Effect of commercial NPK fertilizer on growth and biomass of *Navicula* sp. and *Nannochloropsis* sp.

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Abstract. Microalgae as a source of biodiesel precursor are promising by pointing out several advantages of its cultivation, such as able to be cultivated in non-arable land, high growth rate with high biomass yield, high lipid content, and renewable. Mass cultivation of microalgae requires large amount of nutrients which makes it economically impractical. To overcome this problem, an alternative medium which contains cheaper nutrients sources should be addressed. In this research, the growth and biomass productivity of *Navicula* sp. and *Nannochloropsis* sp. were compared in the commonly used F/2 medium and modified medium containing commercial NPK fertilizer. The results indicated that *Navicula* sp. and *Nannochloropsis* sp. can thrive in modified F/2-NPK medium under continuous illumination, while NPK-only medium didn’t show any significant increase in growth and biomass accumulation for both strains compared to initial cell inoculation. Cell optical density at 750 nm and biomass dry weight of 80% F/2 and 50% F/2 medium were comparable to that in F/2 control medium for both strains, indicating that *Navicula* sp. and *Nannochloropsis* sp. have a similar requirement for nutrients types. Furthermore, higher specific growth rate of *Nannochloropsis* sp. than Navicula sp. seen in both modified medium showed its favorable condition for growth.

1. Introduction

Microalgae which is classified as eukaryotic organism, consists of proteins, carbohydrates, and lipid with certain compositions varied with the type of algae. One of the significant advantages of microalgae over other biomass is their metabolic flexibility which can be regulated for higher desired product yield (lipid, carbohydrates, or protein) [1]. Nitrogen (N) and phosphorus (P) in cultivation media are considered to be the key element for optimal algae growth as it is important for the biochemical backbone of several biomolecules such as chlorophylls, proteins, carbohydrates, and lipids [2]. Nitrogen can be supplied in the form of nitrate, ammonia, or urea [3]. Another important element content such as iron was also studied to significantly affect lipid accumulation in the algae biomass [4]. The existence of other essential micronutrients for optimum algae growth is also studied, which includes Boron (B), Copper (Cu), Zinc (Zn), and Mangan (Mn) albeit in a smaller amount. Some studies revealed that large-scale cultivation of microalgae is economically impractical with a big contribution from the nutrient cost to the overall cultivation cost. For that reason, media selection is one of the key aspects to be considered. Many studies that evaluate low-cost media for algae cultivation demonstrated some promising results highlighting the possibility of low-cost algae cultivation that meets the demand for competitive algae oil price.
Wastewater effluent contains high nitrogen and phosphorus concentration as well as other organic and inorganic compounds in small concentration. Some strains of microalgae such as Scenedesmus obliquus and Chlorella vulgaris have shown its ability to grow in wastewater substrates [5]. Chlorella vulgaris, in particular, was studied to effectively remove groups of the heavy metal element such as Cu, Fe, Zn, Pb, Ni [6]. In the cultivation of S. obliquus, for example, 75% substitution of artificial medium Bold Base Medium with nitrogen and phosphorus-rich wastewater gave promising results with the increased biomass production which also affects the higher lipid production [7]. Coupling microalgae cultivation and wastewater treatment are seen as a good opportunity for economical water treatment as well as applicable clean water supply for the desert area [6,8].

Inorganic fertilizer such as NPK has also been explored as a potential medium for the cultivation of some strains of algae, such as Ankistrodesmus gracilis and C. vulgaris [4,9,10]. The ratio of N, P, and mineral composition in the commercial NPK fertilizer significantly determined the cell growth rate. Even though N and P are known as limiting nutrients, N in excess amount potentially inhibited pigment production which is necessary for photosynthesis [9]. The highest growth rate of C. vulgaris at short cultivation period was obtained from NPK medium at the concentration range of 20-40 mg/L. On the contrary, cultivation in a medium with a higher concentration of NPK (60-80 mg/L) gave longer stability of cell growth until it reached stationary phase which possibly confirmed the inhibition effect of excess N [10]. Besides, some studies also demonstrated that N-limited condition leads to higher lipid production as algae used mostly their energy for lipid production while protein synthesis activity was reduced due to limited nitrogen supply [1].

Cultivation of Nannochloropsis salina using low-cost medium has been studied with agriculture fertilizer and urea as the nutrient replacement of F/2 artificial medium [1]. In recent days, studies on Nannochloropsis are particularly increased as this strain is known to have the potential biodiesel feedstock from its high lipid productivity and photosynthetic efficiency. Under optimal growth, lipid content of Nannochloropsis constitutes 5-20% of cell dry weight, while in stress condition it up to 20-50% cell dry weight [3]. From this study, it is known that the presence of urea as a nitrogen source in low concentration could lead to high biomass and lipid productivity of N. salina while nitrogen supply in the form of ammonia, on the contrary, gave the lowest record of biomass and lipid productivity as the concentration increase [1]. However, high accumulation of urea in media led to a rapid stationary phase of culture and subsequently, the death phase [1]. Compared to the low-cost media, lipid content from the culture grown in F/2 still considered high as low concentration of nitrogen is one of the stress factors that stimulate more lipid production [1].

In this study, cultivation of Navicula sp. and Nannochloropsis sp. under artificial medium mixed between F/2 and commercial NPK fertilizer was carried out to evaluate the feasibility of algae cultivation using the low-cost medium on a laboratory scale. Specific cell growth and biomass productivity of both strains were analyzed in this study.

2. Materials and Methods

2.1. Strain and chemicals

The strain used in this study, Navicula sp and Nannochloropsis sp were obtained from algae culture collections of Research Center for Oceanography - LIPI Jakarta. All the strain cultures were grown and maintained in F/2 medium based on Guillard and Ryther [11] and Guillard [12]. Silica was omitted when culturing Nannochloropsis sp. since it enhanced precipitation. All the chemicals used in this study were purchased from Sigma Aldrich and Merck (USA). The agricultural fertilizer used in this study was commercial NPK fertilizer (Super Trobos, Indonesia) with the composition of macro and micro-nutrient content shown in Table 1. Filtered natural seawater media was obtained from Ancol beach (North Jakarta, Indonesia). Water salinity was measured using Waterproof Salinity Meter (SA 3287 TDS EC, China).
Table 1. Chemical composition of NPK

| Macro-nutrient | Composition (%) |
|----------------|-----------------|
| Nitrogen       | 7.56            |
| Phosphorus (P<sub>2</sub>O<sub>5</sub>) | 2.17 |
| Potassium (K<sub>2</sub>O) | 3.48 |
| Sulfur         | 1.13            |
| Micro-nutrients |                |
| Calsium (Ca)   | 0.07            |
| Magnesium (Mg) | 0.31            |
| Ferum (Fe)     | 0.83            |
| Mangan (Mn)    | 50 ppm          |
| Zinc (Zn)      | 0.1 ppm         |
| Boron (B)      | 0.023 ppm       |
| Cuprum (Cu)    | 0.152 ppm       |
| Other components |                |
| Amino acid, Mineral, Vitamin, Growth hormone | |

2.2. Medium preparation and preculture preparation

Stock solutions of each component from F/2 medium were prepared in water separately such as sodium nitrate, sodium dihydrogen phosphate, trace metal, and vitamin. Main F/2 media firstly was prepared in 5 L Schott bottle with all the components of stock solutions added except vitamin mixed into 5 L seawater media. Subsequently, media pH was adjusted to 7.0 (pH meter Lutron PH-201, China). Main media, bottles and tubing were sterilized using autoclave (Hirayama HVA 85, Japan) at 121°C and pressure 17.5 psi for 15 minutes. Vitamin stock solution was prepared using a syringe filter, then added before inoculation.

Pre-culture of Navicula sp. and Nannochloropsis sp. was prepared first for algae cultivation in F/2 and NPK media. The cells were pre-cultured in F/2 medium until the mid-logarithmic phase was reached (OD<sub>750</sub> 0.6-0.8) (Shimadzu UV-2700, Japan).

2.3. Algae batch cultivation in F/2 and NPK media

In order to find the optimal concentrations of fertilizer used in algae cultivation, a combination of F/2 and NPK fertilizer medium were developed. Two milliliters of NPK fertilizer in liquid concentrate form was diluted into 1,000 ml of filtered seawater before mixed with F/2 medium. The mixture of F/2 and NPK fertilizer medium in percentage (v/v) was made as followed: 1. F/2 as a control group (F/2 100%), 2. F/2 (80%) and NPK (20%), 3. F/2 (50%) and NPK (50%), 4. F/2 (20%) and NPK (80%), and 5. NPK (100%). The total volume for each media is 2 L. All these media were prepared in sterile condition.

Prior to inoculation to fresh medium, pre-cultured cells were centrifuged at 3,500 rpm for 5 minutes using lab-bench centrifuge (Oregon LC-04C PLUS, China) and washed by either F/2 or NPK medium twice. The pre-cultures were inoculated to media and normalized to OD 750 nm of 0.1. Cultures were grown at 20–25°C under continuous illumination of 22,75 μmol m<sup>2</sup> s<sup>−1</sup> (Lutron LM-8000A, Taiwan).
Compressed air was constantly supplied to each culture by low noise air pump at 70 L/min flow rate for 5 bottles and pressure 0.037 Mpa (Resun LP-60, China). Growth rate and biomass accumulation under modified medium was observed for 14 days and compared with F/2 medium.

2.4. Sample analysis

Optical density of cell was measured using a spectrophotometer at 750 nm (Shimadzu UV-2700, Japan). The dry cell weight was measured by filtering 50 ml of each cultures using glass filtration (Millipore, USA) with filter paper (Whatman Glass Microfiber Filters GF/A Diameter 47 mm, China) then dried at at 105°C (Memmert, Germany) overnight, then weighed in mg/mL using analytical balance (Kern ABJ 220-4nm, Germany).

Specific growth rate ($\mu$) was calculated based on the formula in [13]:

$$\mu = \frac{\ln(X_{max}) - \ln(X_i)}{t}$$  \hspace{1cm} (1)

where: $X_i$ = initial biomass concentration (g L$^{-1}$), $X_{max}$ = maximum biomass concentration (g L$^{-1}$), $t$ = cultivation time between $X_i$ and $X_{max}$ (d).

Meanwhile, the productivity of microalgae was calculated using the equation described in [14]:

$$P (g. L^{-1}. d^{-1}) = \frac{X_{max} - \ln (X_i)}{t}$$  \hspace{1cm} (2)

where $P$ = productivity (g · L$^{-1}$ · d$^{-1}$), $X_i$ = initial biomass concentration (g · L$^{-1}$), $X_{max}$ = maximum biomass concentration (g · L$^{-1}$), $t$ = cultivation time related to the maximum biomass concentration (d).

3. Results and Discussions

Microalgae required nutrients to sustain their growth and development. Their cultivation in laboratory need specific medium that supply major and minor nutrients to support optimum growth. Some types of macronutrient such as nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) are essential for the formation of macromolecules, such as carbohydrate, protein and lipid. This study assessed the feasibility of NPK commercial fertilizer to be used as medium for large scale cultivation of microalgae. Two strains of microalgae from different classes were chosen, Navicula sp. from bacillariophyceae (diatom) and Nannochloropsis sp from eustigmatophyceae (green algae lineage).

3.1. Growth and biomass accumulation of Navicula sp. under modified medium were comparable with growth and biomass accumulation under F/2 control medium.

The growth of Navicula sp. cultured in F/2 and modified medium contained NPK fertilizer and F/2 medium is shown in Fig. 1a. As expected, F/2 medium as the control group showed a considerably increased OD$_{750}$ rate compared to all that modified media. The modified medium of 80% and 50% F/2 showed a comparable increased trend toward F/2 control medium (100% F/2 medium). The growth rate was likely inhibited in 20% F/2 medium compared to 100%, 80% and 50% F/2 medium, although no significant differences were observed. The 100% NPK medium without any mixture of F/2 medium showed a delayed increased of growth. The results indicated that growth differences in F/2 control medium and modified medium might be due to different nitrogen sources used in F/2 and NPK fertilizer. As amounts of F/2 medium reduced by 0.5 times of total medium, the growth was presumably delayed. This reduction might also be influenced by the content of trace elements in the medium [15]. Since 100% NPK did not contain any trace element, the cell might require additional nutrients to accelerate its growth.
Figure 1. Growth and biomass accumulation of *Navicula* sp. under F/2 medium as control medium and modified medium contained of F/2 medium and NPK fertilizer. a. Optical density of cell culture under F/2 control medium and modified medium contained of F/2 medium and NPK fertilizer at 750 nm. b. Biomass dry weight under F/2 control medium and modified medium contained of F/2 medium and NPK fertilizer.

The biomass accumulation shown in Fig. 1b had similar trend with the growth rate. The F/2 control medium showed the highest biomass accumulation compared to all modified medium. Meanwhile, 80% and 50% F/2 medium showed comparable yields of biomass dry weight with F/2 control medium. Biomass accumulation of 100% NPK medium confirmed the growth rate result which showed the least biomass yield among of those of F/2 control medium and modified medium. It was previously shown that urea resulted higher cell density compared to sodium nitrate and ammonium chloride as nitrogen source in *Navicula phyllepta* MACC8 [16]. To enhance the growth rate in *Navicula* sp., the use of urea-type fertilizer can be further examined.

3.2. Growth rate of *Nannochloropsis* sp. under modified medium contained of 80% F/2 was slightly higher than growth under F/2 control medium.

The growth of *Nannochloropsis* sp. cultured in F/2 and modified medium contained of NPK fertilizer and F/2 medium is shown in Fig. 2a. Since *Nannochloropsis* sp. does not require silicate for the formation of its cell wall, silicate was omitted from the F/2 control medium and modified medium. Interestingly, the growth rate of *Nannochloropsis* sp. in 80% F/2 medium was slightly higher than those of F/2 control medium and another modified medium. Similar growth trend with *Navicula* sp. was observed in 20% F/2 and 100% NPK medium, which both medium showed a slower than that in 100% F/2, 80% F/2, and 50% F/2 medium. The growth at day 7 in 20% F/2 medium started to enter the stationary phase earlier (figure 1a). It is noted that in 100% NPK medium, the growth rate of *Nannochloropsis* sp. was slightly higher than *Navicula* sp. with OD_{750} of 0.4 at day 14.
This data indicated that *Nannochloropsis* sp. was more likely suitable to grow in NPK medium than *Navicula* sp., as confirmed by the previous study conducted by Bae [15] which analyzed the water used in *Nannochloropsis oceanica* cultured in agricultural fertilizer. The results indicated that the concentration of NH$_4$-N was 154 times higher than that of F/2 medium, with PO$_4$-P was 9 times higher. The higher concentration of ammonia seems to implicate the delayed growth of *Nannochloropsis* sp. in this study. The lack of trace elements used in F/2 medium, such as Co, Cu, Zn, Mo also affected the growth of *Nannochloropsis* sp. The study conducted by Bae [15] showed that the presence of these four elements in the fertilizer medium could successfully increase the *N. oceanica* growth rate by 80% of that in F/2 medium. It was also confirmed by Gonzalez-Rodriguez [17] that each kind of microalgae required different nitrogen sources in the fertilizer to thrive. Since NPK fertilizer used in this study seems likely to contain the nitrogen source that is less-suited to sustain the growth of *Nannochloropsis* sp., other types of nitrogen sources, such as urea and NaNO$_3$ can be supplemented into the medium.

However, biomass accumulation of *Nannochloropsis* sp. shown in figure 2b does not indicate that biomass dry weight in 80% F/2 medium was higher than those in F/2 control and 50% F/2 medium. The differences in biomass accumulation between F/2 control, 80% F/2 and 50% F/2 medium with 20% F/2 and 100% NPK medium were slightly showed after seven days of cultivation (figure 2b). This lagged biomass accumulation might be due to the smaller average diameter of the cells during the lag and logarithmic phase compared with the cell size during the stationary phase which affecting the overall biomass dry weight. On day 14, biomass accumulation of 80% F/2 and 50% F/2 medium were comparable with F/2 control medium. The 100% NPK medium showed the least biomass accumulation among all media, which confirmed the growth rate result in figure 2a.

![Figure 2](image_url)

**Figure 2.** Growth and biomass accumulation of *Nannochloropsis* sp. in F/2 medium as control medium and modified medium contained of F/2 medium and NPK fertilizer. a. Optical density of cell culture under F/2 control medium and modified medium contained of F/2 medium and NPK fertilizer at 750 nm. b. Biomass dry weight under F/2 control medium and modified medium contained of F/2 medium and NPK fertilizer.
3.3. Optical density and biomass dry weight of *Navicula* sp. and *Nannochloropsis* sp. showed a positive correlation, while *Nannochloropsis* sp. possessed a better specific growth rate and biomass productivity compared with *Navicula* sp.

A linear regression was evaluated to obtain a correlation between optical density and biomass concentration. This linear correlation can be used to predict the biomass amount by measuring the optical density [18].

![Figure 3](image-url)

Figure 3. Linear correlation between optical density and dry weight derived from each medium. All points were mean of three biological replicates. a. Linear correlation of optical density and dry weight of *Navicula* sp. Linear correlation of optical density and dry weight of *Nannochloropsis* sp.

Dry weight (y) of both strains and their optical density (x) were positively correlated as shown in Fig. 3. The direct equation with $R^2$ value between optical density and biomass dry weight from both strains was linear according to regression equations, as followed: *Navicula* sp. F/2 100% $y = 1.0765x + 0.3143$ $R^2 = 0.998$, F/2 80% $y = 0.9148x + 0.3079$ $R^2 = 0.9744$; F/2 50% $y = 0.9015x + 0.2823$ $R^2 = 0.9497$; F/2 20% $y = 0.7909x + 0.2544$ $R^2 = 0.839$; NPK 100% $y = 1.0337x + 0.1524$ $R^2 = 0.9118$; *Nannochloropsis* sp. F/2 100% $y = 0.4586x + 0.1097$ $R^2 = 0.9554$; F/2 80% $y = 0.5023x + 0.0879$ $R^2 = 0.9544$; F/2 50% $y = 0.541x + 0.0604$ $R^2 = 0.9558$; F/2 20% $y = 0.5683x + 0.0505$ $R^2 = 0.988$; NPK 100% $y = 0.5682x + 0.0797$ $R^2 = 0.9383$.

*Navicula* sp. in F/2 control medium showed the highest correlation with $R^2$ of 0.998 (figure 3a). *Nannochloropsis* sp. grown under F/2 control and modified medium showed highly correlated optical density and biomass with $R^2$ values above 0.9 (figure 3b). It should be noted that good linearity was obtained when the culture medium was diluted in an appropriate concentration range for the OD measurement. When OD value reach >1, the measurement was retaken after further dilution. High linear correlation between cells dry weight and OD were demonstrated that the modified and NPK only medium can support the health of cell’s growth [3]. It should be also taken into consideration that *Navicula* sp. secreted a substance, called exopolysaccharide (EPS) [19]. During stationary phase, the culture became thicker as the biofilm was normally formed. Thus, the linear correlation was more accurate during logarithmic phase in case of diatom.

Specific growth rate and biomass productivity of *Navicula* sp. and *Nannochloropsis* sp. were compared as shown in figure 4.
It was shown that *Nannochloropsis* sp. possessed better specific growth rate than *Navicula* sp. under F/2 control medium and modified medium (figure 4a). The slower growth of *Navicula* sp. might attribute to the presence of nitrogen and phosphorus types in the fertilizer [19]. Lack of trace elements and vitamins might also play a role in the cause of slower growth of *Navicula* sp. [20]. Since *Navicula* sp. is a benthic diatom, its cells tended to precipitate in the bottom, thus required a good aeration. The diatom physiological characteristic seems to be affecting the overall biomass productivity. As shown in figure 4b, *Nannochloropsis* sp. showed higher biomass productivity compared to *Navicula* sp. under F/2 control medium and modified medium. It can be concluded that culturing *Nannochloropsis* sp. was more suitable than *Navicula* sp. in the laboratory based on its better specific growth rate and biomass productivity. The nitrogen composition in NPK fertilizer acted as a determinant factor to stimulate the growth of microalgae in this study. Since nitrogen is the most essential macronutrient in microalgae after carbon that assimilated in the form of nitrate or ammonium which regulates the metabolism and critical component of important biomolecules, such as proteins, chlorophylls, and DNA [21].

4. Conclusion
The large-scale cultivation of microalgae should be economically feasible, thus a low-cost medium for microalgae cultivation is required to be developed. The standard F/2 medium, which is the common medium for microalgae cultivation is costly and impractical to be used for outdoor mass cultivation. In this study, *Navicula* sp. and *Nannochloropsis* sp. were subjected to grow in modified medium which contained NPK fertilizer. Overall results conclude that *Nannochloropsis* sp. was more appropriate to be cultivated under modified medium than *Navicula* sp. as shown by the specific growth rate. Thus, it promoted overall *Nannochloropsis* sp. biomass productivity which was higher than that in *Navicula* sp. Further development of modified medium should be conducted by supplementing another type of fertilizer, carbon sources, or trace elements into the medium components. Further analysis for supplementary data such as protein, carbohydrate, and lipid content will be conducted in the near future.

Figure 4. Specific growth rate and biomass productivity of *Navicula* sp. and *Nannochloropsis* sp. Each calculated value was mean of three biological replicates. a. Specific growth rate of *Navicula* sp. and *Nannochloropsis* sp. under F/2 control medium and modified medium contained of F/2 medium and NPK fertilizer. b. Biomass productivity of *Navicula* sp. and *Nannochloropsis* sp. under F/2 control medium and modified medium contained of F/2 medium and NPK fertilizer.
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