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Contributed Paper

Hydrocarbon Production and Biodiesel Properties of a Green Microalga *Botryococcus braunii* KMITL 2 Cultivated Outdoor in Open Pond and Closed Photobioreactor

Suneerat Ruangsomboon
Program in Fisheries Science, Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
* Author for correspondence; e-mail: suneerat.ru@kmitl.ac.th

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ABSTRACT

Botryococcus braunii is a promising feedstock for biodiesel production. In general, selection of the most suitable cultivation systems is based on profitable yield and oil quality. The feasibility of using this alga as feedstock for commercial biodiesel production was tested by comparing the cultivation in open pond and closed photobioreactor under the 5 $g \cdot kg^{-1}$ optimum salinity condition previously found, and the results were that the hydrocarbon content (40.7±1.6%) of the alga cultivated in open pond was higher than that cultivated in the photobioreactor and its biomass (2.9±0.1 $g \cdot L^{-1}$) was much higher; its biodiesel properties, however, were nearly identical with cetane values of 48.15-56.99. Drastically different though were the hydrocarbon yields-cultivation in the open pond produced 2.3 times higher yield than the closed photobioreactor. Hence, outdoor cultivation under optimum salinity in open pond was considered suitable for biodiesel production by this algal strain.

Keywords: carbohydrate, carotenoid, cetane, chlorophyll, fatty acid, protein

1. INTRODUCTION

Recently, microalgae have attracted a lot of attention as feedstock for third-generation biodiesel production [1] because they do not need to be reserved as human food and they need less space to cultivate than land plants do. Previous studies have reported alternative feedstock for biodiesel [2-4]. Among the previously investigated microalgae, a green microalga *Botryococcus braunii* has been widely accepted as an alga that gives high hydrocarbon yield with good biodiesel quality, exceeding the cetane number (CN) specification of two international standards, a minimum of 51 and 47, respectively [5-9].

Many attempts have been made to cultivate *B. braunii* in a way that would produce more hydrocarbon and lipid contents [3]. In general, algae produce more lipid content when they are stressed. One method to stress certain strains of *B. braunii* and stimulate their hydrocarbon and lipid production is to cultivate them in a medium with some salinity.

There have also been findings that a suitable salinity level was able to increase *B. braunii* biomass yield [10].

B. braunii strain KMITL 2 was collected and isolated from a reservoir in the central region of Thailand. It was then cultivated and its potential as a source for biodiesel production was tested. Previous preliminary findings on this strain indicated that it was able to produce a high lipid content of 49.9-54.7% [10, 11], and an optimum salinity of 5 $g \cdot kg^{-1}$ was able to [10].

One of *B. braunii* KMITL 2's properties investigated was its biodiesel quality. Biodiesel quality properties such as cetane number, oxidative stability, and cold-flow properties (cloud point and cold-filter plugging point) [12] needed to be determined whether they conformed to the global biodiesel standards. These properties are directly influenced by the strain's fatty acid profile.

In order to cultivate sufficient amount of any algae for commercial production, they need to be cultivated outdoor. Outdoor cultivation can be made in open pond or closed photobioreactor. Cultivation in open pond costs less but runs a risk of contamination while cultivation in a closed photobioreactor costs more but is less likely to get contaminated. There have been reports that cultivation of algae in a photobioreactor provided much greater biomass and oil yield per hectare compared to cultivation in open pond [13].

Different cultivation systems also differently affect the type and amount of algal composition. Biochemical composition of microalgae primarily composes of carbohydrate, protein and lipid, these can be used as a biofuel feedstocks, such as bioethanol, biohydrogen and biogas [14, 15]. Moreover, chlorophyll and carotenoid, valuable for food, pharmaceutical and cosmetic industries [16], were also found in microalgae [17]. Therefore, for culturing the algae as a feedstock for biodiesel production, it is not only necessary to determine the optimum cultivation system for its growth but also the optimum cultivation system for the most suitable biochemical composition for biodiesel production.

In the case of outdoor cultivation, light and temperature fluctuate depending on seasonal change. Thus, to find out which cultivation system is optimum when cultivation location is fixed, both open pond and close-photobioreactor systems must be tested at the same time and place as well as with the same medium volume. The result will indicate the optimum cultivation system for the algae under natural light intensity.

The specific aims of this research were to determine the hydrocarbon production and biodiesel properties of *B. braunii* cultivated outdoor in open pond and closed photobioreactor in order to find the better cultivation method that would produce a higher hydrocarbon yield and better biodiesel properties for biodiesel production.

2. MATERIALS AND METHODS

2.1 Stock Algal Culture

Botryococcus braunii K□ tzing strain KMITL 2 obtained from Klong Boat reservoir, Nakhon Nayok province, Thailand. The GenBank accession numbers was KX470608. The strain was cultured in a chlorella medium, optimum for it growth and lipid production [11], in a laboratory at 25°C in 1 L glass flasks under constant light from 200 mmol photons$\cdot m^{-2} \cdot s^{-1}$ fluorescent lamps and air bubbling, and the culture was used as stock for the next experiment.

2.2 Growth, Chemical Composition and Biodiesel Quality of *B. braunii* Cultured Outdoor under an Optimum Salinity Condition in Open Pond and Closed-photobioreactor

Since previous studied showed that a salinity of 5 $g \cdot kg^{-1}$ gave the highest biomass [10], we decided to compare the growth of the alga cultivated in a closed-photobioreactor and in open pond under the same salinity and environmental conditions. Salinity levels of 5 $g \cdot kg^{-1}$ was made by diluting natural seawater with tap water. After that, chlorella medium was added. The open ponds used consisted of four 300-L fiberglass tanks (90 × 119 × 36 cm) with a water depth of 35.5 cm and flow speed 0.15 $m \cdot s^{-1}$ (Figure 1A). The closed-photobioreactor used was a multi-layer horizontal tubular photobioreactor consisted of 20 continually-circulated, 5.5-cm diameter and 230-cm long transparent acrylic pipes of 300 L total volume with a flow speed of 0.15 $m \cdot s^{-1}$. The distance between the entrance and exit to the degasifier unit of photobioreactor was 46 m (Figure 1B). Constant natural air bubbling was supplied via two bubble air stones (1.5 inch in diameter) in the pond for creating movement of the medium. Tap water (pH 7) was filled up to the original level to replace evaporated water every day.

Figure 1. *B. braunii* cultivated outdoor in open pond (A) and closed-photobioreactor (B).

The algae in both cultivation systems were supplied with 10 $L \cdot min^{-1}$ constant natural air (0.04% CO_2). The initial algal inoculum was 0.20 $g \cdot L^{-1}$. The range of air temperature, water temperature and natural light during algal cultivation were 26.5-37.5°C, 26.1-37.3°C and 65-1,480 mmol photons$\cdot m^{-2} \cdot s^{-1}$ (during day time), respectively. Light intensity was determined at the horizontal surface of the photobioreactor and open pond. Four replicate samples were taken and analyzed for biomass, chlorophyll-a, carotenoid, carbohydrate, and protein every 2 days and for hydrocarbon content and fatty acid composition every 4 days until stationary phase was reached (around 20 days).

The microscopic examination of the culture from both cultivation systems in order to monitor contamination from plankton was performed every 2 days. Contaminants found in open ponds after 10 days of cultivation were ciliated protozoa, whereas no contamination in closed-photobioreactor. However, the number of protozoa was low because of the salinity in

the culture medium. The effect of protozoa on algal dry weight was negligible.

2.3 Determination of Algal Biomass, Pigment, Carbohydrate and Protein

Samples of 10-mL culture suspension were filtered with glass microfiber filter paper (GF/C, Whatmann) and washed with distilled water to remove salt. The collected algal cells on the filter paper were dried at 105°C for 24 h then cooled in a desiccator to room temperature, and the weight of the dry biomass was measured. Chlorophyll-a and carotenoid contents were determined [18]; carbohydrate content was analyzed by a phenol sulfuric acid method [19]; protein content was determined by Lowry method [20]; and specific growth rate (m) was calculated [21].

2.4 Extraction of Hydrocarbon

Microalga culture was harvested by filtering it with 20 µm nylon mesh and washed with distilled water and dried at 40°C then ground into powder with mortar and pestle. N-hexane was used to extract dried biomass, and a Transonic model 460/H (Elma, Singen, Germany) was then used to sonicate the extracted mixture at 70 Hz at room temperature. The extraction procedure was repeated twice. The total extract solution was subjected to silica gel (Silica gel 60, 230-400 mesh, Merck) column chromatography with n-hexane as the mobile phase. All elutes before a yellow band of carotenes were collected. Then, the hydrocarbon extract was dried in a rotary evaporator and weighed.

2.5 Analysis of Fatty Acids

Lipids were extracted from dried biomass with a mixture of chloroform and methanol (1:2 v/v) [22], and the extracted mixture was sonicated at 70 Hz at room temperature. The extraction procedure was repeated twice. Then, direct transmethylation of the lipid extract was used to convert fatty acids into methyl esters, which was then detected by a flame ionization detector (FID) of an Agilent Technologies 6890 N Gas Chromatography system (USA). All of the methods mentioned in this section were already described in more detail in one of our previous works [23].

2.6 Estimation of Biodiesel Quality Properties

Several biodiesel quality properties were calculated by formulas used by previously reported works [24-26]; these properties were saponification value (SV), iodine value (IV), cetane number (CN), degree of unsaturation (DU), long-chain saturated factor (LCSF), and cold filter plugging point (CFPP).

2.7 Statistical Analysis

Four replicates of experimental data were averaged and their standard deviations were calculated by using Microsoft Excel 2010. *Student's t-test was used to* evaluate statistically significant differences between the values at 95% confidence interval ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Growth and Chemical Composition of *B. braunii* Cultivated Outdoor under an Optimum Salinity Condition in Open Ponds and Closed-photobioreactor

Normally, in a closed photobioreactor, oxygen generated from photosynthesis is a particularly serious problem and can decrease the algal biomass. Oxygen concentrations above 35 mg L^{-1} are toxic to most of microalgae species [27]. However, in this study the maximum dissolved oxygen in photobioreactor was 10.9 mg L^{-1} which nearly similar with open pond (10.7 mg L^{-1});

thus, the effect of dissolved oxygen on algal cells in both systems should be similar.

The alga cultivated in open pond exhibited the significantly higher biomass yield of 2.9±0.1 g·L^{-1} (Figure 2A) after 16 days of cultivation and the highest specific growth rate of 0.31±0.02 d^{-1} (Figure 2B) after 4 days of cultivation. The hydrocarbon content and hydrocarbon yield in both treatments tended to increase when cultivation time increased (Figure 2C-D), the significantly higher hydrocarbon content and hydrocarbon yield from the open pond were 40.7±1.6% and 1.1±0.0 g·L^{-1}, respectively. The significantly higher hydrocarbon productivity from the open pond was 87.99±1.11 mg·L^{-1} d^{-1} on the 4th day of cultivation (Figure 2E).

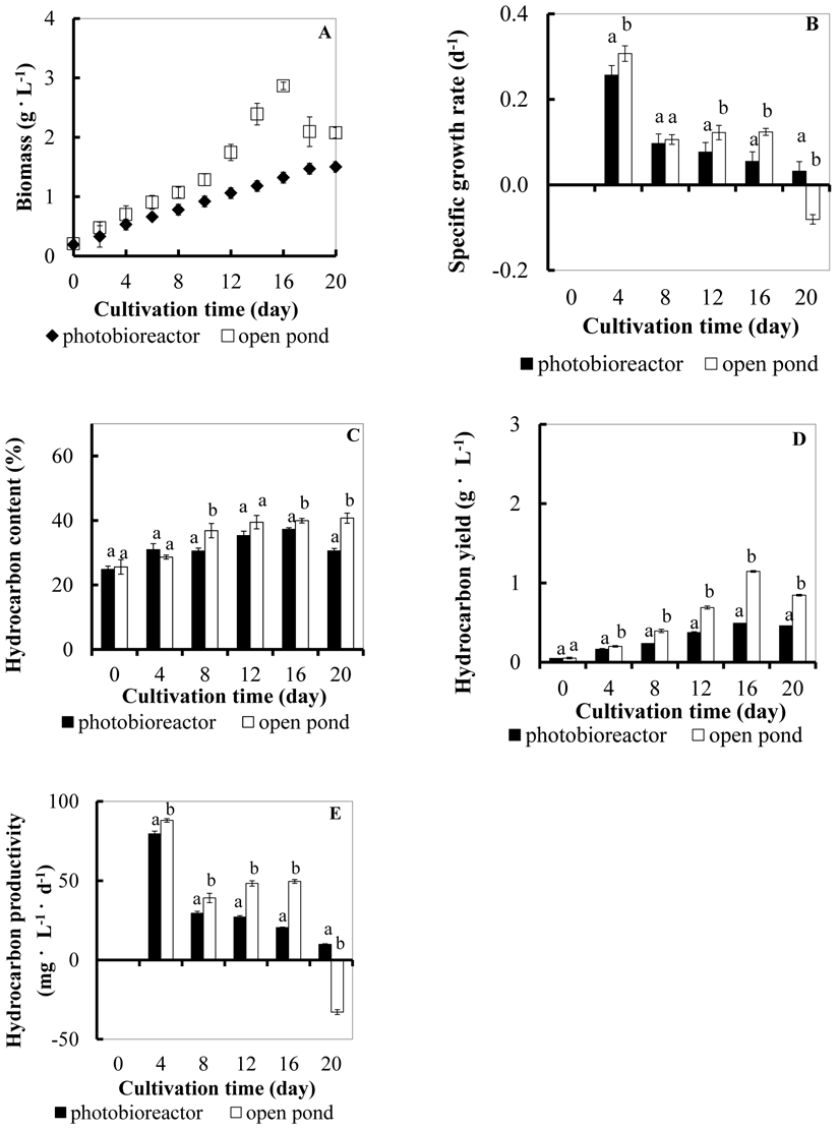

Figure 2. Biomass (**A**), specific growth rate (**B**), hydrocarbon content (**C**), hydrocarbon yield (**D**), and hydrocarbon productivity (**E**) of *B. braunii* cultivated outdoor in open pond and closed-photobioreactor. Different small letters on the bars indicate significant difference between treatment ($p < 0.05$). Error bars represent ± S.D. of four replicates.

As for outdoor cultivation at the optimum salinity level in open pond and closed-photobioreactor, cultivation in the open pond gave 2.2, 1.3 and 2.3 times higher biomass yield, hydrocarbon content and hydrocarbon yield.The algal density in open pond (1,554±27 $g \cdot m^{-2}$) was almost similar to that in the closed-photobioreactor (1,486±7 $g \cdot m^{-2}$). Hydrocarbon content in both cultivation systems tended to increase when cultivation time was increased. The reason for this increase might be because the alga produced more and more hydrocarbon from photosynthesis as the nutrients in the medium were depleted [28].

One of the reasons the alga investigated in this study grew better in open pond than in a closed-photobioreactor might be because there was lower CO_2 input into the reactor. Both cultured systems were supplied with 10 $L \cdot min^{-1}$ natural air, but in the open pond, CO_2 in the air could dissolve into the pond from direct water surface contact, thus they had a higher CO_2 level than that in the closed-photobioreactor. This extra CO_2 might be the main cause of the higher growth rate and biomass yield.

The chlorophyll-a content of *B. braunii* cultivated in open pond (1.68±0.05 $mg \cdot L^{-1}$) was significantly higher than that from the photobioreactor (0.91±0.05 $mg \cdot L^{-1}$) (Figure 3A). Chlorophyll-a content was correlated with biomass, the more biomass the more chlorophyll content (Figure 2A). The carotenoid, carbohydrate and protein contents of alga cultivated in the closed-photobioreactor were significantly higher than those of alga cultivated in the open pond. The highest levels were 0.90±0.03 $mg \cdot L^{-1}$, 117.63±3.85 $mg \cdot g^{-1}$ and 206.79±20.28 $mg \cdot g^{-1}$, respectively (Figure 3B-D). The maximum carotenoid content found in this study was lower than that achieved by *B. braunii* 765 that had a carotenoid content in the range of 13.65-16.68 $mg \cdot L^{-1}$ [29]. Carbohydrate content decreased with cultivation time in opposite to hydrocarbon content which increased (Figure 2C), possibly due to the alga's converting more carbohydrate into lipid and hydrocarbon as the nutrients in the media became depleted [28].

Carbohydrate (3.0-8.2%) and protein (4.9-16.8%) contents of *B. braunii* KMITL 2 cultivated in open pond were lower than those reported by Ashokkumar and Rengasamy [4] at 18±0.9 and 17±0.9%, respectively, where *B. braunii* AP103 was cultivated in an open raceway pond. In this study the KMITL 2 strain showed low carbohydrate and protein contents, so it is not suitable as a feedstock for bioethanol or biomethane production [14, 15]. Before hydrocarbon extraction, the carbohydrate and protein contents were 2.5±0.5% and 12.3±1.4%, respectively. For the residual biomass after hydrocarbon extraction, they were 8.2±0.7% and 32.5±1.9%, respectively. The amount of carbohydrate remained in the residual biomass after hydrocarbon extraction was too low for bioethanol production. However, the protein content in the residual biomass was much higher than the 20 and 22% lower limit of protein source for aquatic and land animal feed, respectively [30]; thus, it can serve as a protein supplement in the animal diets.

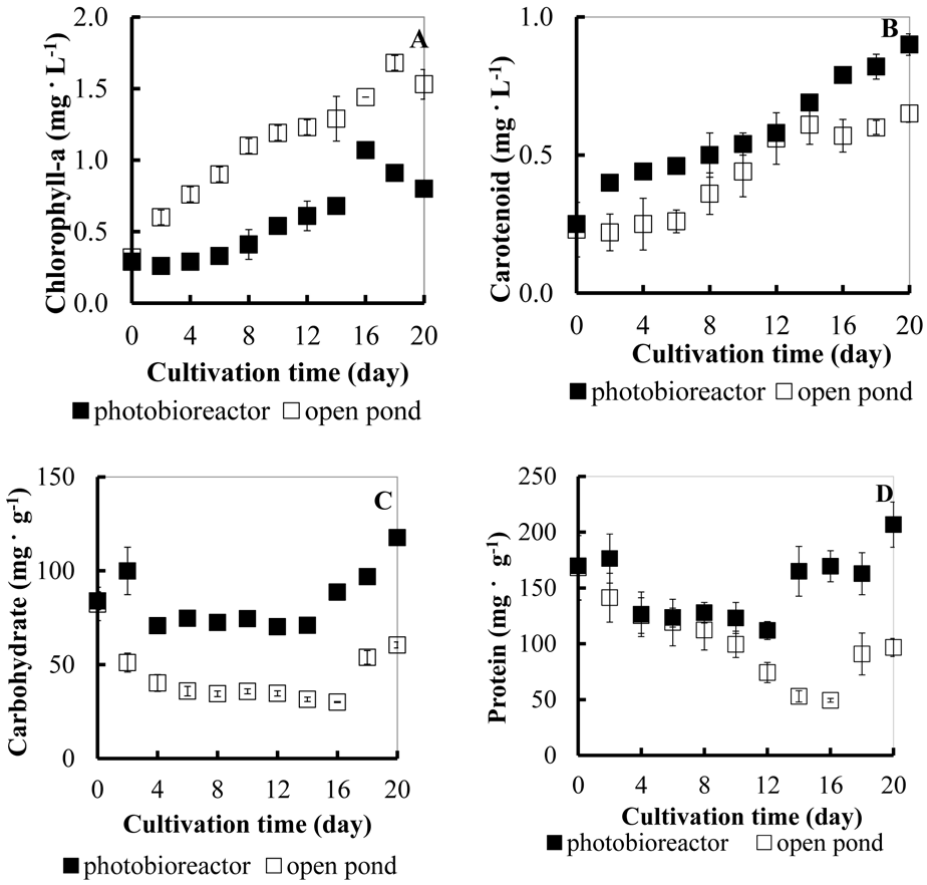

Figure 3. Chlorophyll-a (**A**), carotenoid (**B**), carbohydrate (**C**), and protein (**D**) contents of *B. braunii* cultivated in open pond and closed-photobioreactor. Error bars represent ± S.D. of four replicates.

3.2 Fatty Acid Profile of *B. braunii* Cultivated Outdoor in Open Pond and Closed-photobioreactor at the Optimum Salinity Level

Both fatty acid compositions of the two cultures of *B. braunii* cultivated under the same 5 g·kg^{-1} salinity condition for a period of 20 days in the open pond and in the photobioreactor (Table 1-2) were nearly the same. The most frequently found fatty acid in open pond and a closed-photobioreactor throughout the cultivation period was C16:0 in the range of 28.7-39.1 and 25.5-31.9%, respectively; the percentage of saturated fatty acid were 54.2-64.8 and 54.2-64.1%, respectively; the percentage of unsaturated fatty acid were 35.2-45.8 and 35.9-45.8%, respectively; and the percentage of C16-C18 were 66.7-75.9 and 55.3-74.5%, respectively (Table 1-2). The higher the percentage of C16-18 is the better the lipid of an alga is for biodiesel production because more C16-18 provides higher cetane number and more heat of combustion [25]. *B. braunii*'s fatty acid composition found in this study is similar to that reported in previous works [9].

Table 1. Fatty acid profiles of *B. braunii* cultivated outdoor in open pond.

Fatty acid (%)	Cultivation time (day)					
	0	4	8	12	16	20
C4:0	2.1	3.5	1.1	0.3	0.4	0.3
C6:0	0.4	0.5	0.3	0.4	0.2	0.5
C8:0	4.9	5.2	2.7	1.8	1.2	0.7
C10:0	6.1	6.6	9.2	6.0	12.0	8.2
C11:0	2.7	4.0	2.9	2.2	3.8	1.6
C12:0	0.8	0.2	0.2	0.1	0.0	0.0
C13:0	1.8	1.4	1.2	2.8	1.2	1.5
C14:0	2.2	2.3	1.9	3.1	2.9	2.5
C14:1	0.7	0.6	0.5	1.0	0.8	0.8
C15:0	0.8	0.8	0.8	1.2	1.0	0.9
C15:1	0.7	0.6	0.7	1.0	0.7	0.8
C16:0	29.8	29.5	28.7	38.7	32.1	39.1
C16:1	2.0	1.9	1.4	1.8	0.9	0.6
C17:0	2.6	1.8	1.7	1.8	2.1	3.6
C17:1	2.5	2.4	2.4	3.3	3.1	3.2
C18:0	1.8	1.6	1.7	2.4	2.4	2.7
C18:1n9t	7.0	7.2	5.6	6.2	5.7	6.3
C18:1n9c	7.0	7.3	4.5	4.3	2.3	3.7
C18:2n6t	5.5	5.6	2.2	3.2	2.5	1.8
C18:2n6c	1.1	1.2	0.8	1.1	1.3	1.1
C18:3n3	3.0	2.5	2.1	2.9	4.2	4.7
C18:3n6	7.1	6.5	21.4	8.2	9.9	9.0
C20:0	1.6	1.0	1.2	1.5	2.4	2.1
C20:1	1.8	2.4	1.2	1.4	1.2	1.4
C20:2	0.1	1.1	0.2	0.2	0.2	0.2
C20:3n3	0.2	0.2	0.1	0.1	0.1	0.1
C20:3n6	0.6	0.2	0.1	0.1	0.1	0.2
C20:4n6	0.5	0.1	0.0	0.1	2.2	0.3
C:20:5n3	0.7	0.1	0.1	0.2	1.7	0.1
C22:0	0.2	0.3	0.1	0.1	0.1	0.1
C22:1n9	0.2	0.4	0.3	0.5	0.4	0.4
C22:2	0.4	0.2	0.4	1.0	0.2	0.2
C22:6n3	0.3	0.2	1.9	0.4	0.2	0.3
C23:0	1.0	0.6	0.5	0.5	0.2	0.5
C24:0	0.0	0.0	0.0	0.2	0.0	0.4
C24:1	0.1	0.0	0.0	0.0	0.0	0.2
Saturated fatty acid	58.8	59.3	54.2	63.0	62.2	64.8
Unsaturated fatty acid	41.2	40.7	45.8	37.0	37.8	35.2
Monounsaturated fatty acid	21.9	22.7	16.6	19.5	15.1	17.2
Polyunsaturated fatty acid	19.4	18.0	29.2	17.5	22.7	18.0
C16-C18	69.3	67.4	72.4	73.9	66.7	75.9

Table 2. Fatty acid profiles of *B. braunii* cultivated outdoor in a closed-photobioreactor.

Fatty acid (%)	Cultivation time (day)					
	0	4	8	12	16	20
C4:0	2.1	0.4	1.2	0.1	0.1	0.4
C6:0	0.4	0.3	0.3	0.3	0.1	0.1
C8:0	4.9	0.8	0.8	0.4	0.2	0.4
C10:0	6.1	0.8	4.0	0.5	0.2	0.3
C11:0	2.7	0.8	3.3	2.6	1.1	0.1
C12:0	0.8	1.6	1.3	1.8	2.2	0.1
C13:0	1.8	0.8	2.5	0.9	1.1	2.6
C14:0	2.2	3.0	1.4	3.0	3.0	0.4
C14:1	0.7	0.9	0.5	0.1	1.3	0.4
C15:0	0.8	0.8	0.7	0.7	0.7	1.1
C15:1	0.7	0.6	0.6	0.5	1.2	0.3
C16:0	29.8	29.3	25.5	29.4	31.9	28.5
C16:1	2.0	1.0	0.9	1.1	1.2	1.5
C17:0	2.6	1.5	1.2	1.6	1.0	1.1
C17:1	2.5	1.6	1.5	1.7	2.3	2.3
C18:0	1.8	16.2	15.0	20.9	17.5	15.5
C18:1n9t	7.0	7.2	5.6	5.4	5.2	6.2
C18:1n9c	7.0	1.5	1.4	11.4	8.9	7.1
C18:2n6t	5.5	1.4	2.1	1.8	2.2	1.9
C18:2n6c	1.1	0.4	1.1	0.2	0.7	0.9
C18:3n3	3.0	0.4	0.6	0.7	0.7	0.8
C18:3n6	7.1	13.8	0.4	0.1	0.5	0.9
C20:0	1.6	0.3	1.4	1.0	1.2	1.1
C20:1	1.8	0.5	0.6	0.2	0.6	0.5
C20:2	0.1	0.7	0.9	0.6	0.2	7.0
C20:3n3	0.2	0.5	0.4	0.4	0.2	0.8
C20:3n6	0.6	0.7	0.0	0.1	1.0	0.9
C20:4n6	0.5	0.4	0.7	0.5	0.5	0.4
C:20:5n3	0.7	0.7	0.2	0.0	0.4	0.2
C22:0	0.2	1.5	2.6	0.9	1.8	1.8
C22:1n9	0.2	0.8	0.8	1.2	0.5	0.7
C22:2	0.4	1.1	0.8	1.5	1.1	0.5
C22:6n3	0.3	6.4	13.4	8.3	8.2	12.3
C23:0	1.0	0.6	1.2	0.0	0.2	0.3
C24:0	0.0	0.4	0.0	0.0	0.5	0.2
C24:1	0.1	0.1	5.0	0.1	0.2	0.2
Saturated fatty acid	58.8	59.2	62.4	64.1	62.9	54.2
Unsaturated fatty acid	41.2	40.8	37.6	35.9	37.1	45.8
Monounsaturated fatty acid	21.9	14.3	17.0	21.7	21.5	19.2
Polyunsaturated fatty acid	19.4	26.5	20.6	14.1	15.7	26.6
C16-C18	69.3	74.5	55.3	74.3	72.2	66.7

3.3 Biodiesel Properties of *B. braunii* Cultivated Outdoor in Open Pond and Closed-photobioreactor at the Optimum Salinity Level

The saponification value (SV)-a measure of the average molecular weight (or chain length) of all of the fatty acids present-of the alga cultivated in open pond and the closed-photobioreactor were in the ranges of 212.76-229.81 and 193.64-223.10 (Table 3), respectively. The iodine values (IV) of both types of cultivation were also similar. The IV values of algae cultivated in the open pond and closed-photobioreactor were 58.48-90.72 and 64.74-98.60 g $I_2 \cdot 100\ g^{-1}$, respectively. IV is a measure of the total unsaturation of a biodiesel that is related to its oxidative stability [16]. A biodiesel with high IV is less oxidatively stable than one with a lower IV. The European standard for maximum IV is 120 g $I_2 \cdot 100\ g^{-1}$ [5, 6]. The IV values of *B. braunii* cultivated in open pond and in the closed-photobioreactor throughout the cultivation period were lower than 120 g $I_2 \cdot 100\ g^{-1}$ which meet the standard criteria. The IV values varied unpredictably with cultivation time.

Table 3. Calculated biodiesel properties of *B. braunii* cultivated outdoor in open pond and closed-photobioreactor throughout a 20-day cultivation period.

Cultivation time (day)	SV	IV (g $I_2 \cdot 100\ g^{-1}$)	CN	DU (wt.%)	LCSF (wt.%)	CFPP (°C)
Open pond						
0	223.10	64.74	54.26	60.59	5.80	1.73
4	229.81	59.66	54.84	58.71	5.19	-0.18
8	218.48	90.72	48.15	74.94	5.04	-0.65
12	213.22	58.48	56.99	54.43	7.10	5.82
16	217.83	72.52	52.86	60.49	7.05	5.66
20	212.76	59.68	56.73	53.23	8.19	9.26
Photobioreactor						
0	223.10	64.74	54.26	60.59	5.80	1.73
4	200.07	90.75	50.44	67.29	14.29	28.43
8	203.14	87.91	50.75	58.13	15.41	31.93
12	198.87	66.85	56.70	49.98	15.82	33.21
16	197.83	71.26	55.72	52.79	16.96	36.80
20	193.64	98.60	49.34	72.43	14.75	29.86

As for the cetane number (CN), *B. braunii* cultivated in the open pond and closed-photobioreactor at the optimum salinity level exhibited the highest CN of 56.99 and 56.70, respectively, on the 12th day. CN differentiates biodiesel according to its ignition delay time and combustion quality. A higher CN indicates better ignition and engine performances. As far as biodiesel quality goes, the minimum CN should be at least 47 or 51 according to two global standards [5, 6]. The CNs of *B. braunii* from all experimental treatments were in the range of 48.15-65.18. The highest CNs found in this study were higher than those produced by other *B. braunii* strains reported in other studies to be 52.67 [7], as 55.4 [9], and 51.92-53.47 [8].

Another indicator of oxidative and long-term storage stability is degree of unsaturation (DU)-a lower DU is more stable in long-term storage. DU values of this algal strain cultivated in open pond and closed-photobioreactor varied from 53.23-74.94 and 49.98-72.43%, respectively. These values were much lower than the 76.53-132.08% of green microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa* [26].

B. braunii cultivated in open pond and closed-photobioreactor showed long-chain saturated factor (LCSF) in the range of 5.04-8.19 and 5.80-16.96%, respectively, and cold-filter plugging point (CFPP) in the range of -0.65-9.26 and 1.73-36.80°C, respectively. As for correlated LCSF and CFPP which lower value indicating better low-temperature biodiesel properties [31]. Low-temperature properties depend mostly on saturated fatty acids content, hence unsaturated fatty acid composition exerts negligible effect [24]. The alga cultivated outdoor in the closed-photobioreactor had a high CFPP value than 28°C, so it may cause clogging under low temperature. Further investigation and adjustment are needed if it is desirable to use this kind of cultivation environment.

4. Conclusion

Finally, in terms of suitability for biodiesel production, outdoor cultivation that limited natural gas aeration of *B. braunii* in the open pond was more suitable than cultivation in the closed-photobioreactor under the same optimum conditions because their biodiesel properties, which far exceeded the global standards. Moreover, *B. braunii* cultivated in the open pond gave 2.3 times higher hydrocarbon yield.

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