishal, T. and Dipesh, J. (2013). A. Review : Steam sterilization a method of sterilization. J. Biol. Sci. Opin., 1(2): 138–141.

Paes, C.R.P.S.; Faria. G.R.; Tinoco, N.A.B.; Castro, D.J.F.A.; Barbarino, E. and Lourenço, S.O. (2016). Growth, nutrient uptake and chemical composition of *Chlorella* sp. and *Nannochloropsis oculata* under nitrogen starvation. Lat Am J. Aquat. Res., 44(2): 275–292.

Pisal, D.S. and Lele, S.S. (2005). Carotenoid production from microalga, *Dunaliella salina*. Indian J. Biotechnol., 4: 476–483.

Pozzobon, V., Cui, N., Moreaud, A., Michiels, E., and Levasseur, W. (2021). Nitrate and nitrite as mixed source of nitrogen for *Chlorella vulgaris*: Growth, nitrogen uptake and pigment contents. Bioresour. Technol., 330: 124995.

Prihardianto, M.K.; Chilmawati, D. Subandiyono. (2023). Pola pertumbuhan *Thalassiosira* Sp. pada media Walne dengan rasio N/P berbeda [Growth patterns *Thalassiosira* sp. on Walne media with different N/P ratio]. Sains Akuakult. Trop., 7(2): 196–206.

Putri, D.S. and Alaa, S. (2019). The growth comparison of *Haematococcus pluvialis* in two different medium. Biota Biol. Pend. Biol., 12(2): 90–97.

Sabadel, A.J.M.; Décima, M.; McComb, K., Meyers, M., Barr, N., Gall, M., Safi, K., and Law, C. S. (2022). Amino acid nitrogen stable isotopes as biomarkers of coastal phytoplankton assemblages and food web interactions. Mar. Ecol. Prog. Ser., 690: 1–13.

Santanumurti, M.B.; Puspita, D. and Hudaidah, S. (2023). Performance analysis of *Brachionus plicatilis* production with different densities. IOP Conf. Ser. Earth Environ. Sci., 1273(1): 012031.

Selvika, Z.; Kusuma, A.B.; Herliany, N.E. and Negara, B.F. (2016). The growth rate of the *Chlorella* sp. at different concentrations of coal waste water. Depik, 5(3): 107–112.

Thirugnanasambantham, R.; Elango, T. and Elangovan, K. (2020). *Chlorella vulgaris* sp. microalgae as a feedstock for biofuel. Mater. Today Proc., 33: 3182–3185.

Trinh, K.S. (2023). Comparison of growth of *Chlorella vulgaris* in Flat-plate photobioreactor using batch, fed-batch, and repeated fed-batch techniques with various concentrations of walne medium. J. Tech. Edu. Sc., 18(6): 9–15.

Urry, L.A.; Cain. M.L.; Wasserman, S.A.; Minorsky, P.V. and Reece, J.B. (2017). Campbell Biology, eleven ed. Pearson, New York, 1482pp.

Waluyo, W.W.S.; Suharti, S. and Abdullah, L. (2016). Metode cepat pendugaan kandungan protein kasar pada rumput raja (*Pennisteum purpurhoides*) menggunakan nilai indeks warna daun [Rapid method for estimating crude protein content in king grass (*Pennisteum purpurhoides*) using leaf color index values]. Pastura, 5(2): 78–82.

Wang, X.; Zhao, Y.; Zhao, L.; Wan, Q.; Ma, L.; Liang, J.; Li, H.; Dong, J. and Zhang, M. (2023). Effects of microplastics on the growth, photosynthetic efficiency and nutrient composition in freshwater algae *Chlorella vulgaris* Beij. Aquat. Toxicol., 261: 106615.

Wiryadi, F. and Witono, J.R.B. (2018). Pengaruh aerasi dan penambahan nitrogen terhadap laju pertumbuhan *Nannochloropsis* sp. [The effect of aeration and nitrogen addition on the growth rate of *Nannochloropsis* sp.]. Sem. Nas. Tek. Kim. Kejuang., 2018: 1–6.

Xiao, D.; Zeng, L.; Yao, K.; Kong, X.; Wu, G. and Yin, Y. (2016). The glutamine-alpha-ketoglutarate (AKG) metabolism and its nutritional implications. Amino Acids, 48: 2067–2080.

Yoshimura, M. (2001). Environmental conservation in the fermentation industry. In: "Organisation for Economic Co-Operation and Development, International science and technology co-operation towards sustainable development: Towards sustainable development". Organization for Economic Co-operation and Deveopment. OECD Proceeding, Paris, pp. 335-342.

Yulanda, N.; Hidajati, N.; Achmad, A.B. and Chrismanto, D. (2021). The Effect of molasses addition on physical and chemical quality of corn plant silage given fermented mother liquor. J. Appl. Vet. Sci. Technol., 2(1): 10–14.