Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 1199 – 208 (2024)
www.ejabf.journals.ekb.eg

Laboratory Scale *Nannochloropsis* culture using NaOHpasta of *Nannochloropsis* sp.

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ARTICLE INFO
Article History:
Received: Sept. 2, 2024
Accepted: Sept. 25, 2024
Online: Sep. 29, 2024

Keywords:
Growth,
Nannochlorpsis,
pasta,
NaOH

ABSTRACT

*Nannochloropsis*sp is used as natural feed since it contains high good nutrition for the growth of fish larvae. However, the availability of *Nannochloropsis* sp. is still limited and has become a constraint in aquaculture. This study aimed to address the effect of pasta on the density of *Nannochloropsis* sp. cultured on the laboratory scale from pasta product of massive culture using the combination of agricultural fertilizer and water extract of *Moringaoleifera* leaf. *Nannochloropsis* sp. culture of the laboratory scale used the kw21 fertilizer. The *Nannochloropsis* sp. seeds were taken from *Nannocloropsis* sp. pasta with different concentrations, while the control *Nannocloropsis*sp seeds used the laboratory scale without pasta. The study applied a complete randomized design with 4 treatments (NaOH pasta of 75 ppm, 100 ppm, 125 ppm, and control) and 3 replications. The control treatment (D) used the *Nannochloropsis* sp. of the laboratory scale without pasta. Data analysis utilized the one way-ANOVA, followed by the least significant difference test upon detecting a significant difference. Results indicated the significant effect of the NaOH pasta on *Nannochloropsis* sp. density.

INTRODUCTION

One of the highly important aspects in marine fish hatchery is the availability of natural feed. It is one of the limiting factors for fish and shrimp larvae **(Soto-Sanchez *et al*., 2023; Zahran*et al*., 2023)**. Fish oil has been substituted with *Nannochloropsis* sp. meal as feed for shrimps **(Adissin*et al*., 2010)**. Feed availability is highly dependent upon the amount, type, and feeding time. *Nannochloropsis* sp. is one of phytoplankton, but its massive and continuous production is inhibited by uncontrollable factors, such as high rainfalls that cause water quality changes in culture media, the mortality of *Nannochloropsis* sp., and eventually failures in seed production. The production of *Nannochloropsis* sp. pasta is expected to be a solution to the continuity of seed production.

Nannochloropsis sp. belongs to a unicellular and non-motile marine microalga of phylum Heterokontophyta, class Eustigmatophyceae, and family Eustigmataceae **(Vieler, 2012; Max *et al*., 2016)**. *Nannochloropsis* sp. has cells coated in a unique cell membrane structure containing complex polysaccharides to keep the cells in stable conditions. The

internal cell membrane of *Nannochloropsis* has porous structures with fine-fibered sub-structures and supporting structures connecting this layer to the plasmic membrane **(Scholz, 2014)**.

Nannochloropsis sp. is known to contain eicosapentaenoic acid (EPA) and valuable carotenoid **(Ye *et al.*, 2024)**, high lipid, and ability to grow in diverse environmental conditions. These characteristics make *Nannochloropsis* sp. one of the microalgae producing oil, fatty acid of omega-3, and many high valuable compounds **(Ma *et al.*, 2016)**. Besides, it contains violaxanthin (32% of total carotenoid) and naturally produces vitamin D3, generally found in other microalgae potential as cosmetic material **(Ljubic *et al.*, 2020)**. Similar results were also reported in previous studies **(Khattib *et al.*, 2012; Kim *et al.*, 2019; Ljubic *et al.*, 2020)**.

Nannochloropsis sp. was determined as feed for the Pacific white shrimps, Atlantic salmon, kuruma shrimps, and the European marine bass **(Soto-Sanchez, 2023; Zahran, 2023)**. *Nannochloropsis* sp. meal was used as a meal replacing the fish oil to increase the growth of the aforementioned species **(Lafarga, 2019)**.Through various forms of natural food engineering, either diversification, or natural feed quantity and quality, the massive production of phytoplankton or zooplankton is expected to be able to sustainably meet the food need of the larvae. The phytoplankton culture, especially *Nannochloropsis* sp. has well developed in seeding activities at laboratory, semi-massive, and massive scales.

Kokarkin and Kusnendar (2000) found a practical way to precipitate the microalgal biomass into a solid form as rotifer's natural feed. This is performed through adding NaOH to the culture media to increase the water pH **(Kokarkin & Kusnendar, 1999)**. In high pH conditions, the *Nannochloropsis* sp. cells can adhere and settle. *Nannochloropsis* sp. production in a solid form was carried out by **Muliono (2004)** using 105ppm of NaOHand, yielding a density of 10,000,000 *Nannochloropis* sp. cells, which is a sufficiently good result for *Nannochloropsis* sp. pasta. **Setiawan *et al.* (2019)** who used 100 ppm of NaOH yielded a density of 18,066,666.67 x 10^4cells mL^{-1}. This study aimed to detect the effect of pasta at different NaOHdose applications in the mass culture to be used on the laboratory scale culture. *Nannochloropsis* sp. culture on the laboratory scale was done by adding kw21 fertilizer. The application of 75ppm, 100ppm, and 125ppm pasta is expected to be able to obtain the best dose for durable initial seeds on the laboratory scale culture.

MATERIALS AND METHODS

This study extended from March to July 2024 in Natural Feed Laboratory, State Fisheries Polytechnique, Tual, Southeast Molucca.

1. Equipment and materials

The equipment used in the study were one-litre glass container, 2cm and 0.5cm-diameter plastic tubes, microscope, pipette, flask, digital balance, cover glass, haemocytometer, plastic spoon, satin cloth, digital camera, among others. The study utilized *Nannochloropsis* sp., KW21 fertilizer, moringa leaf, NaOH, distilled water, sterile seawater, alkcohol 70%, freshwater, and chlorine.

2. Pasta production

NaOH dose was dissolved in freshwater, then evenly dropped into a gallon containing 15 liters of *Nannochloropsis* sp., stirred for approximately half an hour, and strongly aerated for 2 hours. The aeration was then terminated for 24h to gradually separate water and *Nannochloropsis* sp. The sediment of *Nannochloropsis* sp. was extracted in water step by step, rinsed three times with clean water, and filtered through cloth, then rinsed again 3 times with clean water before being re-precipitated to extract the final NaOH and obtain the pasta, with pH and salinity being measured. The precipitation of the microalgae was done by adding NaOH into the culture media to increase the water pH **(Kokarkin & Kusnendar, 1999)**. In high pH condition, *Nannochloropsis* sp. cells can adhere and settle.

3. Research design

This study used a complete randomized design. *Nannochloropsis* sp. seeds were taken from mass culture, and then cultured on laboratory scale. The experiment used 4 treatments and 3 replications, as follows: A= 75ppmNaOH; B= 100ppmNaOH; C= 125ppmNaOH, and D= control (*Nannochloropsis* sp. without pasta). Each glass container was filled with seawater with a salinity value of 25ppm. 0.5g of *Nannochloropsis* sp. pasta was taken from each treatment (75, 100, and 175ppm), dissolved in 1mL pure seawater, put into a glass container filled with 1000ml sterile seawater, and adding 1ml of kw21 fertilizer. For control treatment, 300ml of *Nannochloropsis* sp. seeds were added with 700ml sterile seawater. The initial density of each treatment and the cell growth were daily determined under a microscope facilitated with a haemocytometer. *Nannochloropsis* sp. culture in the control media used 100% Kw21 fertilizer as positive control. The 1000ml glass container filled with initial *Nannochloropsis* sp. seeds was placed in the culture cupboard facilitated with 2 units of Philip TL 40 watt as a light source and aerated for oxygen supply. *Nannochloropsis* sp. needs a light intensity of 2500 –5000 lux. The culture media was then covered to prevent contamination. The culture was done for 20 days to attain *Nannochloropsis* sp. able to adapt to the new environment. Water quality measurements focused on temperature, salinity and pH in each observation. *Nannochloropsis* sp. growth estimation followed the details reported in the study of **Isnantsetyo and Kurniastuty (1995)**:

Cell density (cells mL^{-1}) = $n \times 4 \times 10^6$

Where, n = number of conuted cells and 4×10^6 = heamocytometer constant

4. Data analysis

One-way ANOVA was used to know the effect of *Nannochloropsis* sp. pasta application on the density of *Nannochloropsis* sp. cells, and the least significant difference test was applied if a significant effect was detected.

RESULTS AND DISCUSSION

ANOVA indicated that there is no significantly different effect of NaOH application on the cell density of *Nannochloropsis* sp. cultured on the laboratory scale. Nevertheless, an increased abundance of *Nannochloropsis* sp. cell density was observed in each treatment. The highest growth in the exponential phase occurred in treatment B with a density of 61.97 x 10^6±2561,17cellsmL^{-1}. This high growth could result from the appropriate NaOH dose application and *Nannochlorpsis* sp. tolerance so that the nutrient could be well absorbed to push the increased density of *Nannochloropsis* sp., even though the cell density development

ran slowly in this phase since *Nannochloropsis* sp. tends to adapt at the initial phase compared with treatments A and C. The control treatment (D) showed a clear color since *Nannochloropsis* sp. starts adapting to the phase slowliness and grew slowly due to the environmental adaptation**(Isnansetyo & Kurniastuty, 1995)** (Table 1). This finding coincides with that of **Ma (2016)**, who elucidated that the growth of *Nannochloropsis* sp. is highly dependent on the culture media conditions, nutrient absorption, photosynthesis, and the quality of light intensity.

The application of NaOH in each treatment showed that in day-1 till day-10, treatment A yielded a significantly different cell density from treatments B, C, and D, but in day-13 till day-18, treatment D had higher effect than that of treatments A, C, and D (Fig. 1.)

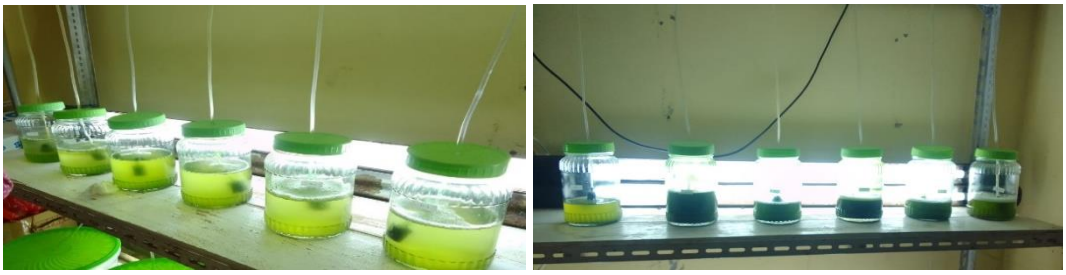

Fig. 1. *Nannochloropsis* sp. culture at laboratory scale.

The harvest of the microalga culture obtained the fresh microalga biomass in the form of *Nannochloropsis* sp. pasta as the following characteristics: green color, no odor, and solid. The pasta was then dried, ground, and collected as *Nannochloropsis* sp. The microalgae grown in KW21 medium had good nutrient for growth. KW21 is a culture medium enriched with nutrients needed by the microalga, such as micronutrients, macronutrients, and vitamins. The microalgae growth is indicated with color change of the culture media to be strongly green up to day-10. It reflects the increased number of the macroalgal cells from 10^6cells $mL^{-1}$to 39.13x10^6 cells mL^{-1}in day-10.

Nannochloropsis sp. is round-shaped, green-colored, not flagellated, and 4– 6μm sized. Fig. (1) demonstrates that there are 4 phases in the culture of *Nannochloropsis oculata*, adaptation, growth, stationary & mortality. Natural feed has become a major factor determining the success of the rotifer seeding activities due to its higher nutrient content compared to those of the artificial feed **(*Astuti et al.*, 2012)**.

The availability of natural feed in a sufficient amount, sustainable, and the right time should be considered. To be able to meet the production target, the phytoplankton culture needs to be done. One of these is *Nannochloropsis oculata* applied for marine fish seeding. *Nannochloropsis* sp. is cultured for *Brachionus plicatilis* feed. *Nannochloropsis* sp. has good vitamin B12 content for the growth of *B. plicalitis*, eicosapentaenoic acid (EPA) of 30.5%, which plays a crucial role as feed for the fish larvae in addition to 42.7% of omega3 HUFAs, 0.89%chlorophyl A, 16% carbohydrate, 0.85% vitamin C and 52.11% protein **(Sen *et al.*, 2005)**. *Nannochloropsis* sp. growth in the culture media can be evidenced through the increased cell size or the increased number of cells. The cell density in *Nannochloropsis* sp.

culture is used to assess the growth patterns of the phytoplankton. Based on the growth pattern, the harvest is done at the time the phytoplankton reaches the population peak.

Table 1. *Nannochloropsis* sp. cell density condition during the study

Day	*Nannocloropsis* sp.density (cells mL^{-1})			
	TreatmentA	TreatmentB	TreatmentC	TreatmentD
0	5.48 x 10^6±24,30	5,48 x 10^6±46,22	5,48 x 10^6±15,28	5,48 x 10^6±109,73
1	9.37 x10^6±137,62	7,30 x 10^6±176,38	7,07 x 10^6±92,07	8,55 x 10^6±154,32
2	5.22 x10^6±80,90	6,69 x 10^6±144,01	6,96 x 10^6±105,86	6,53 x 10^6±82,98
3	6,57 x10^6±161,66	5,74 x 10^6±64,55	5,85 x 10^6±120,46	6,53 x 10^6±82,98
4	4,54 x10^6±46,32	5,07 x 10^6±11,54	5,95 x 10^6±80,01	1,19 x 10^6±379,34
5	6,74 x10^6±33,51	6,10 x 10^6±36,56	5,88 x 10^6±71,12	4,89 x 10^6±28,74
6	5,54 x10^6±41,31	4,97 x 10^6±20,28	8,84 x 10^6±84,74	7,21 x 10^6±97,15
7	5,25 x10^6±492,45	7,41 x 10^6±127,72	7,33 x 10^6±140,55	7,67 x 10^6±110,20
8	6,43 x10^6±119,80	6,65 x 10^6±60,02	9,05 x 10^6±214,91	8,28 x 10^6±40,02
9	7,23 x10^6±61,79	6,69 x 10^6±24,29	9,13 x 10^6±156,21	8,42 x 10^6±144,05
10	9,46 x10^6±39,49	7,92 x 10^6±39,20	8,59 x 10^6±32,76	7,19 x 10^6±13,97
11	8,07 x10^6±59,54	8,12 x 10^6±55,00	8,17 x 10^6±31,80	0
12	9,27 x10^6±17,97	9,78 x 10^6±62,20	9,00 x 10^6±62,20	0
13	33,91 x10^6±1785,44	42,24 x 10^6±2373,05	17,50 x 10^6 x 10^6±419,13	0
14	33,99 x10^6±1862,32	43,40 x 10^6±2370,91	18,57 x 10^6±328,30	0
15	42,69 x10^6±2397,58	56,25 x 10^6±2954,62	39,00±253,92	0
16	42,81 x10^6±2328,72	33,78 x 10^6±784,86	33,78 x 10^6±784,86	0
17	45,44 x10^6±2965,88	61,97 x 10^6±2561,17	30,51 x 10^6±467,59	0
18	51,02 x10^6±2566,79	61,49 x 10^6±2474,91	31,52 x 10^6±189,17	0
19	42,82 x10^6±4674,17	52,98 x 10^6 ±248 x $10^6$8,95	30,51 x 10^6±1543,88	0
20	22,82 x10^6±4674,17	24,98±2488,95	18,51 x 10^6±1543,88	0

Table (1) shows that the mean cell density of *Naanochloropsis* sp. in day 0 is 5.48 x 10^6±24.30 in all treatments. The growth of *Nannochloropsis* sp. fluctuates in each treatment. The cell density is associated with the growth phase, namely lag phase (adaptationor dormant), exponential phase, declination phase, stationary phase, and mortality phase **(Becker, 1994)**.

Based on Table (1), treatment A (75ppm NaOH) yields a cell density fluctuation on day 1 to day 8, and the cells tend to be in an adaptation phase, but the cell density starts increasing on day 9 from 7.23 x10^6±61.79^b to 9.46 x10^6±39.49^a on day 10, and the cell division rises but not so significant then falls down on days 11 & 12. While, on day 13 till day18, the cell density of *Nannochlorpsis* sp. increases significantly from 33.91 x10^6±1785.44^a to 51,02 x10^6±2566,79^a, then starts falling down on day 19 and then dies on day 20.

Treatment B (100 ppm NaOH) showed no signs of growth yet on day 1 till day 9, because the cell is still in the adaptation process. The cell density of *Nannochlorpsis* sp. begins to rise slowly on day 10 from7.92 x 10^6±39.20 to 42.24 x 10^6±2373.05 on day 13 and reaches the peak on day 17, 61.97 x 10^6±2561.17. A slow decrease was noted in the cell density on day18,61.49 x 10^6±2474.91 to 24.98±2488.95 on day 20, and then the cells die. In treatment C (125ppm NaOH), the adaptation phase occurs on day 1. The cell density development fluctuates up to day 7. On the other hand, the cell density grows on day 8 till

day 9 reaching 9.13 x 10^6±156.21, while decreasing again on day 10. In this phase, the cells are still in the adaptation phase to the new environment. On day 13, the cell density of *Nannochlorpsis* sp. starts rising to 17.50 x 10^6 x 10^6±419.13 and reaches the peak ON day 16 as many as 33.78 x 10^6±784.86, then starts decreasing ON day 17 till day 20, leading to the mortality phase. In treatment D, the cell density slightly rises, the growth fluctuates, recording no stability. It can be deduced that the high NaOH application can damage *Nannohloropsis* sp. cells so that they cannot grow well compared with other treatments of A and B using low NaOH dose, which facilitated the way for *Nannochloropsis* sp. to grow and to have cell division.

Treatment D didn't recieved NaOH, and *Nannochloropsis* sp. was cultured on the laboratory scale using KW21 fertilizer. The cell density rises on day 2 till day 5, then decreases in day 6 and leads to gradual mortality up to day 11. Based on these treatments, the highest growth occurs in treatment B, which could result from the nutrient and water quality of the culture media supporting the growth and cell division of *Nannochloropsis* sp. **Garno (1995)** stated that the dissolved nutrients in the water is directly used by *Nannochloropsis* sp., and subsequently increasing the population abundance. According to **Yani *et al*. (2015)**, the use of different NaOH concentrations can influence the growth of *Nannochloropsis* sp. cells.

Water quality

Measurements of water quality parameters are presented in Table (2). Temperature is one of the important parameters influencing the phytoplankton growth. In the present study, the temperature change of the culture media is affected by the light intensity. The optimum temperature for *Nannochloropsis* sp. ranges from 25 to 30°C **(Rustam & Amini, 2015)**. The present study indicated that all water quality parameters measured were in the good range for *Nannochloropsis* sp. This finding has been supported by **Marthia (2020)** and **Isnansetyo and Kurniastuty (1995)**.

Table 2. Mean water quality measurements during the study

Treatment	Parameter		
	Salinity (‰)	**Temperature (°C)**	**pH**
A	25-29	27-32	7.5 – 8.3
B	28-30	27-32	7.5 – 8..
C	28-31	27-32	7.5 - 8.4
D (Kontrol)	29-31	27-32	7.5 – 8.5

CONCLUSION

The effective dose of *Nannochloropsis* sp. pasta production was 100ppm, with the highest growth in treatment B, yielding the cell density of 61.97 x 10^6±2561.17^{a}cells mL^{-1}.

ACKNOWLEDGEMENT

We would greatly appreciate the Ministry of Education, Culture, Research, and Technology, Directorate General of Indonesian Vocational Education, who provide our research team with the Batch I and II Vocation grant of 2024.We also thank the State Fisheries Poly-technique, Tual, for supporting the researcher's activities and all the others who assisted us in this study.

REFERENCES

Afifah, A.S.; Suryawan, I.W.K.; Apritama, M.R.; Prajati, G. and Adicita, Y. (2019). Kinetics of organic and nutrient degradation with microalgae biomass cultured in photobioreactors. 2nd International Conference on Applied Engineering (ICAE) (pp. 1–4). IEEE.

Adissin, T.O.O.; Manabu, I.; Shunsuke, K.; Saichiro, Y.; Moss, A.S. and Dossou, S. (2020)**.** Effects of dietary *Nannochloropsis* sp. powder and lipids on the growth performance and fatty acid composition of larval and postlarvalkuruma shrimp, Marsupenaeusjaponicus. Aquac. Nutr. 26 (1):186–200. http://doi.org/10.1111/anu.12980

Astuti, R.P.; Sagala, S.L.; Gunawan, G. S.; Sumiarsa and Imanto, P.T. (2012). Optimization of feed dosage and frequency in the production of Rotifera (Brachionus rotundiformis). Journal of Tropical Marine Science and Technology. 4(2), 239-246. In Indonesia

Batista, A.P.; Niccolai, A.; Fradinho, P.; Fragoso, S.; Bursic, I.; Rodolfi, L. and Raymundo, A.(2017). Microalgae biomass as an alternative ingredient in cookies: Sensory, physicaland chemical properties, antioxidant activity and in vitro digestibility. Algal research, 26: 161–171.http://doi.org/10.1016/j.algal.2017.07.017

Becker E.W. (1994). Microalgae: Biotechnology and Microbiology. Penerbit: Cambridge University Press, ISBN: 0521350204, 9780521350204. 293 page.

Caporgno, M.P.; Taleb, A.; Olkiewicz, M.; Font, J.; Pruvost, J.; Legrand, J. and Bengoa, C. (2015). Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. Algal Research, 10:232–239.https://doi.org/10.1016/j.algal.2015.05.011

Delgadillo-Mirquez, L.; Lopes, F.; Taidi, B. and Pareau, D. (2016). Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. Biotechnology Reports, 11:18–26.https://doi.org/10.1016/j.btre.2016.04.003

Fithriani, D.; Amini, S.; Melanie, S. and Susilowati, R. (2015). Phytochemistry of totalphenol and antioxidant activity of microalga *Spirulina* sp., *Chlorella* sp., and *Nannochloropsis* sp. Jurnal Pascapanendan Bioteknologi Kelautandan Perikanan, 10(2): 101- 109.https://doi.org/10.15578/jpbkp.v10i2.222 [in Indoensian]

Fukuda, S.Y.; Iwamoto, K.; Atsumi, M.; Yokoyama, A.; Nakayama, T.; Ishida, K. I. and Shiraiwa, Y. (2014). Global searches for microalgae and aquatic plants that can

eliminate radioactive cesium, iodine and strontium from the radio-polluted aquatic environment: A bioremediation strategy. Journal of plant research 127(1): 79–89. https://doi.org/10.1007/s10265-013-0596-9

Garno, Y. S. (1995). Zooplankton exceretion in different feed and Temperature. Proceedings Workshop on Technologi application on Marine Environmental Monitoring, Forcasting and information System. DTL-BPPT, pp.133-139

Hammadi, N. S.; Ankush, M.A.T. ; Taher, M.M.; Al-Dubakel, A.Y. and Muhammed, S.J. (2024). Impact of Phytoplankton on the Growth of Common Carp Cyprinuscarpio L. Larvae. Egyptian Journal of Aquatic Biology & Fisheries. 28(2): 1101 – 1118

Hanif, M. (2016). Designation of macroalgae conversionto biofuel as environmental friendly technological innovation.Jurnal Teknologi Lingkungan, 16(1): 1–8.https://doi.org/10.29122/jtl.v16i1.1605 [in Indonesian]

Hanifzadeh, M.M.; Sarrafzadeh, M.H. and Tavakoli, O. (2012). Carbon dioxide biofixation and biomass production from flue gas of power plant using microalgae. Second Iranian Conference on Renewable Energy and Distributed Generation (pp. 61–64). https://doi.org/10.1109/ICREDG.2012.6190469

Kim H.M.; Jung J.H.; Kim J.Y.; Heo J.; Cho D.H.; Kim H.S.; An, S.; An, I.S. and Bae, S. (2019). The Protective Effect of Violaxanthin from *Nannochloropsisoceanica* against Ultraviolet B-Induced Damage in Normal Human Dermal Fibroblasts. Photochem. Photobiol.95:595–604. https://doi.org/10.1111/php.13030.

Lafarga, T.; Mayre, E.; Echeverria, G.; Vinas, I.; Villaro, S.; Gabriel, A.F.F.; Castellari, M. and Aguilo, A.I. (2019) Potential of the microalgae *Nannochloropsis* and Tetraselmis for being used as innovative ingredients in baked goods. Lwt-Food Sci. Technol. 115:108439. https://doi.org/10.1016/j.lwt.2019.108439

Ljubic, A.; Jacobsen, C.; Holdt, S.L. and Jakobsen J. (2020).Microalgae *Nannochloropsisoceanica* as a future new natural source of vitamin D-3. Food Chem. 320:126627. https://doi.org/10.1016/j.foodchem.2020.126627

Li, Y.; Huang, A.; Gu, W.; Wu, S.; Xie, X. and Wang, G. (2020). Effects of inorganic carbon and light on acetate assimilation by *Nannochloropsisoceanica* (Eustigmatophyceae) in mixotrophic cultivation. Eur. J. Phycol. 55:64–75. https://doi.org/10.1080/09670262.2019.1660808

Ma, X.; Chen, T.; Yang, B.; Liu, J. and Chen, F. (2016). Lipid production from *Nannochloropsis*. Mar. Drugs.14 (4):61. https://doi.org/10.3390/md14040061.

Marthia, N. (2020). PengaruhJenis Media KulturTerhadapKonsentrasiBiomassa*Nannochloropsis* sp. Pasundan Food Technology Journal (PFTJ), 7(3), 97-101.https://doi.org/10.23969/pftj.v7i3.3190

Matamoros, V.; Gutiérrez, R.; Ferrer, I.; García, J. and Bayona, J.M. (2015). Capability of microalgae-based wastewater treatment systems to remove emerging organic

contaminants: a pilot-scale study. Journal of Hazardous Materials, 288, pp. 34–42.https://doi.org/10.1016/j.jhazmat.2015.02.002

Mujtaba, G.; Rizwan, M. and Lee, K. (2017). Removal of nutrients and COD from wastewater using symbiotic co-culture of bacterium Pseudomonas putida and immobilized microalga Chlorella vulgaris. Journal of Industrial and Engineering Chemistry, 49:145–151.https://doi.org/10.1016/j.jiec.2017.01.021

Kumar, K.S.; Dahms, H.U.; Won, E.J.; Lee, J.S. and Shin, K.H. (2015). Microalgae–A promising tool for heavy metal remediation. Ecotoxicology and Environmental Safety ,113: 329–352.https://doi.org/10.1016/j.ecoenv.2014.12.019

Lorenzo, Z. and Fabio, V. (2020). Microalgae of the genus *Nannochloropsis*: Chemical composition and functional implications for human nutrition. Journal of Functional Foods. 68: 103919. https://doi.org/10.1016/j.jff.2020.103919

Pedersen, T.C.; Gardner, R.D.; Gerlach, R. and Peyton, B.M. (2018). Assessment of *Nannochloropsisgaditana* growth and lipid accumulation with increased inorganic carbon delivery. J. Appl. Phycol. 30:2155–2166. https://doi.org/10.1007/s10811-018-1470

Prandini, J.M.; Da Silva, M.L.B.; Mezzari, M.P.; Pirolli, M.; Michelon, W. and Soares, H.M. (2016). Enhancement of nutrient removal from swine wastewater digestate coupled to biogas purification by microalgae Scenedesmus spp. Bioresource Technology, 202: 67–75.https://doi.org/10.1016/j.biortech.2015.11.082

Rustam, Y. and Amini, S. (2015). Cultivation andextraction of microalga oil of *Botryococcusbraunii* and *Nannochloropsis* sp. Bioma, 11(2): 98-111.https://journal.unj.ac.id/unj/index.php/bioma/article/view/1221 [in Indonesian]

Sathasivam, R.; Radhakrishnan, R.; Hashem, A. and Allah, E.F.A. (2029). Microalgae metabolites: A rich source for food and medicine Saudi journal of biological sciences, 26 (4): 709-722. https://doi.org/10.1016/j.sjbs.2017.11.003

Shahid, A.; Malik, S.; Alam, M.A.;Nahid, N. and Mehmood, M.A.(2019). The culture technology for freshwater and marine microalgae. In: Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment. pp 21–44. Springer, Singapore.

Sen, B.; Kocer, M.A.T; Alp, M.T.; Erbas, H. (2005). Studies on Growth of Marine Microalgae in Batch Cultures: III. *Nannochloropsis oculata* (Eustigmatophyta). *Asian Journal of Plant Sciences, 4(6): 642-644.* DOI: 10.3923/ajps.2005.642.644

Setiawan, A.; Rusyani, E. and danNurjannah, S. (2019). The endurance test of Nannochloropsis sp. paste isolated from Lampung Mangrove Centre (LMC). In IOP Conference Series: Earth and Environmental Science. 314 (1): 012032). IOP Publishing.

Scholz, M.J.; Weiss T.L.; Jinkerson, R.E.; Jing, J., Roth R.; Goodenough, U.; Posewitz, M.C. and Gerken, H.G. (2014). Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. Eukaryot. Cell. 13:1450–1464. https://doi.org/10.1128/EC.00183-14.

Shene, C.; Chisti, Y.;Vergara, D.; Burgos-Diaz, C. and Rubilar, M. (2016). Bustarnante M. Production of eicosapentaenoic acid by *Nannochloropsisoculata*: Effects of carbon dioxide and glycerol. J. Biotechnol. 239:47–56. https://doi.org/10.1016/j.jbiotec.2016.10.006.

Soto-Sanchez, O.; Hidalgo, P.; Gonzalez, A.; Oliveira, P.E.; Arias, A.J.H. and Dantagnan, P. (2023). Microalgae as raw materials for aquafeeds: Growth kinetics and improvement strategies of polyunsaturated fatty acid production. Aquac.Nutr. 2023:5110281. https://doi.org/ 10.1155/2023/5110281

Timira, V.; Meki, K.; Li, Z.; Lin, H.; Xu, M. and Pramod, S.N. (2021). A comprehensive review on the application of novel disruption techniques for proteins release from microalgae. Critical Reviews in Food Science and Nutrition, 62 (16):4309-4325.https://doi.org/10.1080/10408398.2021.1873734

Usha, M.T.; Chandra, T.S.; Sarada, R. and Chauhan, V.S.(2016). Removal of nutrients and organic pollution load from pulp and paper mill effluent by microalgae in outdoor open pond. Bioresource technology, 214: 856–860.https://doi.org/10.1016/j.biortech.2016.04.060

Vieler, A.; Wu, G.; Tsai, C.H.; Bullard, B.; Cornish, A.J.; Harvey, C.; Reca, I.B.; Thornburg, C.;Achawanantakun, R. and Buehl, C.J. (2012). Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsisoceanica* CCMP1779. PloSGenet. 8 (11):1003064. doi: 10.1371/journal.pgen.1003064

Ye.Y.; Liu, M.; Yu, L.; Sun, H. and Liu, J.(2024). *Nannochloropsis* as an emerging algal chassis for light-driven synthesis of lipids and high-value products.Mar Drugs. 22(2):54 https://doi.org/10.3390/md22020054.

Yani, A. S.; Murwani, E.; Rusyani, E. (2015) Culture Nannochloropsis sp. And Making Pasta Nannochloropsis sp. NaOH Dosage Using Different In TheCenter Of Raising Marine Fisheries (BBPBL) Lampung. Proceedings of the National Seminar on Food Self-Sufficiency of the Lampung State Polytechnic. ISBN 978-602-70530-2-1 page 588-595. In Indonesia

Yen, H.W.; Ho, S.H.; Chen, C.Y. and Chang, J.S. (2015). CO2, NOx and SOx removal from flue gas via microalgae cultivation: A critical review. Biotechnology Journal, 10 (6):829–839. https://doi.org/10.1002/biot.201400707

Zahran, E.; Elbahnaswy, S.; Ahmed, F.; Ibrahim, I.; Khaled, A.A. and Eldessouki, E.A. (2023). Nutritional and immunological evaluation of *Nannochloropsisoculata* as a potential Nile tilapia-aquafeed supplement. BMCVet. Res. 19(1):65. https://doi.org/10.1186/s12917-023-03618-z.