Selection and Cultivation of Microalgae for CO₂ Biofixation

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Abstract. Climate change has become the main focus of the world which caused various negative impacts throughout the earth. Carbon dioxide is one of the greenhouse gases that effect on climate change and environmental pollution. One of the promising opportunities to reduce CO₂ emissions in the atmosphere is bio fixation by microalgae. Many species of microalgae have the ability to capture carbon dioxide as its carbon source for the photosynthetic process. This research aimed to cultivate many species of microalgae in the carbon dioxide incubator and study its CO₂ bio-fixation capability. Microscopic analysis was occurred to identify microalgae morphology. The optical density measured representing growth rate of microalgae. *Tetraselmis* *sp.* and *Navicula* *sp.* are suitable for CO₂ biofixation due to the good adaptation to the existence of CO₂ in the medium.

1. Introduction
The rapid development of the human population and uncontrolled energy consumption affected to the worldwide. Over consumption of energy has been identified as the primary cause of the global warming and environmental pollution [1]. The emissions of CO₂ mainly derived from fossil fuels and industrial activities, as a major greenhouse gas in the atmosphere has greatly increased. It contributes largely to the climate change and environmental pollution [2-4]. International efforts to reduce CO₂ emissions in the atmosphere have been made by Kyoto Protocol in 1997 and Paris COP 21 in 2015. One of the promising opportunities to reduce CO₂ emissions in the atmosphere is bio fixation by microalgae. Microalgae are microscopic organisms that typically grow suspended in water, which some of them are living by photosynthetic cycle as plant [5]. Nowadays, microalgae are consumed as food supplement, pharmaceuticals, animal feed, aquaculture and cosmetics. Some microalgae are able to remove the pollutant and able to produce lipids [6].

Many species of microalgae have the ability to capture carbon dioxide as its carbon source for the photosynthetic process. Previous study have shown the aqua water and seawater microalgae cultivation on the photobioreactor to capture carbon dioxide [7]. CO₂ emissions has been reduced up to 78% by bio-scrubber technology using microalgae [8]. Microalgae consumes 368 mg/L carbon dioxide as its carbon source for the metabolism everyday [9]. The growth of microalgae can be affected by several factors, such as composition of medium, culture conditions, and bioreactor type. Effect of culture salinity and CO₂ concentration effected to the growth rate of *Chlorella vulgaris* in the
freshwater and tofu waste water [6]. High concentration of carbon in the medium caused high lipid content of microalgae. The final product of CO₂ fixation of microalga is biomass, that can be used as food, pharmaceuticals, energy, and animal feed. This work aimed to cultivate many species of microalgae in the carbon dioxide incubator and study its CO₂ bio-fixation capability.

2. Materials and Methods

2.1. Microalgae and cultivation medium

Five different of microalgae species, *Chlorella Pyrenoidosa*, *Chlorella vulgaris*, *Nannochloropsis*, *Navicula* sp. and *Tetraselmis* sp., were used in this study. This inoculums of microalgae were obtained from BPAP Jepara, that cultivated using Walne’s medium. 500 ml algae was inoculated in 1 L Erlenmeyer flasks containing Walne’s medium under sterile condition. The algae was incubated in a carbon dioxide incubator with 5% carbon dioxide and relative humidity 70%, under the white LED light. The cultivation temperature was 27 ± 5°C.

2.2 Microalgae Analysis

The morphology of microalgae was identified microscopically using Olympus Microscope CX 23. Growth rate of algae was known from its optical density OD which measured for 10 days. Measurement of algae OD was carried out using Hitachi Double Beam Spectrophotometer UH5300 at optimum wavelength as shown in table 1.

| Microalgae                  | Wavelength (nm) |
|-----------------------------|-----------------|
| *Chlorella pyrenoidosa*     | 256             |
| *Chlorella vulgaris*        | 298             |
| *Nannochloropsis* sp.       | 314             |
| *Navicula* sp.              | 698             |
| *Tetraselmis* sp.           | 782             |

3. Results and Discussion

3.1 Microscopic Observation of Microalgae

The microalgae was identified by morphological using microscopic observation. Morphological features of microalgae was recorded photographically at 100X magnification using Olympus CX23 microscopy. Microscopic observation from figure 1 showed that the microalgae has a green spherical unicellular with 2-10 μm diameter and many structural elements similar to plants.
Figure 1. Microscopic Observation of Microalgae (a) *Chlorella vulgaris* (b) *Chlorella pyrenoidosa* (c)*Nannochloropsis* sp. (d)*Navicula* sp. (e)*Tetraselmis* sp.

*C. vulgaris, C. Pyrenoidose, Nannochloropsis* sp. and *Tetraselmis* sp. are chlorophyta that live in the sea, freshwater, and brackish water. *C. vulgaris and C. pyrenoidosa* are unicellular cosmopolitan organisms that are spherical with a diameter of 2-8 microns, having large amounts of chlorophyll a and b [10]. *C. vulgaris* also have carotenoid and xanthophyll pigments. *C. vulgaris* lives in marine waters and *C. pyrenoidosa* is commonly found in freshwater. Both species have ability to reproduce very quickly and produce more lipid and less protein [11]. *C. vulgaris* has a nutritional content of 55% protein, 5.8% fiber, 10.2% lipids and 23.2% rat carbohydrates [12]. Then, *Nannochloropsis* sp. is a non-motile spherical green algae that is only 4-6 microns in diameter. It has a cell wall made of cellulose components. *Nannochloropsis* sp. is cosmopolitan and can grow at salinity 0-35 ppt. The optimum salinity for growth is 25-35 ppt, 25-30°C is the optimal temperature range [13]. *Navicula* sp. is the Bacillariophyceae and it has the insertion of a gritty substance in the cell wall and also cylindrical in shape with concentrated chloroplasts in the central part.

3.2. Growth Rate of Microalgae

Figure 2. Optical Density of Microalgae

Figure 2. shows the optical density of microalgae during ten days of cultivating. Optical density measurements were used to quantify the microalgae or biomass in samples. It also used to measure the growth of microalgae. Generally, growth of microalgae can be segregated into four phases which are lag phase, log phase or exponential, stationary phase and finally death phase. Lag phase is the initial phase of cultivation in which the microalgae adapts to the surrounding such as medium, pH,
temperature and lighting. Subsequently, the microalgae begin to undergo active cell division and the biomass of the culture will increase usually in exponential order. From the graph above, it shown that *Tetraselmis* sp. reached its maximum value on the 7th day. Another species of microalgae in this study reached its highest OD on the day 4. Increased in optical density indicates that microalgae had passed trough lag phase and reached the exponential phase. Therefore, stationary phase begins which biomass increase. This is due to the equal rate of the cell division and cell death. This phase mainly occurs due to depletion of nutrients in the medium. Lastly, the microalgae death rate will be higher than the cell division rate, hence the graph shows decrease in biomass [14].

Growth of microalgae is determined by some factors, such as nutrition salinity, temperature, light, pH, and CO$_2$ supply. Considering the stoichiometric ratio, the amount of CO$_2$ present in the air (0.03%) is not enough to provide the necessary gas pressure in the cultures to promote high productivity. Moreover, the supply of CO$_2$ to microalgae cultures allows increasing biomass productivity, but the reduction of pH, resulting from the increase of CO$_2$ availability in the medium, can inhibit the growth of some species of these microorganisms [15]. In this study microalgae was cultivated in the incubator, which the amount of CO$_2$ is 5%. *Tetraselmis* sp. and *Navicula* sp. are suitable for CO$_2$ biofixation due to the good adaptation to the existence of CO$_2$ in the medium. It known from the higher optical density than another species of microalgae.

3.3. Effect of Walne Concentration to The Microlage Growth Rate

![Figure 3](image-url)

**Figure 3.** Optical Density of (a)*Navicula* sp. (b)*Tetraselmis* sp. in different Walne concentration
Based on the previous chapter, Navicula sp. and Tetraselmis sp. had good ability on capturing carbon dioxide. Effect of nutrient concentration to the microalga growth was observed in this study by added different composition of Walne to the medium. Navicula sp. had grown in 24 hours of cultivation, then continued with exponential growth around the 4th day. Figure 3.a shown that the highest optical density of Navicula sp. was obtained on the 3rd day by giving 3 mL. Meanwhile Tetraselmis sp. had slowly grown until 4th day, then decreased gradually. This species had exponentially grown until 5th day and obtained the highest optical density by giving 1 mL Walne to the medium. The Walne’s medium composes of NaNO₃ (100.0 g/l), disodium EDTA (C₁₀H₆Na₂O₈) (45.0 g/l), H₂BO₃ (33.6 g/l), NaH₂PO₄·H₂O (20.0 g/l), FeCl₃·6H₂O (1.3 g/l), MnCl₂·4H₂O (0.36 g/l), trace metal solution (1.0 mL) including, ZnCl₂ (2.1 g), CoCl₂·6H₂O (2.0 g), (NH₄)₆MoO₂⁴·4H₂O (0.9 g), thiamine (0.2 g), cyanocobalamin B₁₂ (0.01 g) [16]. In this study, the concentration of carbon dioxide also enhanced up to 10%. Increasing of Walne and carbon dioxide concentration will increase carbon sources for microalgae that formed carbonic acid in the medium. The reduction of pH resulting from carbonic acid in the medium inhibited microalga growth. Hence, optimum concentration of carbon dioxide and Walne are needed for microalga growth.

4. Conclusion
The microalgae in this study has a green spherical unicellular with 2-10 μm diameter. Tetraselmis sp. reached its maximum value on the 7th day. Another species of microalga in this study reached its highest OD on the day 4. Tetraselmis sp. and Navicula sp. are suitable for CO₂ biofixation due to the good adaptation to the existence of CO₂. Effect of nutrient concentration to the microalga growth was observed in this study by added different composition of Walne to the medium. Navicula sp. had grown in 24 hours of cultivation, then continued with exponential growth around the 4th day. The highest optical density of Navicula sp. was obtained on the 3rd day by giving 3 mL. Meanwhile Tetraselmis sp. had slowly grown until 4th day, then decreased gradually. Hence, optimum concentration of carbon dioxide and Walne are needed for microalga growth.

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