Cultivation of ‘Botryococcus Braunii’ Microalgae for Hydrocarbons Production and CO₂ Bio-fixation

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Abstract CO₂ fixation for climate change mitigation actions through photosynthesis can be carried out by growing intensively managed microalgae. The capability of microalgae in the CO₂ fixation is favourable compared to trees or crops, yielding three to five times more biomass per land area than typical plants. Another interesting feature of microalgae is microalgal farms can use arid, semi-arid land or highly saline water, which is practically not suitable for agriculture and other biomass production activities. In addition, algae biomass contains fibre, fatty acid, lipid, protein, polysaccharide, and hydrocarbon. These materials could be extracted for biofuels or biochemicals. Biofuels derived from microalgae are environmentally friendly and carbon neutral. However, microalgal farming seen only for producing biomass as sources of fuels and chemicals probably uneconomical. The yields of microalgal farm to produce fuels/chemicals are also low. Therefore, microalgal farm with the objective of solely producing fuels/chemicals is economically unfeasible. If it is designed for CO₂ mitigation measures and all at once for fuels/chemicals production, such endeavour could be economically justifiable. This paper presents results of a research on microalgae cultivation for producing biomass and hydrocarbons for biofuels that simultaneously fixing the CO₂ emissions from coal power plant effluent through photosynthesis process, which also for the implementation of circular economy concepts. A strain of ‘Botryococcus Braunii’ microalgae, which known can produce high hydrocarbons and lipids throughout its growth period has been used. The research identified and evaluated the effect of cultivation medium and CO₂ concentration to the growth rate, biomass production, lipid and hydrocarbon content and type. The microalgae were cultivated in bioreactor supported with airlift system for supplying CO₂/air and for mixing process. Results of the research show that modified Chu13 (MChu13) is appropriate cultivation medium for the growth, hydrocarbon/lipid production, and CO₂ fixation from B. brauni. The magnitude of biomass production in MChu13 is 0.145 g/L·9days, while in modified BG11 is 0.118 g/L·9days, Bold Bassal Medium (BBM) is 0.09 g/L·9days, and Bristol is 0.023 g/L·9days. This biomass production rate is in line with the CO₂ fixation rate. In MChu13 the dry weight of B. brauni is 0.268 g/L·7days while in Bristol only 0.229 g/L·7days.

Keywords: biofixation, Botryococcus braunii, circular economy, CO₂ emissions, hydrocarbon, lipid, microalgae, mitigation measures, photosynthesis, airlift fermenter, vertical grow reactor

1. Introduction

In recent years, atmospheric carbon dioxide level is increasing on global scale due to GHG emissions, which might be derived from increased activities in combustion of fossil fuels, industrial processes, petroleum productions, etc. Some measures undertaken for reducing these emissions, among of them are decreasing the fuel consumption by more efficient use of energy, increasing the utilization of low or
no carbon emission fuels, end of pipe pollution control, CO\(_2\) emissions fixation, etc. Regarding the CO\(_2\) fixation, one of the options is photosynthesis by growing intensively managed microalgae. The capability of microalgae in CO\(_2\) fixation is favourable compare to tree or crops, yielding three to five times more biomass per land area than typical crops or plants [1]. Other interesting feature of microalgae is that microagal farm can use arid, semi-arid land, or highly saline water, which are practically not suitable for agriculture and other biomass production activities. In addition, algae biomass contains high concentration of important materials such as protein, polysaccharides, dyes or pigments, fibre, fatty acids, lipids, and bio-hydrocarbons. These materials could be extracted and converted to biofuels, biochemicals, or food [2]. Fuels derived from microalgae farming are environmentally friendly and can be considered as carbon neutral, in which the net of CO\(_2\) emissions from such fuel is zero [3]. However, microagal farming seen only as CO\(_2\) abatement measure is probably uneconomical. The yields of microalgal farm to produce fuels/chemicals are low. Therefore, microalgal farm with the objective of solely producing fuels/chemicals is economically unfeasible. If it is designed both for CO\(_2\) emissions abatement measure and for fuels and chemicals productions, such endeavour could be economically justifiable and considered as circular economy [4].

Research on microalgal farming for producing fuels/chemicals that simultaneously capturing and fixing the CO\(_2\) emissions is still ongoing. The research focuses on searching of strains that appropriate for this specific objective (biofuels/biochemicals production as well as CO\(_2\) fixation) and finding the optimum farming condition (medium characteristics, CO\(_2\) concentration, and other related parameters to the growth rate of microalgae, biomass weight, hydrocarbon content and type). Previous research on chlorella microalgae farming was carried out in simulated saline water with CO\(_2\) concentration of the air supply was similar to the flue gas of coal power plant. The research is intended to produce lipids that can be converted to biodiesel. However, results of the research show that the lipid content in the biomass of the chlorella is still low, i.e. less than 10% of the dry weight [5]. Therefore, a research that focusses on finding other strain, which contained high lipids or hydrocarbons are needed. A strain of ‘Botryococcus Braunii’ is used in the research with several reasons. Firstly, B. braunii is known as one of microalgae that can produce high hydrocarbons and lipids throughout its growth period. Secondly, B.braunii can grow in a high saline water while other microalgae usually grow in fresh water, and therefore B.braunii can grow solely for hydrocarbons/lipids production without any disturbance from other microalgae. Thirdly, capability of B.braunii as microalgae in the CO\(_2\) bio-fixation is favourable compare to tree or crops, yielding three to five times more biomass per land area than typical plants. This paper presents results of research on B.braunii microalgal cultivation for producing hydrocarbons throughout its growth period that simultaneously producing biomass for CO\(_2\) fixation [6].

2. Experimental procedures

2.1. Material, Equipment, and Procedures
The experimental work was carried out using a set of fermenter unit named Airlift fermenter with 10 L volume made of transparent glassware (see Error! Reference source not found.). Transparent glassware was used due to allow incoming sunlight to fermenter for the photosynthesis process. In this research, florescent lamp was used to represent the sunlight. The illumination was supplied by 6 cool-white fluorescent-lights to keep the illumination of 150 \(\mu\) E/m\(^2\)/s where the power capacity of each lamp is 8/25 watt/lumen [6].

2.2. Experimental Design and Analysis of Data
Experiment activities were designed into five stages, i.e. (i) isolation of monoculture B.braunii, (ii) preparation of culture media and stock culture of B.braunii, (iii) observation of B.braunii growth in several cultivation media, (iv) development of the growth curve and measurement of dry-cell weight of the B.braunii biomass in each cultivation medium at the same growing time, and (v) analysis of lipids production and hydrocarbons content in the B.braunii culture biomass at the same growing time.
2.2.1. Isolation and Activation of the Botryococcus braunii

B. braunii microalgae used in this study is originated strain of Indonesia. Search for this strain has been carried out in typical places where they might be found, namely swamps area, lakes, freshwater pond, rivers, ditch, and other slightly saline water (ephemeral lakes, brackish water, saltwater swamps, etc.). This search has been concentrated in the areas closed to oil and gas fields, in which about 40 samples have been collected from oil and gas field area (Bongas in West Java, East Kalimantan, East Java), oil and gas receiving terminal (Tanjung Priok Jakarta). Samples from freshwater lake (Situpatenggang and Kawah Putih) in West Java were also collected. From those 40 samples, B. braunii was found in samples from Bongas, Tuban, and Tanjung Priok. In this research, B. braunii from Bongas in West Java was used [6].

Isolated B. braunii appropriate for this research were obtained by separating B. braunii from a mix culture. Separation of B. braunii from other organisms was challenging, particularly from pediasastrum (simplex/duplex), scenedesmus econis, staurastum anatinum, and anabaena sp. Floating microalgae colonies collected from the mix culture were diluted in serial dilutions (6 times dilution) on Bristol medium (see Table 1). Within two weeks, single colonies were separated from these cultures and isolated as single cell in a gelatine slant to obtain unialgal isolates.

Table 1 Composition of Bristol (940 ml of distilled water + stock solution) [7,8].

| Ml | Stock solution | gram/400 ml H₂O |
|----|----------------|-----------------|
| 10 | NaNO₃          | 10              |
| 10 | CaCl₂·2H₂O     | 1               |
| 10 | MgSO₄·7H₂O     | 3               |
| 10 | KH₂PO₄         | 3               |
| 10 | KH₂PO₄         | 7               |
| 10 | NaCl           | 1               |

Before commencing experiment stages, B. braunii culture needs to be cultivated on starter medium to activate the strain and make it more adaptable to the changes of nutrients, components of cultivation medium, CO₂ concentration, air supply flowrate, etc. The activation was carried out in 1 L glass bottle supported by airlift for supplying air (containing CO₂ and O₂). Selected unialgal culture with high B. braunii colonies was diluted ten times (10% isolates unialgal in 90% water). The adaptation process in the activation culture ordinarily takes place for 6-7 days until the growth phase of B. braunii has become stationary. Starter medium is used for B. braunii cultivation, in which four starter mediums are used, i.e. BBM, Bristol, BG-11, and MChu13. The MChu13 medium is used in this research with varied concentrations of potassium nitrate, potassium dihydrogen phosphate, magnesium sulphate and ferric citrate. The selection of appropriate medium was carried out base on the growth rate, biomass weight, and chemicals content (lipid or hydrocarbon).
2.2.2. *Preparation of Growth Culture Media and Cultivation of ‘Botryococcus braunii’* Cultivation for *B. braunii* must be conditioned to meet intrinsic requirements of most organisms that may be physical (temperature, light, pH), chemicals (carbon, magnesium, nitrogen, phosphor, potassium, sulphur). These materials were used for structural and protoplasmic synthesis of the algae. The required concentration of these materials is presented in Error! Reference source not found. Required nutrient compositions for algal cultivation are listed in the following section.

1) Algal biomass (50%) consists of carbon, sufficient carbon is needed for cultivation. In freshwater (low salinity) and neutral pH, carbon is supplied as inorganic substance (gaseous CO₂). In most photoautotrophic, carbon is supplied as bicarbonate or organic carbon (sugar or acetate).
2) Nitrogen is essential for algal growth (10% of algal biomass consists of nitrogen), which supplied as nitrate, ammonia, and urea. Changes in nitrogen content could influence metabolic pathways.
3) pH of algal cultivation is controlled under neutral or slightly acidic to avoid element precipitation.
4) Trace elements (iron, manganese, boron, cobalt, copper, molybdenum, and zinc) are essential trace elements, which are mostly supplied in small quantities. Sodium (in any form) is not essential and specific requirement for calcium is contradictory.

Other elements (potassium, magnesium, phosphate, sodium, sulphate), organic components, and growth-promoting substances (vitamins, nucleic acid) are required as nutrient for algal cultivation.

| Component       | Required Concentration, mg/L | US Patent Plant 6, 169 Concentration, mg/L H₂O |
|-----------------|-----------------------------|-----------------------------------------------|
| Ca (NO₃)₂·4H₂O  | 50 – 250                    | 26.5                                          |
| NH₄Cl or NH₄HCO₃| 10 – 150                    | –                                             |
| MgSO₄·7H₂O      | 10 – 100                    | 25                                            |
| K₂HPO₄ or KH₂PO₄| 0 – 50                      | 10                                            |
| H₃BO₃          | 0 – 1                       | 0.6                                           |
| Na₂EDTA        | 0 – 25                      | 7.7                                           |
| ZnCl₂          | 0 – 10                      | 0.624                                         |
| CaCl₂·2H₂O     | 0 – 10                      | –                                             |
| NaMoO₄·2H₂O    | 0 – 10                      | 0.252                                         |
| CoCl₂·6 H₂O    | 0 – 10                      | 0.420                                         |
| FeCl₃·6 H₂O    | 0 – 10                      | 2.5                                           |
| MnCl₂·4 H₂O    | 0 – 10                      | 0.36                                          |
| CuCl₂·2H₂O     | 0 – 10                      | 0.268                                         |
| MOPS Buffer    | –                           | 3.14                                          |

2.3. *Experimental design and analysis of data*

Using 10 L batch airlift fermenter, *B. braunii* was cultivated in 6 L medium, in which isolated algal cultures were illuminated under 150 μ E/m-2s-1, in ambient condition (± 27- 30°C, 1 atm), and pH was adjusted (by acid or base) to the range from 6.5 to 7.5. Each culture has equal treatment, such as aeration using available air in the airlift fermenter, volume of initial cultivation (10% v/v) at the same time and place. Cultivation media for this experiment are the same as those in starter medium, i.e. BBM, Bristol, BG-11, and MChu13 with compositions such presented in Error! Reference source not found.. The aeration and agitation rate in the cultivation reactor must be maintained constantly using airlift system at 18 L/minutes gas rate. Reynolds number should exceed 3000 to remain turbulent flow to prevent sedimentation of microalgal biomass in the cultivation reactor while the flow velocity was kept around 0.3-0.4 m/s to avoid the cell damage. During cultivation, mix gas (air–CO₂) were introduced in the fermenter. The variation of the mix gas rate is discussed in Sub-chapter 3.2.

| Composition | Bristol (gr/L) | MChu 13 | BG11 | BBM |
|-------------|---------------|---------|------|-----|
| K⁺          | 0.168         | 0.28    | 0.08 | 0.165|
| NO₃⁻        | 0.25          | 0.2     | 1.5  | 0.25 |
| PO₄³⁻        | 0.093         | 0.04    | 0.04 | 0.0915|
### 3. Results and Discussions

#### 3.1. The Effect of Cultivation Medium to the Growth of Botryococcus braunii

The research has identified the effect of cultivation media to the growth of B. braunii. The observation of the effects of each medium to the growth of B. braunii was carried out under the four growth phases, i.e. lag phase, exponential phase, stationary phase, and death phase. The growth does not occur in lag phase, therefore the growth rate is zero for the lag phase. Subsequently, the microalgae begin to be an active cell and the density will increase as a function of time during exponential phase. The growth rate in this phase is constant. After the exponential phase, the culture enters the stationary phase where the cell concentration remains constant at its maximum value and cell death. The growth phases of B. braunii in each medium composition is presented in Figure 2.

![Figure 2 The Growth curve of B.braunii.](image)

In all microalgae growth phase, exponential phase begins after the first 160 hours. However, Figure 2 shows that the lag phase does not occur. It seems that B. braunii has adapted to the surrounding environment, i.e. medium, pH, temperature and lightning. The maximum growth rate of B. braunii for each medium are occurs when the cultivation carried out during 150 hours and the gradient of the curves reduces after 150 hours. The rapid growth shows a significant increase of cell density. After B. braunii reaches stationary phase, the curves are indicated constant and continued with the constant density. The density is still increasing or decreasing due to the balance of catabolism and anabolism cells. In addition, nutrient levels in microalgae are decreasing that will stimulate the cells to form a fatty acid which is the precursor for the formation of hydrocarbons.

It also can be seen in Figure 2 that MChu13 leads to the highest growth of microalgae cell. Refer to Table 3, each of cultivation media has specific compounds that can affect the growth of B. braunii. It can be seen that BG11, BBM and Bristol are dominated by $K^+$, $NO_3^-$, $PO_4^{3-}$, $Mg^{2+}$, $Fe^{3+}$, $SO_4^{2-}$, $Cl^-$, $Ca^{2+}$. 

| Composition | Bristol (gr/L) | MChu 13 | BG11     | BBM     |
|-------------|---------------|---------|----------|---------|
| Na$^+$      | 0.275         | -       | 1.54     | 0.275   |
| Mg$^{2+}$   | 0.075         | 0.1     | 0.075    | 0.073   |
| Fe$^{3+}$   | 0.0005        | 0.01    | 0.006    | 0.005   |
| $SO_4^{2-}$ | 0.075         | 0.1     | 0.075    | 0.078   |
| Cl$^-$      | 0.0765        | 0.19    | 0.09     | 0.073   |
| $CO_3^{2-}$ | -             | -       | 0.02     | -       |
| Ca$^{2+}$   | 0.025         | 0.08    | 0.036    | 0.024   |
| EDTA        | -             | -       | 0.001    | 0.045   |
| Citric Acid | -             | 0.1     | 0.006    | -       |
| Ferric citrate |           | 0.01   |          |         |
NO$_3^-$ and Na$^+$. There were additional essential components of EDTA in BBM and BG11, high content of PO$_4^{3-}$ in Bristol, and specific essential components in MChu13. The MChu13 does not contain sodium, which contributes to the transformation of nitrogen into ammonia in algal binding molecules. However, B.braunii does not require a source of nitrogen such as ammonia. The presence of ammonia will decrease photosynthetic rate, carbohydrate formation, and growth rate.

Table 4 Biomass dry weight of B.braunii at various medium.

| Medium                  | Dry weight of B.braunii (g/L.9days) |
|-------------------------|-------------------------------------|
| MChu13                  | 0.145                               |
| Bristol                 | 0.023                               |
| BG11                    | 0.118                               |
| Bold Bassal Medium (BBM)| 0.090                               |

The growth of B.braunii were also represented as the growth of biomass dry weight. Table 4 shows the effect of cultivation medium to the dry weight of B.braunii. It can be seen from Table 4 that the highest biomass dry weight of B.Braunii was produced in MChu13. It is obviously that MChu13 medium is the best medium for cultivation medium of B.braunii. The highest growth of microalgae cell and biomass weight was obtained from MChu13 (Figure 2 and Table 4).

In this research, airlift fermenter is used to measure the growth rate of B.braunii and the ability of B.braunii to absorb CO$_2$ was identified by introducing more CO$_2$ in the cultivation reactor under the two variations of the growth medium, i.e. MChu13 and Bristol. Bristol was selected as comparison because this medium has been used for B.braunii cultivation in previous study so that required parameter for the maximum growth of B.braunii using the previous study. The growth of B.braunii cell for both medium, Bristol and MChu13 in airlift fermenter are presented in Figure 3.

Figure 3 The growth curve of B.braunii in airlift fermenter.

The results of this research show that the best growth is still provided by MChu13 medium. Bristol medium showed that the cell number of B.braunii increased in-line with increasing of dissolved CO$_2$ during the 75 hours cultivation duration with the same rate as MChu13. However, the Bristol medium indicates an earlier time of death phase. In comparison, the MChu13 shows that the cultivation is still continuous increasing until the death phase, i.e. 187.5 hours. The cell growth for B.braunii in Bristol was 0.019 cells/(mL.9hr) while in MChu13 was 0.22 cells/(mL.9hr). The MChu13 also results the best dry weight of B.brauni, i.e 0.268 g/L.7days while Bristol only 0.229 g/L.7days.

The cell growth and dry weight in MChu13 was higher than those in Bristol because the MChu13 has Fe and Mg, which is an essential component that supports the energy transfer for the formation of cell biomass [10]. However, Bristol contain PO$_4^{3-}$ slightly higher compare to other medium. The PO$_4^{3-}$ is essential to support biosynthesis of phospholipids, nucleic acids, and particular coenzyme, especially
nucleotide compounds (ATP, GTP, NAD, NADP, FAD) that are needed as electron carriers in energy transfer [11].

3.2. The effect of Dissolved CO\textsubscript{2} in Each Medium on Botryococcus braunii Growth

In the growth phase, introducing CO\textsubscript{2} into cultivation culture determines the logarithmic phase of the growth but does not determine the lag phase. During cultivation, mix gas (air – CO\textsubscript{2}) were introduced in the fermenter with variation of mix gas rate in this experiment is presented in Table 5.

| Table 5 Effect of dissolved CO\textsubscript{2} on the length of the B.braunii growth phase. |
| Run | CO\textsubscript{2} rate, L/minute | CO\textsubscript{2} concentration, % | Lag | Logarithmic |
|-----|----------------|-----------------|-----|------------|
| 1   | 0              | 0.03            | 48  | 32         |
| 2   | 1.4            | 7.8             | 48  | 49         |
| 3   | 2.1            | 11.7            | 48  | 49         |
| 4   | 2.9            | 16.1            | 48  | 49         |

Notes: Total mix gas (air – CO\textsubscript{2}) was constant at the rate of 18 L/minute

The determination of the supply rate of CO\textsubscript{2} has considered (i) typical CO\textsubscript{2} concentration in flue gas of fossil fuel combustions, which will be used as the source of CO\textsubscript{2} for supplying the B.braunii cultivation (ii) the ability of B.braunii in absorbing CO\textsubscript{2}, and (iii) the solubility of CO\textsubscript{2} in the medium where B.braunii grow (freshwater). In each run of experiments, the algae were cultivated during 7 days until the growth achieved stationer phase. Error! Reference source not found. shows that introducing CO\textsubscript{2} into algal cultivation does not control microalgae adaptation, which was shown by the phase lag. The logarithmic phase's length shows that introducing CO\textsubscript{2} encounter photo-oxidation reaction. The B.braunii can still grow with longer time since CO\textsubscript{2} emissions increase carbon sources of the medium. The result of this experiment indicates that B.braunii can grow well by absorb CO\textsubscript{2} concentration of 0.03-16.1%. The increased of CO\textsubscript{2} rate the result obtained the algae was tolerant up to CO\textsubscript{2} rate of 2.9 L/minute depend on flue gas of fossil fuel combustion as source of CO\textsubscript{2}.

3.3. Hydrocarbon Content of Botryococcus braunii

B.braunii has known contains high hydrocarbon that could achieve 40-86% of its dry weight biomass although the typical range is 25-40%. An active colony produces unbranched olefins (C27:2, C29:2, C29:3, and C31:2) that could achieve 32% of its dry weight biomass. Resting-state colonies produce unusual branched olefins (C\textsubscript{n}H\textsubscript{2n-10}; n = 30 – 37) as terpenoid origin. These olefins are named as Botryococenes components. The fraction of these components are 27-80% in natural cultivation.

This research shows that B.braunii contains unusual branched olefins, i.e. C\textsubscript{n}H\textsubscript{2n-10}; n = 32, 33, 34. Analysis of the hydrocarbon components is only for qualitative characteristics as the dry weight biomass produced from this experiment is too low (2–3.5-gram dry weight) for quantitative analysis. The B.braunii usually need a relatively long period of mass doubling time, which exceeds seven days. The measurement of hydrocarbon composition and production level would require a long experiment time. In this preliminary short time research, such measurement has not been performed.

3.4. Other Requirement Parameters for the Production System of Microalgae

Generally, in addition to a carbon source, microalgae's growth depends on i) temperature: the temperature parameter affects the diffusion rate and the activity of the microalgae's inner molecules. The lower temperature causes the molecule movement and diffusion process to obstruct, while the higher temperature causes denaturation of proteins where these microalgae intracellular enzymes will be damaged; ii) pH: pH is an essential parameter in the cultivation process of Botryococcus braunii; the appropriate pH is maintained within range 7-9. Out of the optimum pH range will affect the equilibrium of the biomass formation reaction and form unwanted deposits; iii) circulation flow rate: the cultivation is also carried out by aeration of air to supply oxygen.

Oxygen is a terminal oxidizer in electron transport systems and the essential element in ATP formation for intracellular growth. Apart from that, aeration also plays a role in the homogenization of culture and the diffusion of CO\textsubscript{2}. The more ratio of the surface area to the volume of air bubbles will
increase the CO₂ diffusion rate into the culture; iv) Lighting: the experiment utilizes external lights to provide appropriate light intensity in the photosynthesis process of the microalgae. The light is used to form NADPH and ATP, which will be used in dark reactions. In the dark reaction, building blocks of glucose and other organic compounds will be formed.

It is also expected that the growth of Botryococcus braunii would be affected by the presence of other constituents in each medium where the microalgae live. The MChu13’s predominance among the different autotrophic medium (BBM, Bristol, and BG11) is its high Fe dan Mg content [11]. Fe dan Mg is an essential component that supports the energy transfer events so that the formation of cell biomass was getting better.

4. Conclusions
In this research, B.braunii were successful cultivated in an Airlift Fermenter with several mediums of cultivation. The main objective of the cultivation is to produce biohydrocarbon and CO₂ biofixation. Among cultivation mediums used in this research, MChu13 was identified as the best for B.braunii cultivation with the cell growth of 0.22 cells/(mL.9hr) and high biomass productivity of 0.145 dry weight (g/L.9days) while in Bristol medium the cell growth is only 0.019 cells/(mL.9hr) and biomass productivity is only 0.023 dry weight (g/L.9days).

The optimum growth of other medium (Bristol) is only during the period of 75 hours after that period the microalgae will be in death phase. During the cultivation, cell population of the B.braunii increases in-line with the increasing of dissolved CO₂. After 75 hours cultivation period, the cell density decreases rapidly and the culture eventually collapses. As comparison, the microalgae growth optimum in MChu13 is in the period of 187.5 hours. The research shows that MChu13 is the best medium for CO₂ fixation. The dry weight biomass production is 0.268 g/L.7days in MChu13 medium while in Bristol is only 0.229 g/L.7days.

The CO₂ emission from fossil fuel-based power plant contributes significantly to the national GHG emissions of energy sector. The emission has been increased significantly during the past decade due to the increasing number of fossil fuels power plants in Indonesia. This paper presents results of a research on microalgae cultivation for producing biomass and hydrocarbons for biofuels that simultaneously fixing CO₂ emissions from coal power plant effluent through photosynthesis process, which also for the implementation of circular economy. B.braunii microalgae could be one of mitigation measures to reduce the CO₂ emissions due to the capability of microalgal to utilize CO₂ as a carbon source for their growth, which also at the same time serves as bio-fixation of CO₂ emissions. B.braunii can grow well by absorbing the CO₂ of about 0.03-16.1%. The increased of CO₂ rate the result obtained the algae was tolerant up to CO₂ rate of 2.9 L/minute depend on flue gas of fossil fuel combustion as source of CO₂. In addition, B.braunii microalgae could also produces alternative biofuels (gasoline and biodiesel).

B.braunii usually contain high hydrocarbon (40-86% of its dry weight biomass), an active colony produces unbranched olefins (C27:2, C29:2, C29:3, C31:2) that could achieve 32% of its dry weight biomass. A resting-state colony produce unusual branched olefins (CₙH₂ₙ₋₁₀; n = 30-37) as terpenoid origin that could achieve 27-80% in natural cultivation. These olefins are Botryococccenes components. This research shows that B.braunii contains unusual branched olefins, i.e. CₙH₂ₙ₋₁₀; n = 32, 33, 34.

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References
[1] Hauck J T, Olson G J, Scierka S J, Perry M B, and Ataai M M 1996 Effects of simulated flue gas on growth of microalgae Abstracts of Papers. American Chemical Society, 212 Meeting, Pt.1, Fuel 118, Orlando, FL 25–29 August 1996
[2] Hossein A A, Nahid R, Raul G, Abbas A, and Jose R M 2019 Microbial Cell Factories p18
[3] Cossarizka and Limawan 2005 *The Effect of Dissolved CO$_2$ in The B. Braunii Cultivation and CO$_2$ Bio-fixation* (Bandung, Indonesia: Institute Teknologi Bandung)

[4] Dewi RG 2003 *Proc. Int. Conf. Effect of Dissolved CO$_2$ on Growth and Hydrocarbon Content of Micro Algae ‘Botryococcus Braunii’* Osaka Gas Foundation of International Cultural Exchange

[5] Jay M, Kawaroe M, Effendi H 2017 *Earth and Environmental Science*

[6] Dewi R G 2009 *Symp., on 16th Asean Regional Symposium on Chemical Engineering Integrated Up Lift Vertical Grow Bioreactor for Microalgae Cultivation in the Context of Biofixation of CO$_2$ Emission and Production of Biofuel* December 1-2th, 2009, Manila Hotel, Philippines

[7] Droop M R 1967 *Br. Phycol. Bull* 3 295–297

[8] Hegewald E 1985 *Cramer, Braunswchweig* 41 219-271

[9] Nomura A M and Calif D M 1986 (Berkely : The University of California)

[10] Khalid A, Mohamed T, Brian H M, Stella S, Andrew S B, and Eric M A 2015 *European Journal of Phycolgy* vol 51

[11] Anindita R and Jayanthy M T 2010 *Studi Pertumbuhan Botryococcus braunii Pada Berbagai Medium Pertumbuhan dalam Reaktor Terintegrasi Airlift-Vertical Growth yang Ditunjukkan untuk Biofikasi CO$_2$ dan Produksi Biofuel* (Bandung, Indonesia: Institute Teknologi Bandung)

[12] Wolf F R 1983 *Applied Biochemistry and Biotechnology* 8

[13] Srikaton D 2009 *Integrated Up Lift Vertical Grow Bioreactor for Microalgae Cultivation in the Context of Biofixation of CO$_2$ Emission and Production Biofuel* (Bandung, Indonesia: Institute Teknologi Bandung)

[14] Vonshak A 1986 *Handbook for Algal Mass Culture*, Richmond A, CRC Press (Florida) 117- 146.

[15] Pengfei C, Yan W, Qiyong Y and Tianzhong L 2017 *International Journal of Agricultural and Biological Engineering* 10 134–141