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Improving the Energy Balance of Hydrocarbon Production Using an Inclined Solid–Liquid Separator with a Wedge-Wire Screen and Easy Hydrocarbon Recovery from *Botryococcus braunii*

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Received: 27 July 2020; Accepted: 6 August 2020; Published: 10 August 2020



Abstract: The green colonial microalga *Botryococcus braunii* produces large amounts of hydrocarbons and has attracted attention as a potential source of biofuel. When this freshwater microalga is cultured in a brackish medium, the hydrocarbon recovery rate increases; furthermore, the colony size becomes large. In this study, the effects of such changes on the energy balance of harvesting and hydrocarbon recovery were studied via filtrate experiments on an inclined separator and extraction from a concentrated slurry. The inclined separator was effective for harvesting large-colony-forming algae. The water content on the wire screen of slit sizes larger than 150 μm was <80% and a separation rate of >85% could be achieved. The input energy of the harvesting using the brackish medium with this separator was $\approx 44\%$ of that using the freshwater medium with vacuum filtration, while the input energy of the hydrocarbon recovery using the brackish medium was $\approx 88\%$ of that using the freshwater medium with pre-heating before *n*-hexane extraction. Furthermore, the energy profit ratio of the process in the brackish medium was 2.92, which was ≈ 1.2 times higher than that in the freshwater medium. This study demonstrated that filtration techniques and hydrocarbon recovery from *B. braunii* with a low energy input through culture in a brackish medium are viable.

Keywords: microalgae; inclined solid–liquid separator; hydrocarbon recovery; biofuel; energy balance; harvesting

1. Introduction

Botryococcus braunii (*B. braunii*) is an autotrophic alga that can potentially be employed as a biofuel resource because this alga produces hydrocarbons during cell division and secretes them in an oleophilic extracellular biopolymer. First, *B. braunii* produces and stores hydrocarbons up to 86% of its dry weight [1,2]. Although hydrocarbons are produced in species of all alga pyhla, their contents are low and the lipids that most microalgae can store with a high content rate are triglycerides or fatty acids [2,3]. Meanwhile, *B. braunii* produces hydrocarbons that can be classified based on their type [4]; for example, the hydrocarbons accumulated by the A, B, and L races of *B. braunii* are *n*-alkadienes and *n*-alkatrienes, mainly C_{30} to C_{37} triterpenoids, and tetraterpenoids, respectively. Among the specified races, race B has been widely studied for liquid biofuel production because it stores hydrocarbons at relatively high volumes and can be easily decomposed into suitable fuels for internal combustion engines via hydrocracking [5]. Second, *B. braunii* produces hydrocarbons during cell division [6].

Most microalgae start to produce lipids under environmental stress conditions, such as nutrient depletion. This feature allows for easier culture management under continuous conditions on a large scale and a high algal density can be maintained to prevent contamination; however, the growth rate of *B. braunii* is slower than other oil-producing microalgae. This may be because not only saccharides or proteins but also high energy hydrocarbons similar to fossil fuel are produced with cell division. Third, *B. braunii* secretes hydrocarbons in the oleophilic extracellular biopolymer that connects cells undergoing cell division [7,8]. Other algae store lipids in the cell interior and hence extra processes involving degradation of the cell wall are required to effectively recover them [9,10]. Therefore, biofuel production from *B. braunii* can potentially save extra input energy and production costs during the culture, lipid-recovery, and fuel-conversion processes.

Various methods have been reported for hydrocarbon recovery from *B. braunii*. Efficient hydrocarbon recovery may be achieved using an amphiphilic solvent, such as a methanol/chloroform mixture or dimethyl ether [11,12]. Hydrothermal liquefaction or supercritical carbon dioxide extraction, in which organic solvents are not required, is also reportedly effective for hydrocarbon recovery from *B. braunii* [13,14]. These methods require high-temperature or high-pressure conditions; otherwise, the recovery of some polar solvents from the water phase is difficult. Meanwhile, non-polar solvent extraction, for example, *n*-hexane extraction of soybean oil, can also be applied to *B. braunii* because this alga stores hydrocarbons in the oleophilic extracellular biopolymer. However, the direct recovery rate from wet slurries is low, which is a limitation of this methodology [15]. Cells and thin layers containing hydrocarbons are entirely enclosed by fibrillar structures, i.e., the colony sheath, on the retaining wall [16,17]. This sheath mainly consists of saccharide components that prevent the entry of non-polar solvents into the colony interior [18]. Various pretreatments, such as drying [19], homogenization [20], and pre-heating [21], have been reported to improve hydrocarbon recovery using *n*-hexane, but these methods also require thermal or electrical energy as an input for treating algal slurries. Previously, we reported that the hydrocarbon recovery rate could be improved from *B. braunii* cultured in a brackish medium (BM) with a salinity of 3 g L⁻¹ without growth inhibition [22]; this improvement was attributed to a shortening in the colony sheath and low fibrilliform density via a shift in algal metabolism from the biosynthesis of the colony sheath consisting of polysaccharides that surround the algal colonies to produce osmolytes of disaccharides and obtain salt tolerance [18]. When the salinity in the medium rises above 3 g L⁻¹, the hydrocarbon recovery rate is improved more but the growth is inhibited. It is considered that this salinity is the limit of salt tolerance for the culture of freshwater microalga *B. braunii*. This is different from the approach in which a high osmotic shock is used to disrupt cell walls to extract lipids [23]. Saga et al. evaluated the energy profit ratio (EPR) of the hydrocarbon recovery process with a pre-heating treatment by changing the ratio of *n*-hexane to the algal slurry or the water content of the slurry [24]. EPR is the index used to evaluate the energy investment efficiency of energy production equipment and is obtained by dividing the production energy by the fossil energy input. Culturing in the BM can decrease the input energy required for hydrocarbon recovery compared to a pre-heating process.

Moreover, algal culture in a BM increased the colony size, with the median particle diameter of colonies increasing from ≈60 μm in a freshwater medium (FM) to over 200 μm in a BM [22]. Increases in the colony size of *B. braunii* are achieved using a high light intensity [25] or in the presence of 40 mM glucose [26]. Our light condition (100 μmol m⁻² s⁻¹) is classified into low density compared with Zhang and Kojima [25]. Tanoi et al. reported that it was suggested that the high osmotic pressure from glucose in a culture medium affects the colony size [26]. They inferred the colony size was related to the change in polysaccharides or other matrix components, such as algaenan, via the culture conditions created by the high light intensity or osmotic pressure. The BM in this study contained 30 mM NaCl of the main components and an ion concentration of NaCl such that the osmotic pressure was doubled. The osmotic pressure was not significantly different from the literature. The brackish medium caused a shift in the algal metabolism of saccharides, as mentioned above. We consider that the changes in saccharide metabolism via the high osmotic pressure in the BM affected the colony size. Although

B. braunii is a colonial and large alga compared to other unicellular algae, it is difficult to harvest concentrated algal slurries before hydrocarbon recovery. Centrifugation, flocculation, or filtration via cloth is generally used for harvesting algae [27]. In this context, inclined solid–liquid separators with wedge-wire screens are often used for sewage treatment. In this apparatus, wastewater comes into contact with an inclined screen with slits in the tangential direction; the filtrate passes through the screen and the concentrated slurry or solid particles are continuously recovered on the screen. This separator requires only pump power for harvesting and ensures easy maintenance. No case has yet been made for introducing the inclined separator for harvesting *B. braunii*. The harvesting of large-colony-forming *B. braunii* cultured in the BM via this separator would lead to obtaining under 80% water content of slurry-like sewage treatment and decreasing the input energy required for harvesting.

In this study, the effect of the hydrocarbon recovery rate and colony size of *B. braunii* cultured in a BM on the EPR was investigated. We first evaluated the harvesting process based on filtrate experiments using an inclined solid–liquid separator with a varying slit size for the different colony sizes of *B. braunii* cultured in different media. Second, hydrocarbon recovery was evaluated based on solvent extraction experiments on a concentrated algal slurry because we previously performed the solvent extraction only for low algal concentrations in a culture fluid. Lastly, the EPR was analyzed based on the data from these experiments and the literature.

2. Materials and Methods

2.1. Microalgae Cultivation

B. braunii race B (Showa strain) was cultured in a 10 L culture bag aerated with 1% CO₂ at 25 °C under illumination (100 μmol m^{−2} s^{−1}) with a 12 h light–dark cycle for 30 days. Two types of culture media were used: a modified Chu13 medium as the freshwater medium (FM) and a brackish medium (BM) with a salinity of 3 g L^{−1}, which was prepared by diluting commercial artificial seawater (Daigo's Artificial Seawater SP for Marine Microalgae Medium, Wako Pure Chemical Industries, Japan; total salinity, 36 g L^{−1}) [22]. In our previous study, we found that this salinity did not inhibit algal growth or the hydrocarbon production rate (0.031 g-hydrocarbons L^{−1} d^{−1} in both the FM and the BM) [22]. Nutrients and trace metals, such as KNO₃, K₂HPO₄·3H₂O, and FeNaEDTA, were already present in the Chu13 medium, but not in the artificial seawater. Therefore, they were added to the brackish medium at levels similar to those in the Chu13 medium.

2.2. Filtration Using an Inclined Solid–Liquid Separator with a Wedge-Wire Screen

In this test, culture liquids with an algal density of 1.0–1.5 g L^{−1} were used. The algal density was measured after the algal culture liquid was filtered through a glass fiber filter (GF/A 110 mm diameter, Whatman, Germany), which was rinsed with deionized water to wash the salt in the culture media and dried at 105 °C for 24 h. The concentrated algal slurry on the screen was recovered and the water content was also measured by drying at 105 °C for 24 h. Algal fluid (20 L) was allowed to flow into the first screen of the separator at a flow rate of 3 L min^{−1} from a tank positioned at a height of 350 mm above the separator. Figure 1 shows the side view of the inclined solid–liquid separator. The filtrate was passed through the wire screen and collected. The wedge wires were triangles pointing toward the filtrate and were lined vertically relative to the flow direction. The shape of the triangular cross-section led to minimizing the contact area and clogging of the screen when solids passed through the screen. The separator consisted of screens positioned at two different angles, where the inclination angles of the first and second screens were 66° and 51°, respectively. The concentrated slurry could be recovered after the culture liquid passed through the second screen. The size distribution of the algal colonies in this study was measured using a laser diffraction particle-size-distribution analyzer (MT–3300EXII, Nikkiso, Japan). Based on the measured colony size, the slit size of the wedge-wire screen was determined to be 10, 20, 30, 50, and 75 μm for samples cultured in the FM, and 50, 100, 150,

200, and 300 μm for samples cultured in the BM. The dry-matter separation rate (R_s) of the separator was calculated as follows:

$$R_s = M_s TS_s / M_c TS_c \quad (1)$$

where M_s and M_c (kg) are the masses of the recovered algal slurry and algal culture liquid, respectively, and TS_s and TS_c (kg kg^{-1}) are total algal solids in the recovered algal slurry and algal culture liquid, respectively.

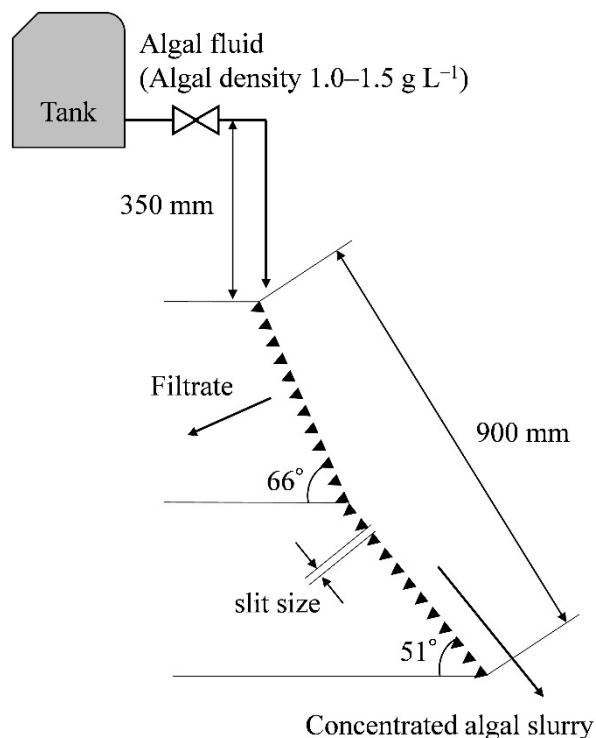


Figure 1. Side view of the inclined solid–liquid separator consisting of two wedge-wire screens at different angles.

2.3. Hydrocarbon Recovery from Concentrated Algal Slurries

The alga was concentrated using vacuum filtration through a 20 μm nylon mesh and the obtained slurry was rinsed with deionized water. The water content and total solids (TS) content in the concentrate were adjusted by the degree of the vacuum and measured using a moisture analyzer (MX-50, A&D, Japan). Solvent extraction from 10 g of the wet algal slurry was conducted in a 250 mL Teflon vessel. In this process, the algal slurry was dispersed in *n*-hexane (weight of *n*-hexane/dry weight of algal slurry = 6/1) and the mixture was stirred at 800 rpm. The sediment was rinsed using the same amount of *n*-hexane twice to reflect the actual counter-current multistage (three-stage) extraction. The hydrocarbon mass dissolved in the recovered solvent was measured using programmed-temperature gas chromatography with flame ionization detection (GC-FID) by using a capillary column (GC-2014 with Rtx-1 capillary column, Shimadzu, Japan). The temperature program was as follows: the column was maintained at 50 $^{\circ}\text{C}$ for 1 min, heated from 50 to 220 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C min}^{-1}$, maintained at 220 $^{\circ}\text{C}$ for 3 min, heated from 220 to 260 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C min}^{-1}$, and equilibrated at 260 $^{\circ}\text{C}$ for 3 min [28]. The hydrocarbon mass was calculated from the sum of the peak areas corresponding to hydrocarbons relative to the peak areas for known amounts of standard hydrocarbons extracted and purified from freeze-dried algal samples; this process is described below.

The hydrocarbons contained in the alga were extracted from dry algal samples. The extraction from dried *B. braunii* using *n*-hexane or *n*-heptane was carried out to measure the content of external hydrocarbons [15] rather than the total lipids in the alga [29,30]. Freeze-dried algal samples were

soaked in *n*-hexane. The extraction process was repeated until the yellow pigments that co-existed with the hydrocarbons in the extracellular matrices were lost. Subsequently, the extracts were combined and subjected to silica-gel column chromatography. The fraction of pure hydrocarbons obtained after the chromatography was weighed and a standard curve was constructed for GC-FID [22]. GC-electron impact mass spectrometry analysis (GCMS-QP2010, Shimadzu, Japan) with a capillary column (InertCap 1MS, GL Science, Japan) was carried out under the same temperature program as the GC-FID analysis. The major eight components that are typical of methylated squalene (C30–34 botryococcene) were identified in accordance with Atobe et al. [28]. The number of peaks and hydrocarbon elution patterns were similar between the GC-FID and GC-MS. The hydrocarbon recovery rate was expressed as the ratio of the amounts of hydrocarbons recovered from the wet algal slurry to the hydrocarbon content.

2.4. Energy Profit Ratio Calculation

Figure 2 compares the hydrocarbon production from the BM and the FM (with pre-heating before the hydrocarbon extraction). Alga cultured in the BM entered the hydrocarbon recovery process directly after the inclined solid–liquid separator or vacuum filtration. In contrast, alga cultured in the FM was introduced into the hydrocarbon recovery process through vacuum filtration and pre-heating the algal slurry containing 92% water at 95 °C. The thermal pretreatment dissolved all amphiphilic fibrils of the colony sheath into the hot water. The slurry was rinsed twice after pre-heating and subjected to vacuum filtration because the released fibrils formed emulsions with *n*-hexane and prevented hydrocarbons extraction [24]. Although the thermal pretreatment was effective for *B. braunii* cultured in BM, the pretreatment with BM was not considered in order to show the energy profit ratio and hydrocarbon recovery rate from the concentrated slurry that had a different colony surface structure. Under these conditions, it was assumed that the water content reduced from 92% to 70%. The input energy can be categorized into thermal energy and electrical energy for mechanical operations (e.g., for the pump). In this study, the receiving and efficiency in Japan were set at 36.9%. Assuming a rotary vacuum belt filter for the vacuum filtration (TSK belt filter, TSK, Japan), the filtration time was set at 20 s based on the dipping rate and rotational frequency of the drum (Table 1). The algal filtration throughput over 20 s was determined at a gauge pressure of 40 kPa using the vacuum leaf test (Leaf Tester, Miyamoto, Japan); in this case, a 20 µm nylon mesh was used as the filter cloth. The electrical energies of the pump (2) and belt filter (3) were calculated using the following equations:

$$E_s = \rho V g H / \Phi \quad (2)$$

$$E_b = P_g V / \Phi \quad (3)$$

where E_s and E_b (W) represent the electric power required for transferring the algal culture liquid (1.0 g L^{−1}) to the filtration unit and vacuum pump of the belt filter, respectively; ρ (kg L^{−1}) is the culture density (1.0 kg L^{−1}); V (L s^{−1}) is the throughput flow rate; g (m s^{−2}) represents acceleration due to gravity; H (m) is the pump head; P_g (Pa) is the gauge pressure; and Φ (-) indicates the pump efficiency (0.7). The EPR was calculated using the following relationship on the basis of 1 kg of dry algae (4):

$$\text{EPR} = \text{HHV of recovered hydrocarbons} / \text{Input energy} \quad (4)$$

Here, HHV (MJ) represents the higher heating value. It was calculated from the elemental composition using Dulong's formula, as shown in Equation (5) [31]:

$$Q = 0.3383C + 1.442(H - O/8) \quad (5)$$

where Q (MJ) is the heating value. The elemental compositions of the *B. braunii* and the purified hydrocarbons were analyzed using two elemental analyzers (NCH-22F, Sumika Chemical Analysis Service, Ltd., Japan and EMGA-920, Horiba, Ltd., Japan). The mixing power of the slurry was measured

using a mixing torque meter (ST-300II, Satake Chemical Equipment, Japan) and the slurry was prepared by mixing *B. braunii* with a 70% water content and *n*-hexane (*n*-hexane/dry weight of algal slurry = 6/1).

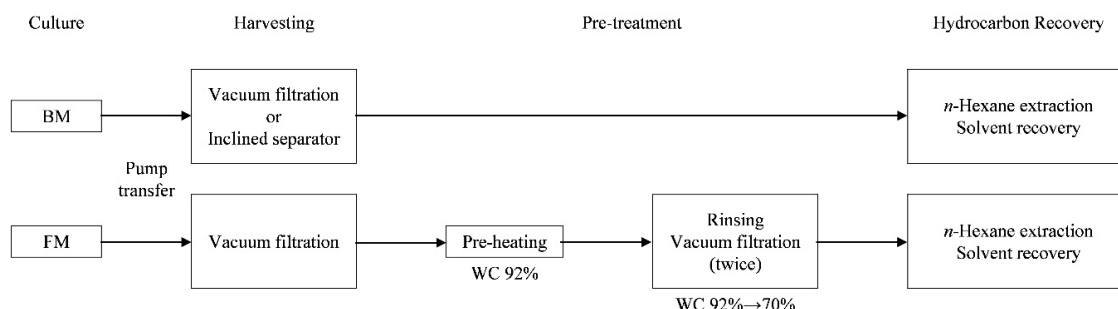


Figure 2. Hydrocarbon production from algal cultures in brackish medium (BM) or freshwater medium (FM) with pre-heating before the hydrocarbon extraction. WC: Water content.

Table 1. Parameter values used to calculate the energy profit ratio.

Item		Units	Value	Ref.
Rotary vacuum belt filter	Drum diameter	m	4.2	TSK
	Drum length	m	7.6	
	Rotational speed	rpm	1.0	
	Dipping rate	-	0.33	
	Motor output	kW	10	
	Gauge pressure	kPa	40	
Pump head		m	10	Set in this study
Algal throughput by one belt filter unit	FM	kg s ⁻¹	0.23	Leaf test
	BM	kg s ⁻¹	0.58	Leaf test
Elemental composition of <i>B. braunii</i>	C	%	71.4	Elemental analyzer
	H	%	10.3	
	N	%	1.21	
	O	%	13.6	
Elemental composition of the hydrocarbons	C	%	87.4	Elemental analyzer
	H	%	12.1	
Higher heating value	<i>B. braunii</i>	MJ kg ⁻¹	36.6	Dulong's formula
	Hydrocarbons	MJ kg ⁻¹	47.0	
	<i>n</i> -hexane	MJ kg ⁻¹	46.2	
Hydrocarbon content	FM	kg kg ⁻¹	0.35	[22]
	BM	kg kg ⁻¹	0.35	[22]
Hydrocarbon recovery rate	Pre-heating	%	95.0	[24]
	BM		95.0	From this study
Pinch temperature for pre-heating		°C	20	[19]
Specific heat	<i>B. braunii</i>	kJ kg ⁻¹ K ⁻¹	1.9	[19]
	Water	kJ kg ⁻¹ K ⁻¹	4.18	
Latent heat	<i>n</i> -hexane	MJ kg ⁻¹	0.34	[19]
	Water	MJ kg ⁻¹	2.25	
<i>n</i> -hexane recovery rate			99.9	[19]
Evaporated water loss/residual hexane		kg kg ⁻¹	154	[19]
Stirring power on extraction from slurry		W kg ⁻¹	15	Torque meter
Receiving and efficiency	Japan	%	36.9	

Table 1 shows the values of the parameters used in this study to calculate the EPR. The hydrocarbon contents of the alga cultured in both media were set at 35.0% [22]. In this previous report, the brackish medium led to a tendency toward a high hydrocarbon content with no significant difference rather than no effect. However, a slight difference in hydrocarbon contents has a huge effect on the energy profit ratio. Hence, the hydrocarbon contents were set at the same ratio in this study in order to show the difference of input energy in each process. The elemental compositions of the *B. braunii* cultured in the BM were supposed to be equal to the alga in the FM regarding hydrocarbon contents, although the saccharide metabolites were particularly changed by the BM.

3. Results and Discussions

3.1. Harvesting Using the Inclined Solid–Liquid Separator with Wedge-Wire Screens

Figure 3 shows the size distributions of the algal colonies used in this study. Table 2 shows the results of harvesting using the inclined solid–liquid separator with wedge-wire screens of different slit sizes selected based on the measured colony size (Figure 3). Figure 4 shows the apparatus used in this experiment and *B. braunii* recovered from the BM culture on the screen. High separation rates of >90% were achieved with a slit size of less than 30 μm in the FM, but the water content in the slurry recovered on the screen was >99.0%. This was because the water did not pass through the wire screen due to its high surface tension. When the slit size was larger than 50 μm , the separation rate decreased sharply and the water content was found to be >99.0%. At a slit size of 75 μm , almost all the alga passed through the screen and could not be recovered on the screen. In conclusion, the alga in the FM could not be concentrated by the inclined separator due to its small colony size. Pressure injection from the nozzle might be needed to recover small colonies and a low slurry content from the wedge-wire screen with a slit size of less than 30 μm . In contrast, this separator was effective for separating the algae cultured in the BM. Although the water content of the slurry on the screen with a slit size of 50 μm was 96.6%, the water content at slit sizes larger than 150 μm was below 80%. Moreover, unlike in the case of the FM culture, there was no sharp decrease in the separation rate from the BM. A separation rate of >85.0% was achieved with a slit size of less than 150 μm and the water content of the slurry on the screen did not change; this rate decreased only when the slit size was larger than 150 μm . The water content after centrifugation was about 99% and after the vacuum belt filter, it was about 75% [32], which was similar to the inclined separator in this study. This separator had the same effect as the vacuum belt filter without using any electrical energy input when the *B. braunii* cultured in the BM was used.

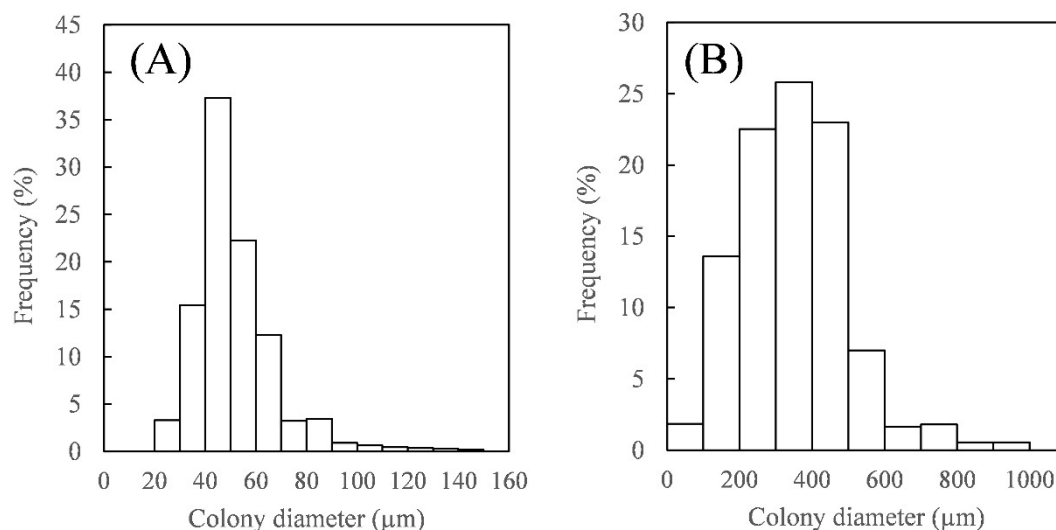


Figure 3. Size distributions of algal colonies cultured in (A) the freshwater medium and (B) the brackish water medium.

Table 2. Effect of the slit size of the wedge-wire screen on the separation rate and water content of the algal slurries recovered from different media. FM: Freshwater medium, BM: Brackish medium. At a slit size of 75 μm , almost all the algae cultured in the FM passed through the screen, and hence the water content and the separation ratio could not be determined (ND).

Medium	Slit Size (μm)	Aperture Ratio (%)	Separation Rate (%)	Water Content (%)
FM	10	2.0	96.3	99.9
	20	3.8	93.0	99.7
	30	5.7	91.3	99.3
	50	4.8	51.3	99.1
	75	7.0	ND	ND
BM	50	4.8	96.0	96.6
	100	9.1	92.6	83.0
	150	13.0	86.7	78.9
	200	16.7	77.8	77.4
	300	16.7	66.2	78.8



Figure 4. Harvesting of *B. braunii* cultured in the brackish medium (BM) using an inclined solid–liquid separator with a wedge-wire screen (150 μm slit size).

Moreover, we considered that the colony increase was caused by the changes in a factor related to saccharide metabolism that accelerated or inhibited the colony division via high osmotic pressure in the BM; however, the obvious reason, as mentioned in the introduction, was not found to be the case. The colony organization of *B. braunii* is like a flower bouquet in which the cell apex is directed toward the surface of the colony and matrix that stores hydrocarbons fills the space between the cells [18]. Cells in the center of the large formed colony clearly stored the hydrocarbons in cells and matrices, as seen using fluorescent microscopy and freeze-substitution electron microscopy. *B. braunii* produces hydrocarbons in cell division. This was not directly evidenced by the cell viability in the inner colony; however, the growth and hydrocarbon contents in the BM with 3 g L^{−1} was equal to that from the

FM [22]. This also shows that the colony increase was not caused by the simple accumulation of lipids. As another possibility, the improvement of light transmittance via colony enlargement may have affected the photosynthetic efficiency of cells outside the colony, although cells in the inner colony were dead. Further analysis of the colony structure and metabolome is required in the next challenge in order to confirm whether the colony enlargement was the cause.

Figure 5 shows the input electrical energy required for each harvesting process. The inclined separator with the FM (Figure 2) was not considered because the alga cultured in the FM could not be recovered by the filtration process described earlier. The total input energies of the BM harvesting with the inclined separator, the BM with the vacuum filtration, and the FM with the vacuum filtration were 0.14, 0.21, and 0.32 MJ kg⁻¹, respectively. Using the BM, the input energy could be reduced by 56% when compared to that using the FM. Two points must be particularly mentioned here. The first is that the pump transfer consumed a large proportion of the input energy. In this study, the pump head was set at 10 m on the basis of a general centrifugal pump. When the algal slurry was transferred from the edge of a wide pond to the harvesting apparatus, the pump head exceeded the selected value in some cases. However, the inclined separator did not require electrical energy and was portable, unlike the vacuum-filtration setup. This means that the algae could be harvested at the edge of the pond. The second point is that in this study, it was assumed that during the vacuum filtration, no cracks were formed in the residue on the filter cloth. The occurrence of cracks increased the input energy of the vacuum pump due to air suction [33]. Therefore, the BM-inclined separator combination required lower input energy than the FM-based approaches. The operating costs would reduce as the input energy reduces compared with vacuum filtration. The introduction motive of the inclined separator is the low initial and management costs if the target of the filtration can be recovered. This is because the wire screen is easy to wash due to being made of stainless steel and requires no motor. The cost calculation should be considered by obtaining the maximum throughput via filtrating further amounts of algae in the next step.

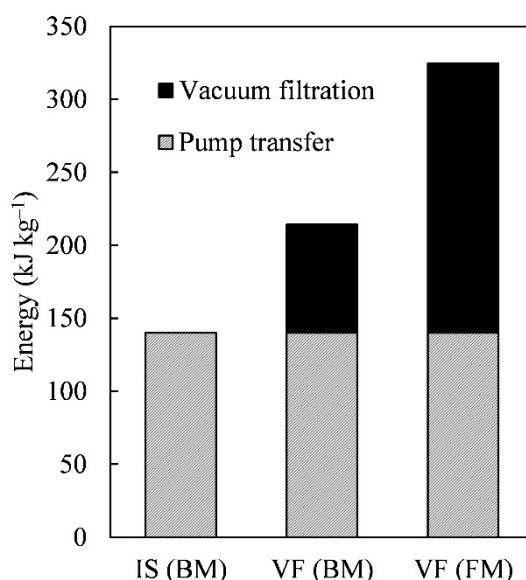


Figure 5. Input electrical energy per unit of dry algae in the algal harvesting from cultures in different media using an inclined solid–liquid separator (IS) or using vacuum filtration (VF). FM: Freshwater medium, BM: Brackish medium.

3.2. Hydrocarbon Recovery Rate from the Concentrated Algal Slurry

Figure 6 shows the effects of the water content in the algal slurry and culture medium on the hydrocarbon recovery rate. A hydrocarbon content of >95% could be recovered in 4 h when the water content in the slurry cultured in the BM was 70%. Furthermore, the hydrocarbon recovery rate

increased with a decrease in the water content. In the case of the FM, the recovery rates were only 20% or 47% within 9 and 12 h, respectively. Saga et al. reported that a hydrocarbon content of >95% could be recovered from a pre-heated *B. braunii* slurry (same strain as that used in this study) within 2 h under similar solvent conditions [24]. Furthermore, it took only 45 min to recover 90% of the hydrocarbon content from 60%-water-content algal pellets. Pre-heating was found to exert a more significant effect on the hydrocarbon recovery than the BM culture. The extraction time for a 95% hydrocarbon recovery was almost 4 h in the BM system but only 2 h was required with the FM and pre-heating.

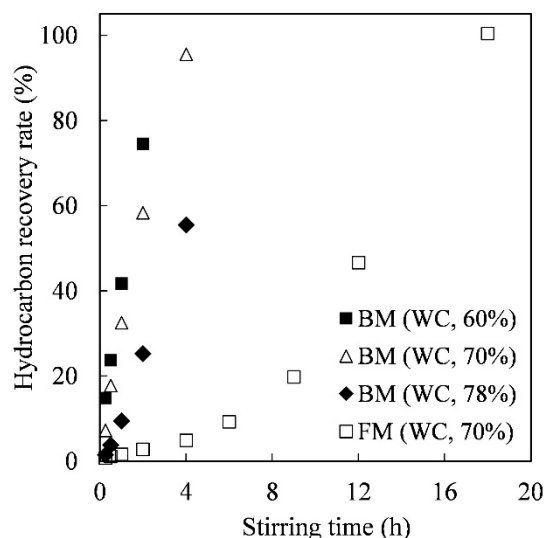


Figure 6. Hydrocarbon recovery rates from concentrated algal slurries of different water contents cultured in different media. FM: Freshwater medium, BM: Brackish medium, WC: Water content.

Figure 7A shows the input energy in the hydrocarbon recovery process and the recovered hydrocarbon energy from dry algae per unit mass. The HHV of the recovered hydrocarbon per unit weight of dry algae was 15.6 MJ kg^{-1} and the input energy recovery from the FM and BM cultures were 5.69 and 4.98 MJ kg^{-1} , respectively. This difference was attributed to the exclusion of pre-heating in the BM process and a low stirring power. Hexane loss contributed to the HHV of the *n*-hexane in the residue without being evaporated and recovered. In addition, extraction for long periods had little effect on the EPR. However, if the extraction time for a 95% hydrocarbon recovery was >16 h in the FM without pre-heating, as shown in Figure 6, the input energy for the stirrer was $>2.3 \text{ MJ kg}^{-1}$ and larger than that for pre-heating. In this context, one point is to be particularly observed. The evaporation of water from the residue was required to recover 99.9% of the *n*-hexane content using liquid–liquid extraction. The proportion of the input energy for the evaporation of water was the largest. Therefore, the balance between the amount of the evaporated water and residual *n*-hexane or using solvents that evaporate more easily than *n*-hexane should be considered in the future for liquid–liquid extraction from microalgae.

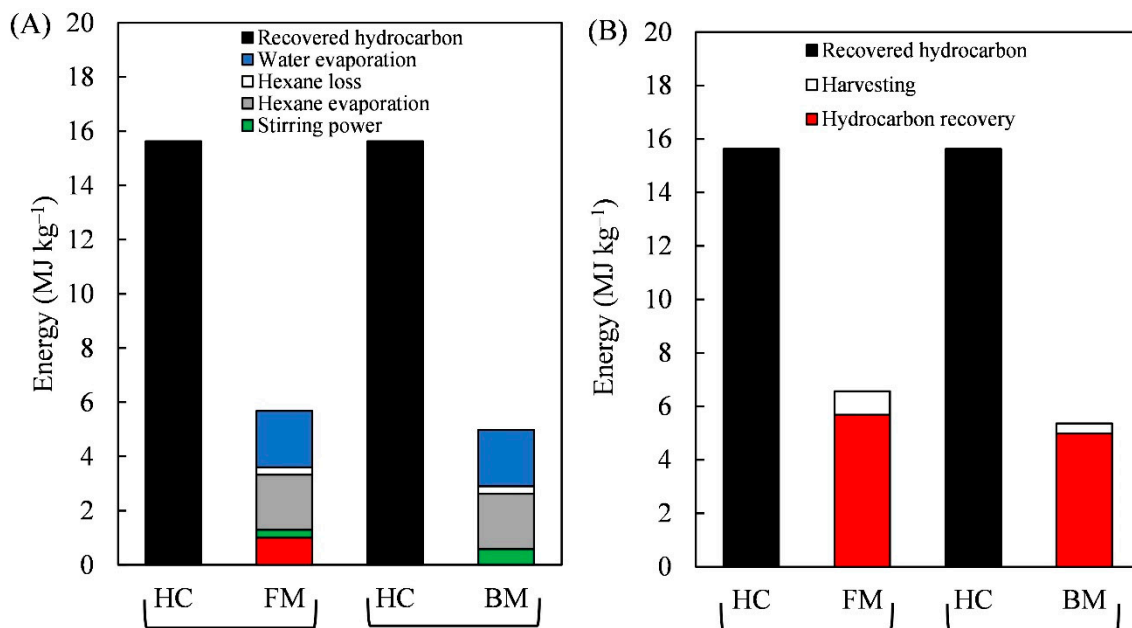


Figure 7. Input energy per unit of dry algae of hydrocarbon recovery (A) and the process from harvesting to hydrocarbon recovery (B) from algae cultured in different media. HC: Hydrocarbon, FM: Freshwater medium, BM: Brackish medium.

3.3. The Energy Profit Ratio from Harvesting to Hydrocarbon Recovery

Figure 7B shows the total input energy from harvesting to hydrocarbon recovery and the HHVs of the recovered hydrocarbons. In this experiment, the inclined separator was used for harvesting algae cultured in the BM. The total input energies in the FM and BM processes was 6.57 and 5.36 MJ kg⁻¹, respectively. Meanwhile, the EPR of the BM process was 2.92, which was ≈ 1.2 times higher than that of the FM process (2.38). The input energy for harvesting was much lower than that for the hydrocarbon recovery with both the FM and BM. When reviewing the energy balances in the biofuel production from microalgae, in most models, it was found that harvesting and drying accounted for the largest proportion of the energy consumption [34]. This was thought to be due to the incorporation of the drying process into the harvesting process. *B. braunii* grows in large colonies, has good filterability, secretes hydrocarbons in the oleophilic extracellular biopolymer, and does not require drying or cell disruption. These unique characteristics lead to a decrease in the ratio of the energy of the harvesting process to the total input energy. Finally, the input energy of the culture process was omitted in this study. This was because the input energy of the culture process in open ponds had various estimations in reports and we observed that there was no difference in the growth rate and hydrocarbon productivity between the BM and FM in our previous study [22]. In this study, the utilization of the extraction residue was not considered. The lipid-extracted microalgae could be utilized as a mediator and substrate in microbial fuel cells [35], and the quorum-sensing molecules extracted from sludge in this cell enhanced the productivity of the algal culture [36]. Thus, a further improvement of the energy balance is required via the energy transformation of the extraction residue. Moreover, in the future, culture development by taking advantage of the large algal colony size in the BM should be considered.

4. Conclusions

In this study, the effects of a *B. braunii* culture in a BM on the EPR of hydrocarbon recovery and harvesting were analyzed via the extraction from concentrated slurries and filtration using an inclined solid–liquid separator, respectively. The inclined solid–liquid separator with a wedge-wire screen, which was used for filtration, did not require electrical energy and was effective for large-colony-forming algae cultured in the BM. The water content in the slurry on the wire screen at slit sizes larger than

150 μm was <80% and a separation rate of >85.0% could be achieved at slit sizes less than 150 μm . In contrast, in the case of the FM culture, the water content in the slurry was found to be >99.0% at any slit size due to its small colony size and high surface tension. The input electrical energy of the harvesting process using the BM with the inclined separator was 0.14 MJ kg^{-1} , which was ≈ 0.44 times higher than that observed using the FM with vacuum filtration (0.32 MJ kg^{-1}). The EPR of the process in the BM was 2.92, which was ≈ 1.2 times higher than that in the FM with pre-heating before the liquid–liquid extraction (2.38). The brackish medium of this study can be prepared in the filed scale because only seawater was added into the culture medium. This study showed that the filtration techniques and hydrocarbon recovery with a low energy input were viable. However, we consider that the main bottlenecks of biofuel production from microalgae are the stable culture technology and culture costs. In the next step, culture technology will be developed by taking advantage of the large algal colony size through the elucidation of the mechanism of colony enlargement.

Author Contributions: Conception and design of the study: K.F., F.H., M.Y., Y.K., and K.I. Collection and analysis of the data: K.F., F.H., and M.Y. Data interpretation, manuscript drafting, and final approval of the article: K.F., F.H., M.Y., Y.K., and K.I. All authors have read and approved the final version of the manuscript.

Funding: This work was supported by JSPS (Japan Society for the Promotion of Science) KAKENHI (grant number JP 19K15939).

Conflicts of Interest: The authors declare no conflict of interest.

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