

Increased lipids production of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel synthesis through the optimization of growth medium composition arrangement by using bicarbonate addition

Dianursanti*, Zenitha Lintang Agustin, and Dwini Normayulisa Putri

Chemical Engineering Department, Faculty of Engineering, University of Indonesia, Kampus Baru UI, Depok, West Java, 16424

Abstract. *Chlorella vulgaris* and *Nannochloropsis oculata* are a highly potential microalgae to be used in pilot-scale of biodiesel synthesis. The essential content from these microalgae is the fatty acid of lipid which is the main target for the feed and biodiesel industries. One of the key factor in improving lipid microalgae are the arrangement of nutrients in the growth medium. Research on the regulation of nutrients using bicarbonate (HCO_3^-) as an additional inorganic carbon source has been done by many studies, but the yield of lipids obtained has not been much. The aim of the study was to improve the lipid yield of *Chlorella vulgaris* and *Nannochloropsis oculata*. Variation of $[\text{HCO}_3^-]$ which added to Walne medium were 25 ppm and 75 ppm, while the Walne medium without the addition of bicarbonate acts as control. The results showed that $[\text{HCO}_3^-]$ 75 ppm could increase *Chlorella vulgaris* biomass by 0.9162 g/l with 17.0% wt, while *Nannochloropsis oculata* produced the greatest lipid content in $[\text{HCO}_3^-]$ 25 ppm of 20.3% wt and the largest biomass on $[\text{HCO}_3^-]$ 75 ppm of 1.7233 g/l.

1 Introduction

Transportation and electricity are the most crucial daily needs and must be met to support human welfare. On the other hand, the increase population can causes an increasing of these sector demand and raising various problems in the energy field. To respond and address these issues, researchers are always looking for and developing renewable energy sources. The best candidate of this potential source is microalgae because it can produce a large number of lipid in a small area everyday, so it better than crop plants [1].

The most commonly used microalgae as a renewable energy sources for biodiesel synthesis are *Nannochloropsis* and *Chlorella* [2]. Both microalgae can produce a high lipid content in its cells, i.e 22.7-29.7% of the *Nannochloropsis* dry weight and 5-58% of the *Chlorella* dry weight [3]. One of the most common problems is the acquisition of biomass and lipid microalgae that do not meet production targets for feed and biodiesel industries.

Based on the potential of these microalgae, University of Indonesia is actively researching and developing *Nannochloropsis* and *Chlorella* to contribute in the production of biodiesel, especially in Depok, West Java. One of effort to increase the lipid microalgae production is re-optimize the regulation of growth medium using bicarbonate based on previous studies, so that the University of Indonesia has valuable assets to play a role in biodiesel synthesis.

The selection of bicarbonate is considered to be an important point for increasing the lipid production of *Nannochloropsis* and *Chlorella* in the upstream biodiesel synthesis study. According to Devgoswami, the solubility of bicarbonate (HCO_3^-) is higher than carbon dioxide (CO_2), so it can be directly absorbed by the microalgae [4]. In addition, bicarbonate acts as a carbon source that is closely related to photosynthesis and the accumulation of lipids in cells [5].

2 Materials and Methods

2.1 Microalgae and Growth Medium Compositions

The microorganisms used during this study were *Nannochloropsis oculata* and *Chlorella vulgaris* microalgae. The isolate is cultivated in the Laboratory of Bioprocess Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Indonesia, Depok, West Java. Isolates of both types microalgae are always maintained and reproduced in the range of logarithmic-stationary growth phases.

The growth medium of *Nannochloropsis* and *Chlorella* used in this study was the Walne medium in seawater. Walne medium composition can be seen in Table 1 [6].

* Corresponding author: danti@che.ui.ac.id

Table 1. Walne Medium Composition

Nutrient solution		Trace element solution		Vitamin solution	
Materials	per litre	Materials	per litre	Materials	per litre
NaNO ₃	100,0 g	ZnCl ₂	21,0 g	Thiamine.HCl (vit. B1)	1,0 g
H ₃ BO ₃	33,6 g	CoCl ₂ .6H ₂ O	20,0 g	Cyanocobalamin (vit. B12)	0,05 g
Na ₂ EDTA	45,0 g	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	9,0 g		
NaH ₂ PO ₄ .H ₂ O	20,0 g	CuSO ₄ .5H ₂ O	20,0 g		
FeCl ₃ .6H ₂ O	1,3 g				
MnCl ₂ .4H ₂ O	0,36 g				
trace element solution	1 ml				
vitamin solution	100 µl				

2.2 Stock Solution of NaHCO₃

The NaHCO₃ solution is used as an additional carbon source solution in the Walne medium. The purpose of making NaHCO₃ stock solution in this study was to provide homogeneous conditions on each treatment and to obtain significant results in the work culture. The NaHCO₃ stock solution made for this study was 300 ppm which can be prepared by dissolving 300 mg of NaHCO₃ in 1000 ml of distilled water. Dilution for working culture using a mole ratio between NaHCO₃ and HCO₃⁻.

2.3 Working Culture and Culture Condition

The working culture of *Nannochloropsis* and *Chlorella* uses glass-reactor bottles with a capacity of 1500 ml. Variations of [HCO₃⁻] which added to Walne medium were 25 ppm and 75 ppm, whereas treatment without addition of [HCO₃⁻] was used as a control. The composition of the working culture in this study can be seen in Table 2.

Table 2. Working Culture Compositions

[HCO ₃ ⁻] treatment (ppm)	<i>Nannochloropsis oculata</i>			<i>Chlorella vulgaris</i>		
	0	25	75	0	25	75
Inoculum (ml)	500	500	500	500	500	500
NaHCO ₃ solution addition (ml)	0	125	375	0	125	375
Walne medium (ml)	1000	875	625	1000	875	625

With a working culture composition that looks like above, the *Nannochloropsis* and *Chlorella* culture are set with initial optical density (OD) of ±0.4. The set of reactor then placed near the white lamp with light intensity that has been set for each microalga, i.e 3000-

-3500 lux for *Nannochloropsis* and 2000 lux for *Chlorella*. The both culture then harvested for lipid extraction after reaching the stationary phase.

2.4 Harvesting Biomass

After reaching the stationary phase, *Nannochloropsis oculata* and *Chlorella vulgaris* cultures are harvested. The working culture is harvested by centrifugation at 4000 rpm for 15 minutes to obtain biomass [7]. The precipitated biomass then separated from the supernatant and transferred to a container. Container with wet biomass then dried naturally. The dry weight of biomass (X) can be determined by the following equation:

$$X (g/l) = \frac{\text{weight final (g)} - \text{weight initial (g)}}{\text{sample volume (l)}} \quad (1)$$

2.5 Lipid Extraction

The lipid extraction method used in this study is a modification of the Bligh & Dyer method [7]. Bligh & Dyer modification method can be done by means of wet biomass mixed with chloroform: methanol (2:4), then sonicated for 20 minutes. Then, the mixture was added with chloroform:aquades (2:2) and re-sonicated for 20 minutes. The mixture then centrifuged for 15 minutes to form three layers. The bottom layer is the extracted lipid. The extracted lipid then placed into a vial, which has been known the empty weight, for the solvent evaporation. After all the solvent has evaporated, the vial is then weight. Microalgal lipid (Y_L) can be determined by the following equation:

$$Y_L (\%) = \frac{\text{weight final (g)} - \text{weight initial (g)}}{\text{sample biomass (g)}} \times 100\% \quad (2)$$

3 Results and Discussions

3.1 Results

3.1.1 Growth and Biomass Production

* Corresponding author: danti@che.ui.ac.id

The cultivation of *Nannochloropsis oculata* and *Chlorella vulgaris* with variations of $[\text{HCO}_3^-]$ addition in the Walne medium reached the stationary phase at 288-336 hours. Growth of both microalgae can be seen in Figure 1. Based on Figure 1, the control culture of *Nannochloropsis oculata* and *Chlorella vulgaris* experienced the lowest growth, while the $[\text{HCO}_3^-]$ 75 ppm experienced the highest growth.

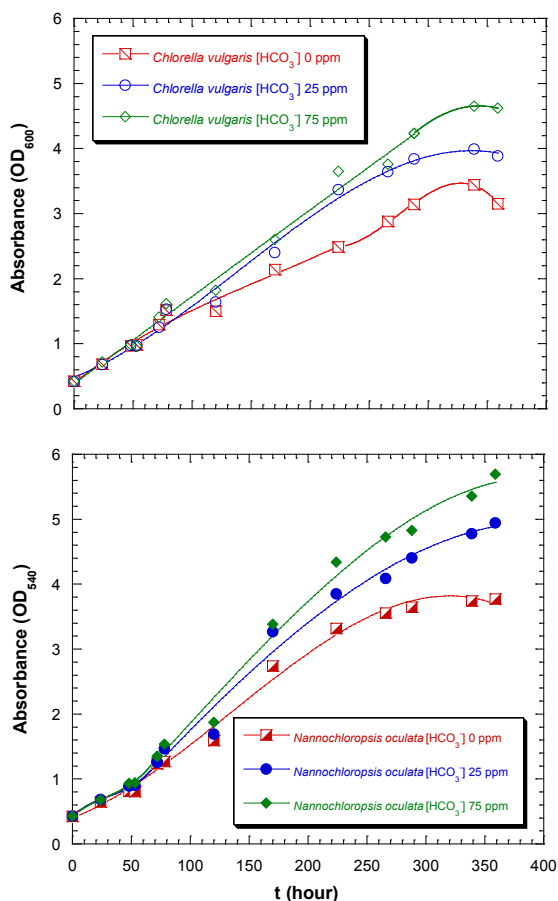


Fig. 1. Growth of *Nannochloropsis oculata* and *Chlorella vulgaris* $[\text{HCO}_3^-]$ treatments

Dry biomass of *Nannochloropsis oculata* and *Chlorella vulgaris* can be seen in Figure 2. According to Figure 2, the control treatment from *Nannochloropsis oculata* and *Chlorella vulgaris* also had the lowest biomass of 0.8954 g/l and 0.743 g/l, whereas the highest biomass was owned by $[\text{HCO}_3^-]$ 75 ppm treatment of 1.7233 g/l and 0.9162 g/l. Increased growth and biomass on the addition of $[\text{HCO}_3^-]$ into the culture medium has also been demonstrated in Devgoswami et al. [4], Ibrahim [8], Lin et al. [9], White et al. [10], and Agustin [11].

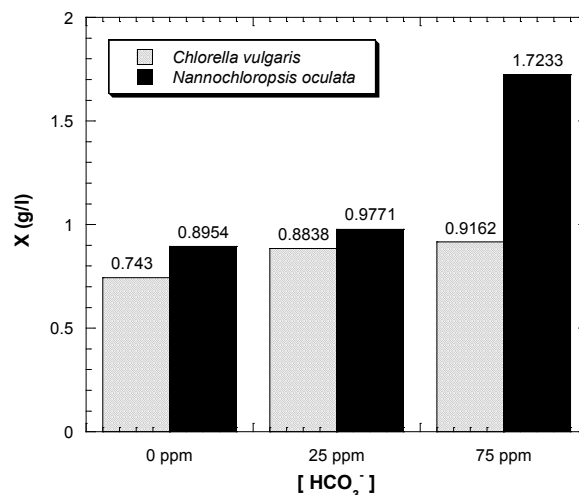


Fig. 2. Dry biomass of *Nannochloropsis oculata* and *Chlorella vulgaris* $[\text{HCO}_3^-]$ treatments

3.1.2 Lipid Production

Yield lipid of *Nannochloropsis oculata* and *Chlorella vulgaris* can be seen in Figure 3. According to Figure 3, the lowest lipid is controlled by the control treatment of both cultures, i.e. 12.1% dry weight of *Nannochloropsis oculata* and 11.8% dry weight of *Chlorella vulgaris*. The highest lipid was obtained from cultures of *Nannochloropsis oculata* with $[\text{HCO}_3^-]$ 25 ppm of 20.3% dry weight and *Chlorella vulgaris* with $[\text{HCO}_3^-]$ 75 ppm of 17% dry weight. Increased lipid in cultures that given of $[\text{HCO}_3^-]$ also been demonstrated in Devgoswami et al. [4] and Agustin [11].

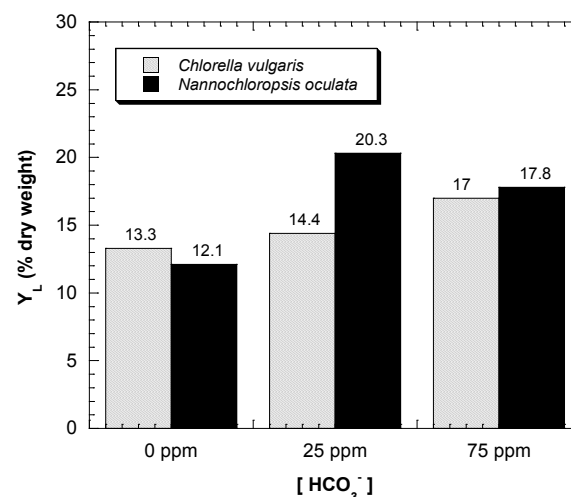


Fig. 3. Yield lipid of *Nannochloropsis oculata* and *Chlorella vulgaris* $[\text{HCO}_3^-]$ treatments

3.2 Discussions

The lowest growth in control culture was caused by no addition of carbon source that can enhance the process of photosynthesis, so cultures rely only on air derived from aeration. This is in accordance with Gardner et

al., that culture with the availability of low carbon sources will lead to low cell growth because photosynthesis is inhibited [12]. When $[\text{HCO}_3^-]$ is added to the cultivation medium, the bicarbonate (HCO_3^-) will induce the process of photosynthesis. The induction occurs in photosystems II (PS II) of light reactions and resulting in a large number of NADPH and ATP [5].

In addition, bicarbonate (HCO_3^-) also serves as a carbon to replace source of CO_2 in the medium and in the dark reaction of photosynthesis because the solubility of bicarbonate (HCO_3^-) in water is greater than that of CO_2 . When $[\text{HCO}_3^-]$ is added to the cultivation medium, the carbon source will increase to bind to the Rubisco enzyme (Ribulose-1,5-biphosphate), resulting in G3P synthesis (glyceraldehyde-3-phosphate or triose-3-phosphate) at The Calvin cycle will increase. Increased of G3P will increase the formation of glucose and lipids, because 28 G3P molecules will produce 14 molecules of glucose and 1 molecule TAG C18 [13]. According to Agustin, glucose is used as a source of energy for cell growth, so that biomass increases [11].

Furthermore, the remaining bicarbonate (HCO_3^-) in the medium still serves to induce lipid synthesis to form energy reserves because the nutrient conditions reduced when culture entered the stationary phase. Bicarbonate (HCO_3^-) will induce the formation of malonil-CoA which acts as a substrate in lipid synthesis. Carbohydrates will be broken down into acetyl-CoA which is a precursor of respiration and lipid synthesis. Acetyl-CoA then interacts with bicarbonate (HCO_3^-) to form malonil-CoA. According to Foster, increased production of malonil-CoA will cause acetyl-CoA for the Krebs cycle then diverted to lipid synthesis, since the enzyme activity in the Krebs cycle is inhibited [14].

4. Conclusion

The results showed that $[\text{HCO}_3^-]$ 75 ppm treatment could increase *Chlorella vulgaris* biomass by 0.9162 g/l with 17.0% wt, while *Nannochloropsis oculata* produced the largest biomass on $[\text{HCO}_3^-]$ 75 ppm treatment of 1.7233 g/l and the greatest yield lipid content in $[\text{HCO}_3^-]$ 25 ppm treatment of 20.3% wt. The addition of bicarbonate into the Walne medium with the composition are set in such way on this study has been able to induce the production of biomass and lipids of *Nannochloropsis oculata* and *Chlorella vulgaris* significantly compared with control treatments and prior studies.

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