Cultivation of 'Botryococcus Braunii' Microalgae for Hydrocarbons Production and CO_2 Bio-fixation

Abstract The CO_2 fixation for climate change mitigations through photosynthesis can be carried out by growing intensively managed microalgae. The capability of microalgae in the CO_2 fixation is favourable compare to trees or crops, yielding three to five times more biomass per land area than typical crops or plants. Another interesting feature of microalgae is microalgal farms 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 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 CO_2 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 CO_2 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 CO_2 concentration to the growth rate, biomass production, lipid and hydrocarbon content and type. The microalgae were cultivated in fermenter (bioreactor) supported with airlift system for supplying CO_2/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 CO_2 fixation from Botryococcus braunii. 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 lined with the CO_2 fixation rate.

Keywords: *biofixation, Botryococcus braunii, circular economy, CO_2 emissions, hydrocarbon, lipid, microalgae, mitigation measures, photosynthesis, uplift fermenter, vertical grow reactor*

1. Introduction

In recent years, atmospheric carbon dioxide level is increasing on global scale due to 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

microalgal 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, microalgal 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 strains that contain high lipid or hydrocarbons are needed. This paper presents results of a research on microalgal cultivation, in which a strain of Botryococcus braunii (B.braunii) microalgae is used 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 level of 150 μ E/m^{2}s where the power capacity of each lamp is 8/25 watt/lumen [6].

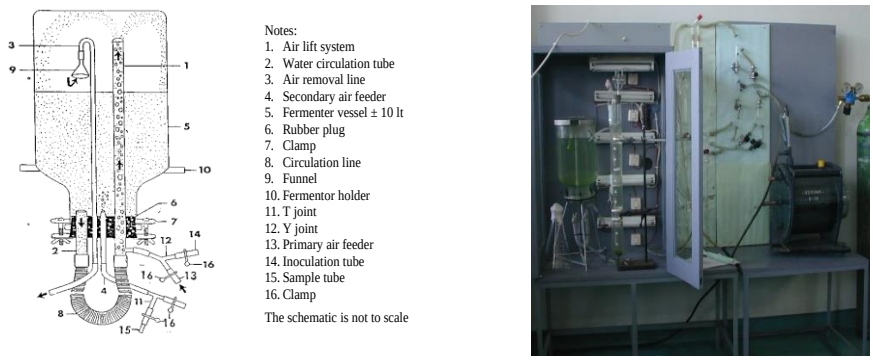


Figure 1 Schematic of Airlift Fermenter System [6]

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 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 pediastrum (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 slants to obtain unialgal isolates.

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

Ml	Stock solution	gram/400 ml H_2O
10	$NaNO_3$	10
10	$CaCl_2.2H_2O$	1
10	$MgSO_4.7H_2O$	3
10	K_2HPO_4	3
10	KH_2PO_4	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, CO_2 concertation, air supply flowrate, etc. The activation was carried out in 1 L glass bottle supported by airlift for supplying air (containing CO_2 and O_2). 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.**.

Requirements in developing nutrient recipes for algal cultivation are described as the following section.

1) Algal biomass (50%) consists of carbon, therefore sufficient carbon is important for cultivation. For freshwater (low salinity) and neutral pH, carbon is supplied as inorganic substance (gaseous CO_2). 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). It can be supplied as nitrate, ammonia, and urea. Changes in nitrogen supply 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.

Table 2 Required components of the B.braunii cultivation medium [9]

Component	Required Concentration, mg/L	US Patent Plant 6, 169 Concentration, mg/L H_2O
Ca $(NO_3).4H_2O$	50 – 250	26,5
NH_4Cl or NH_4HCO_3	10 – 150	–
$MgSO_4.7H_2O$	10 – 100	25
K_2HPO_4 or KH_2PO_4	0 – 50	10
H_3BO_3	0 – 1	0.6
Na_2EDTA	0 – 25	7.7
$ZnCl_2$	0 – 10	0.624
$CaCl_2.2H_2O$	0 – 10	–
$NaMoO_4.2\ H_2O$	0 – 10	0.252
$CoCl_2.6\ H_2O$	0 – 10	0.420
$FeCl_3.6\ H_2O$	0 – 10	2.5
$MnCl_2.4\ H_2O$	0 – 10	0.36
$CuCl_2.2H_2O$	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.**.

Table 3 Composition of Botryococcus braunii cultivation medium [6]

Composition	Bristol (gr/L)	MChu 13	BG11	BBM
K^+	0.168	0.28	0.08	0.165
NO_3^-	0.25	0.2	1.5	0.25
PO_4^{3-}	0.093	0.04	0.04	0.0915
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		

In addition, 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–CO_2) were introduced in the fermenter. The variation of the mix gas rate is discussed in Sub-chapter 3.2.

3. Results and Discussions

3.1. The effect of Cultivation Medium to the Growth of Botryococcus braunii

The research identified the effect of cultivation media to the growth of B.braunii. The observation of the growth of B.braunii were 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, in which the growth rate is zero. Subsequently, 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 of B.braunii in each medium composition is presented in Figure 2

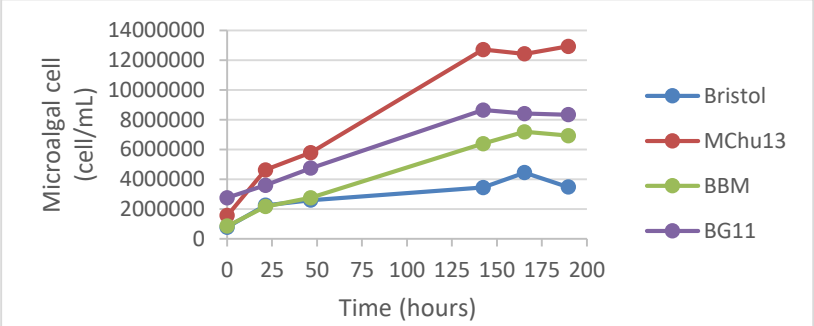


Figure 2 The Growth curve of B.braunii

In most microalgae growth, the exponential phase usually 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 that were continued with the constant density. Although 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 affects 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+}. 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 the photosynthetic rate, the carbohydrate formation, and the 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.branuii 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.

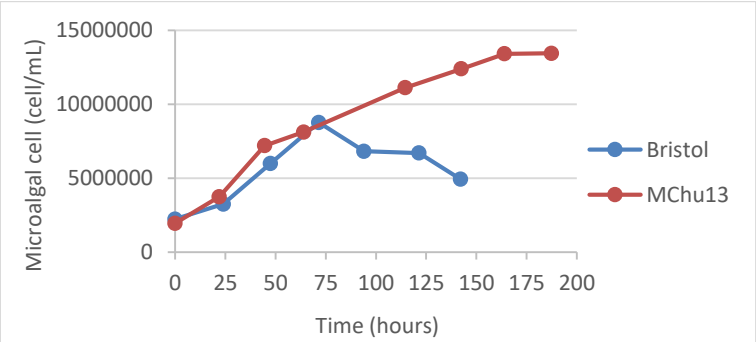


Figure 3 The Growth curve of B.braunii in airlift fermenter

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 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 $PO4^{3-}$ slightly higher compare to other medium. The $PO4^{3-}$ 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_2 in Each Medium on Botryococcus braunii Growth

In the growth phase, introducing CO_2 into cultivation culture determines the logarithmic phase of the growth but does not determine the lag phase. During cultivation, mix gas (air–CO_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_2 on the length of the B.braunii growth phase

Run	CO_2 rate, L/minute	CO_2 concentration, %	The Growth Phase	
			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_2) was constant at the rate of 18 L/minute

The selection of the CO_2 rates has considered (i) typical CO_2 concentration in flue gas of fossil fuel combustions, which will be used as the source of CO_2 for supplying the B.braunii cultivation (ii) the ability of B.braunii in absorbing CO_2, and (iii) the solubility of CO_2 in the medium where B.braunii grow (freshwater). In each run of the experiment, the algae were cultivated during 7 days for the growth

purposes till the growth achieved the stationer phase in the microalgae growth curve. **Error! Reference source not found.** shows that introducing CO_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_2 encounter photo-oxidation reaction. The B.braunii can still grow with longer time since the CO_2 increases the carbon sources of the medium.

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_nH_{2n-10}; n = 30 – 37) as terpenoid origin. These olefins are named as Botryococcenes 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_nH_{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_2. The more ratio of the surface area to the volume of air bubbles will increase the CO_2 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

The CO_2 emission from fossil fuel power plant contributes significantly to national GHG emissions in energy sector. The emissions 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 the CO_2 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 CO_2 emissions due to the capability of microalgal to utilize CO_2 as a carbon source for their growth, which also at the same time serves as bio-fixation of the CO_2. In addition, B.braunii microalgae could also produces alternative biofuels (gasoline and biodiesel).

In this study, B.braunii were successfully cultivated in variations medium in an Airlift Fermenter with the main objective to produce bio-hydrocarbon and bio-fixation. Among medium used in this

research, MChu13 medium is identified as the best for B.brauni cultivation with the growth level of 0.019 cells/(mL.hr) and high biomass productivity of 0.0327 g/L day.

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 of microalgae B.braunii, the cell population of the B.braunii increases in-line with the increasing of dissolved CO_2. 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 164 hours. The biomass production using the Airlift fermenter in MChu13 is 0.145 g/L.9days while in Bristol is 0.023 g/L.9days, BG11 is 0.118 g/L.9days, and Bold Bassal Medium is 0.09 g/L.9days.

In addition, the MChu13 medium is also the best medium for CO_2 fixation with the dry weight biomass production is 0.229 g/L.7days in Bristol medium while in the MChu13 medium is 0.268 g/L.7days. The biomass production using in MChu13 is 0.145 g/L.9days, while in Bristol is 0.023 g/L.9days, BG11 is 0.118 g/L.9days, and Bold Bassal Medium is 0.09 g/L.9days.

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References

[1] Hauck JT, Olson GJ, Scierka SJ, Perry MB, and Ataai MM 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 RG 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 MR 1967 *Br. Phycol. Bull* **3** 295–297
[8] Hegewald E 1985 *Cramer, Braunschweig* **41** 219-271
[9] Nomura A M and Calif D.M 1986 (Berkely : The University of California)
[10] Khalid A, Mohamed T, Brian HM, Stella S, Andrew S B, and Eric M A 2015 *European Journal of Phycology* vol **51**
[11] Anindita R and Jayanthy MT 2010 *Studi Pertumbuhan Botryococcus braunii Pada Berbagai Medium Pertumbuhan dalam Reaktor Terintegrasi Airlift-Vertical Growth yang Ditunjukkan untuk Biofiksasi CO_2 dan Produksi Biofuel* (Bandung, Indonesia: Institute Teknologi Bandung)
[12] Wolf FR 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, R*ichmond 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