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Research Report

Catalytic Conversion of *Botryococcus Braunii* Oil to Drop-in Fuel under Mild Reaction Conditions

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ABSTRACT The non-oxygenated triterpenic hydrocarbons (mainly $C_{34}H_{58}$) produced by the Bot-22 strain of *Botryococcus braunii* are an extremely promising feedstock for automobile fuels. However, their application requires the development of a cracking technique for conversion of these oils into a fuel that can be used for automobiles. This work aimed to develop an on-site process to convert the oil extracted from Bot-22 (Bot-oil) to a fuel that satisfies the specifications for diesel fuels, a so-called drop-in fuel. The ability to operate at low temperatures was a key requirement for this on-site conversion to ensure a low-cost system with structural compactness and simple construction. Experimental analysis of Bot-oil catalytic conversion under mild conditions was performed. The Bot-oil conversion reaction proceeded at 260°C and 1 atm with a yield of 76%. The physical properties of the converted oil satisfied the grade No. 2, specified by the Japan Industrial Standards diesel fuel specification (JIS K 2204). However, the results of a combustion test using a single-cylinder engine showed that the cetane number, which is one of the most important chemical properties of diesel fuel, is below the JIS required value of 45. Based on analysis using a chemical reaction simulation, the low ignitability of the converted oil under engine conditions was attributed to residual components involving double-bond structures in the converted oil. Therefore, further development of the conversion technology is required to produce a drop-in fuel using a simple on-site process.

EXEYWORDSII Botryococcus Braunii, Fuel Conversion System, Catalytic Reaction, Drop-in Fuel, Cetane Number

1. Introduction

Algae oils represent a promising alternative feedstock for biofuels.^(1,2) Most algae produce and accumulate oils as triglycerides,^(3,4) which are generally transformed into fatty acid methyl esters (FAME) or hydrogenated biodiesel (HBD). However, several problems with using FAME as an automobile fuel have been reported, such as unacceptable quality variations, quality degradation by high temperatures, and detrimental effects on gum and resin parts. Although the chemical properties of HBD are suitable for its use as an automobile fuel, the HBD production process must still be improved to reduce the cost by lowering the consumption of hydrogen.

Botryococcus braunii produces and accumulates oils composed of non-oxygenated hydrocarbons.^(5,6) Thus, this species is of significant interest as a candidate for the production of fuel for automobiles. For the oil extracted from B. braunii (Bot-oil), the Bot-22 strain of B. braunii produces triterpenic hydrocarbons (mainly $C_{34}H_{58}$) with purity over 90%.⁽⁷⁾ However, the physical properties of Bot-oil are unsuitable for use as a fuel for current automobiles, thus necessitating the development of cracking technologies that will enable the Bot-oil to attain the required fuel properties. Several studies of Bot-oil cracking have been reported. Hillen et al.^(8,9) performed hydrocracking of Bot-oil to produce transport fuels, whereby their process converted oils with a gasoline fraction of 67% and provided a yield of 80% at conditions of 400°C and 20 MPa. Kitazato et al.⁽¹⁰⁾ investigated the catalytic cracking of Bot-oil to obtain gasoline. The converted oils had a gasoline fraction of 62% with an octane number of 95 at 500°C. These processes required the use of large-scale facilities because both cracking reactions were performed under severe conditions. Moreover, the converted oils obtained from both processes contained other components such as light cycle oil (LCO) and heavy cycle oil (HCO) with gasoline fractions. Furthermore, even if the gasoline components are separated from the converted oils, it will be difficult to use these products as gasoline because most commercial types of gasoline are formed by blending several components with cracking components. Therefore, these converted oils could not be used as a drop-in fuel, which is defined as a fuel that can be used without any processing and can be directly supplied to a fuel-tank.

The aim of this research was to develop a process for the conversion of Bot-oil to a fuel that satisfies the diesel fuel specifications, a so-called drop-in fuel, using an algae fuel system based on the following concept. Both the algae pond and fuel production facility are to be located on the premises of a factory or thermal power plant to effectively use the exhaust gases emitted from them. The CO₂ in the exhaust gas will be used for algal growth, and the heat of the exhaust gas will be used as the conversion energy for fuel production. The produced fuel will be consumed in the surrounding area. This concept also has the advantage of saving energy required to gather decentralized algae oils and promotes local production for local consumption. It will be expected that this system can achieve high efficiency with a minimal load on the environment due to the use of waste energy as the conversion energy in this process. Moreover, a system that operates under mild conditions can typically run at a lower cost. Thus, low-temperature operation is a key feature of this on-site conversion process.

In this study, the catalytic conversion of Bot-oilunder mild conditions was performed to obtain the highest product yield, and the fuel properties of converted oils were compared to the grade No. 2, specified by the Japan Industrial Standards diesel fuel specification (JIS K 2204). Among these specifications, the cetane number (CN) is one of the most important descriptors of diesel fuel quality. The CN of the converted oil was predicted by a simple alternative method using a constant-volume combustion chamber with a small amount of sample. Finally, diesel combustion was conducted using a single-cylinder engine to confirm the suitability of the converted oil for use as diesel fuel.

2. Materials and Methods

2.1 Algal Strain and Oil Extraction

The Bot-22 strain of *B. braunii* was isolated from a reservoir located in the Okinawa prefecture, Japan. The Bot-22 cells were grown in a 10 L carboy under continuous fluorescent light (photon flux of $300 \ \mu mol/m^2s$) with constant aeration (supplemented with 1% CO₂) at 25°C using AF-6 medium.⁽¹¹⁾ When the cell concentration reached 2–3 g/L, the culture was transferred to a round-shaped open pond with a working volume of 300 L. This pond was illuminated with a 1 kWh metal halide lamp for 24 h and was aerated and mixed with 1% CO₂ at 25°C in AF-6 medium.

The oil fraction was extracted from air-dried *B. braunii* cells by rinsing several times with n-hexane, and then the hydrocarbon fraction was purified using a silica gel column. The eluate from the purification process was used as the oil for catalytic conversion.

2.2 Algal Oil Properties

The properties of the Bot-oil obtained from the extraction process were the same as those reported in a previous study.⁽⁷⁾ The main component of the Bot-oil was $C_{34}H_{58}$, and the purity of hydrocarbons including isomers and $C_{33}H_{56}$ was over 99%. Analysis of the oil using gas chromatography-time of flight mass spectrometry (GC-TOFMS) revealed neither N nor O atoms. Moreover, the pour point of the Bot-oil was below -35° C, which is quite low. The molecular structure of $C_{34}H_{58}$ is shown in **Fig. 1**. $C_{34}H_{58}$ comprises a long main carbon chain with multiple short-chain branches with most double bonds located in the short-chain branches.

2.3 Catalytic Conversion

The technique used for catalytic conversion was based on the use of waste heat as the conversion



Fig. 1 Molecular structure of $C_{34}H_{58}$ (main component of Bot-oil).

energy. It is a challenge to obtain high reaction efficiency with this technique, even if the reaction rate tends to be low for operation under mild conditions. To achieve a high product yield within the diesel fuel range, it is crucial to prevent excessive decomposition that leads to the formation of undesirable products with low carbon numbers. Therefore, the following two measures were employed. (1) The reaction should proceed under conditions of a low decomposition rate. (2) Decomposition products should be removed from the reaction field. The first measure is beneficial for the process under mild conditions while the second measure improves the reaction rate. A catalytic flow reactor with sequential product separation to adopt these measures was developed.

The reaction proceeded in the liquid phase in this reactor. The reactor with a gas separation space enabled separation of gaseous products from the liquid reaction field to be easily achieved and prevent excessive product decomposition. The reaction rate with this reactor could also be adjusted by changing the oil flow rate. The reaction rate at low temperature can be enhanced by promoting the diffusion of reactants to the catalysts by the oil flow and product separation. Preliminary experiments confirmed that the use of gaseous product separation increased the amount of the converted oil by 50%.

2.3.1 Indexes of Conversion Performance

The reacted Bot-oil is cracked into various components from a gas to liquid. The converted oil was defined as the liquid components of cracking products:

The amount of reacted Bot-oil and the yield of converted oil are indicators of the conversion performance. The former is a measure of the reaction rate because it is the amount of the oil consumed during a given time period, whereas the latter is a measure of the reaction efficiency. The amount of reacted Bot-oil was calculated as the difference between the total mass of the oils before and after the reaction. The converted oil yield was defined as: Yield of converted oil [%] = Converted oil [g] / Reacted Bot-oil [g] ×100 (2)

2.3.2 Conversion System

Figure 2 shows a schematic diagram of the flow-reactor conversion system. The reactor has two inlets and outlets located one above the other. The catalyst was installed at the bottom of the reactor and heat was supplied from the lower surface. A given amount of Bot-oil was fed into the reactor continuously from the lower inlet and some of the oil was converted by catalytic cracking in the liquid phase. Gaseous products flowed out of the reactor from the upper outlet with N₂ sweep gas supplied from the upper inlet. Unconverted oils flowed out from the lower outlet and were returned to the oil inlet for reuse. Some of the high-boiling point components in the converted oil products from the upper outlet were removed at the condenser and were also returned to the oil inlet. The converted oil from the condenser was condensed and separated by a gas-liquid separator. The adjustable parameters in this experiment, i.e., the feed rate of the Bot-oil and N₂, the amount of in-reactor Bot-oil and N₂, the amount of catalyst, and the reaction temperature, were optimized to maximize both the reaction efficiency and the quality of the converted oil. Based on our preliminary experiments, the experimental conditions selected are listed in Table 1. Catalytic reaction using this reactor was conducted for 6 h at atmospheric pressure. An aluminum-containing mesoporous silica (Al-FSM) prepared in-house⁽¹²⁾ was used as the cracking catalyst. The catalyst was processed into the form of pellets with diameters in the range of 1-2 mm.

2.4 Measurement of Converted Oil Properties

The physical properties of the converted oil were compared with the JIS No. 2 diesel fuel specifications. The properties measured in this study are related to the fundamental engine performance. Density was measured using a specific gravity meter (DA-110, Kyoto Electronics Manufacturing Co., Ltd.) and kinematic viscosity was measured with an Ubbelohde viscometer in the range of 2 to 10 cSt (Shibata Scientific Technology Ltd.). Distillation properties were measured using an in-house constructed distillation apparatus based on the JIS K2254 specification that required a sample of only 3.5 g for each measurement. The distillation ratio was evaluated by mass rather than by volume.

The composition of the converted oil was determined using gas chromatography with a flame ionization detector (GC-FID; CG-14B, Shimadzu Corporation). The compositions were then classified into three groups based on the carbon number (< C9, C9-C23, > C23). Among these, C9-C23 products are suitable for use as diesel fuels.

2.5 Predicted Cetane Number Determined Using a Constant-volume Combustion Chamber

The CN is an index of ignition quality and is the most important chemical property of a diesel fuel. The CN is usually determined by a standard test method specified in JIS (JIS K 2280) and ASTM (ASTM D613). Although this is the officially authorized test method, it has some disadvantages, especially the requirement for a special engine and a large amount of test fuel. While the cetane index (CI) is commonly used as a substitute for the CN in the case of petroleum-derived diesel fuels, it is unclear whether the CI agrees with the CN in the case of converted oils because it is known that the CI cannot be applied to synthetic fuels and fuels with a cetane improver. Several alternative indicators have been developed based on the constant-volume combustion chamber (CVCC) technology: the derived cetane number (DCN),⁽¹³⁾ the CN by fuel ignition analyzer method (FIA-CN), and the estimated cetane number (ECN). Nevertheless, the development of an evaluation method that is applicable to all fuels is still a subject of ongoing research.(14,15)

In this study, the CN of the converted oil was predicted from the ignition delay measured using an in-house constructed CVCC. This test procedure was essentially the same as the previously mentioned



Fig. 2 Schematic diagram of flow-reactor conversion system.

Temperature [°C]	200 - 260	
Pressure [atm]	1.0	
Reaction period [hour]	6	
W/F ratio (catalyst/Bot-oil) [g/g]	0.083	
LHSV-F ^(*) [1/hour]	160	

 Table 1
 Experimental conditions for catalytic conversion.

(*) LHSV-F: Liquid hourly space velocity of fuel (= Fuel flow rate [g/h] / Catalyst mass [g]) CVCC methods, except that it was possible to conduct the tests using a small test sample of 50 cc. A schematic diagram of the experimental apparatus and the experimental conditions applied in this work are shown in Fig. 3 and Table 2, respectively. This system generated a high-temperature and pressure field by combustion of a premixed $H_2/N_2/O_2$ mixture in the chamber. The premixed gas was O₂ rich to ensure that the burned gas had the same 21% O₂ concentration as air. Following pre-combustion, the temperature and pressure decreased with time due to heat loss from the chamber, and the test fuel was injected into the chamber after an arbitrary time period. The test fuel was ignited with some delay time (τ). The ignition delay data of the test fuel was measured at various temperatures and pressure conditions by changing the injection timing. Preliminary measurements were performed for the ignition properties of four standard fuels with known CNs (CN = 30, 40, 45 and 50). Moreover, the mathematical relationship between CN and τ , where

 $\tau = f(CN)$, was established using the least-squares method, as shown in **Fig. 4**. The predicted CN (PCN) of the test fuel was estimated by comparing the τ data with that calculated by the formula for a standard fuel. It was confirmed that the PCN of a diesel fuel obtained using this method was consistent with the FIA-CN.

2. 6 Observation of Engine Combustion Using a Single-cylinder Engine

Engine combustion tests were conducted to investigate the suitability of the converted oil for use as a diesel fuel, because PCN evaluation was conducted at a temperature and pressure that were lower than the temperature and pressure in actual engine operation conditions. An optically accessible single-cylinder engine⁽¹⁶⁾ was used to observe the fuel spray and flame behavior in parallel with the engine performance. **Figure 5** shows a schematic diagram of the apparatus, and the engine specifications and



Fig. 3 Apparatus for measurement of the ignition delay using a constant-volume combustion chamber.

Table 2	Experimental conditions for measurement of the ignition delay
	using a constant-volume combustion chamber.

Chamber size [mm]	$\Phi 80 imes 30$	
Injection pressure [MPa]	80	
Hole diameter [mm]	0.1	
Injection period [ms]	1.0	
Injection time after pre-combustion [ms]	200 - 500	

experimental conditions are listed in **Table 3**. The engine test was conducted for the middle load condition. The fuel was injected twice for the pilot and main injections. The tested fuels were as follows: a converted oil, which has the highest PCN; and three standard fuels for which the CN values were 15, 30 and 45, respectively. The heat release rate was calculated from the average of the in-cylinder pressure history for 20 cycles. The shadow-graph visualization method using a bottom-view system was applied for observation of the fuel spray and flame behavior using a high-speed video camera (FASTCAM SA1.1, Photron). The frame speed of the high-speed camera was 20000 fps.

3. Results and Discussion

3.1 Catalytic Conversion

Preliminary feasibility studies were conducted using a batch reactor (non-flow reactor) with sequential product separation to confirm the effect of the reaction temperature and the weight ratio of the catalyst to in-reactor Bot-oil (W/F) on the conversion performance.⁽¹⁷⁾ For the reactor with product separation, products with a higher carbon number are more difficult to separate from the liquid-phase reaction field because they tend to have higher boiling points. These non-separated products continue to react until their



Fig. 4 Ignition delay data for standard fuels (Plots: experimental data, Lines: least-squares curve fits based on $\tau = f(CN)$).



Fig. 5 The bottom-view type optically accessible single-cylinder engine with an elongated piston.

 Table 3 Engine specifications and experimental conditions.

Bore × Stroke [mm]	86 × 96
Compression ratio	16.2
Engine speed [rpm]	1200
Fuel injector [mm × holes]	$\Phi 0.098 \times 9$
Injection pressure [MPa]	40
Fuel amount [mm ³ /st]	25
Injection pattern	$10 15 \\ -1 -1 -1 \\ -20^{\circ} TDC$
Intake O ₂ [%]	21

boiling point approaches the reaction temperature. Therefore, the reaction temperature and W/F are highly important parameters in determining the reaction performance. Figure 6 shows the reaction performance as a function of (a) the reaction temperature and (b) the W/F ratio. For the reaction temperature, the reaction proceeded from a temperature of 200°C. The amount of reacted oil increased with the temperature, while the growth of the yield slowed at temperatures higher than 220°C. The ratio between C9 and C23 products reached a peak at 240°C. A high temperature reaction increased the reaction rate and the yield but decreased the fuel quality due to the increased amount of products with high carbon numbers. Conversely, conducting the reaction at a low temperature decreased the reaction rate and the yield due to excessive decomposition caused by product stagnation. For the W/F ratio, an increase in the number of reaction sites by the use of high W/F ratios could increase the amount of reacted oil but would also lead to excessive decomposition of the products, thus generating low molecular weight

hydrocarbons. Conversely, at low W/F ratios, the amount of reacted oil and the yield decreased due to the lack of catalyst. The reaction in the flow reactor showed the same trend. At 260°C, the obtained yield of the converted oil was 76%. In the case of a reactor without a condenser, as shown in Fig. 2, the yield increased to 81%. However, due to some high-boiling components, the kinematic viscosity and the 90% distillation temperature (T90) also increased to values that were beyond the range required by the diesel fuel specifications.

3.2 Properties of Converted Oil

The properties of the converted oil (at 25°C) were compared to those of a diesel fuel and the original Bot-oil,⁽⁷⁾ as shown in **Table 4**. The value for T90 was determined from the distillation property data. Moreover, the physical properties of the converted oil were comparable to those of the diesel fuel. It was especially obvious that the kinematic viscosity of the



Fig. 6 Reaction performance as a function of (a) the reaction temperature and (b) the W/F ratio.⁽¹⁷⁾

converted oil became much lower than that of the Bot-oil. The converted oil also exhibited high fluidity under the low temperature conditions, and the pour point was below -35° C.

The PCN of the converted oil was estimated to be equivalent to 46, while the PCN of the original Bot-oil was predicted to be below 35. This prediction is based on the properties of squalene ($C_{30}H_{50}$), which has a similar molecule structure to that of Bot-oil and has a kinematic viscosity of 15 cSt and an FIA-CN value of 35. Therefore, the converted oil is considered to be substantially improved relative to the original Bot-oil.

3.3 Diesel Combustion of Converted Oil

The combustion properties of the converted oil were evaluated using a single-cylinder engine. **Figures 7** and **8** show the heat release rate as a function of the crank angle, and the observed in-cylinder spray and flame behavior, respectively. For the converted oil, a single heat release peak was obtained. The initially injected pilot fuel did not ignite until the main fuel injection time and subsequently combusted with the main injection fuel. For standard fuels with CNs of 30 and 45, two heat release rate peaks were obtained. The pilot fuel was ignited before the main fuel injection, and the main fuel that was injected in a high-temperature atmosphere formed by the pilot fuel combustion subsequently ignited and combusted rapidly. The time of the first heat release was more advanced in the case of the fuel with the higher CN. These results indicate that the actual CN of the converted oil was lower than 30 and higher than 15 because no heat release appeared, which meant that no ignition occurred for a standard fuel with the CN of 15.

 Table 4
 Comparison of physical properties for JIS No. 2, converted oil, and Bot-oil.

	JIS No. 2	Converted oil	Bot-oil
Density (15°C) [kg/m3]	< 0.86	0.84	0.84
Kinematic viscosity (30°C) [cSt]	> 2.5	3.3	58
90% distillation temperature (T90) [°C]	< 350	350	> 400
Cetane Index (Cetane number)	> 45	46 (PCN)	-
Sulfur content [wt%]	< 0.001	0.0	0.0
Pour point [°C]	<-7.5	<-35	-35



Fig. 7 Comparison of the heat release rate as a function of the crank angle.

Fig. 8 In-cylinder images of fuel spray and flame behavior with converted oil and a standard CN45 fuel.

3. 4 Approaches to Improving the CN of Converted Oil

The CN of a fuel is determined by both physical and chemical processes during the time period from fuel injection to ignition. The physical properties of a fuel affect the injection, atomization, evaporation processes, and the mixing of the fuel with the surrounding mixture. However, the chemical properties affect the combustion reaction. Examination of the visualization images shown in Fig. 9 suggest that the spray length of the converted oil determined by the physical properties was similar to that of standard fuels, whereas that of the original Bot-oil was long. It could be confirmed that the low CN of the converted oil is caused by the low quality chemical properties and is not due to the physical properties. Therefore, it is necessary to analyze the chemical reaction to investigate the origin of the poor chemical properties.

In this study, it was observed that there was a difference between the CNs of the converted oil obtained using the CVCC and the CNs obtained using the engine, although no such difference was observed for diesel fuel. The main difference between these apparatuses is the combustion conditions, i.e., the pressure and temperature. For the engine, the temperature ranged from 750 to 850 K and the pressure range was from 5 to 6 MPa. For the CVCC, the temperature range

Fig. 9 Spray images in CVCC.

was from 900 to 1050 K and the pressure ranged from 1 to 2 MPa, so that the CVCC measurements were performed under higher temperature and lower pressure conditions than those with the engine.

The converted oil is comprised of various components of hydrocarbon types with different hydrocarbon numbers. The reaction properties of the same types of hydrocarbons are generally almost the same, even if the carbon number is different. Therefore, the effect of temperature and pressure on the ignitability for various hydrocarbon types was investigated by kinetic reaction analysis. Based on the results for the hydrocarbon compounds of the converted oils under several experimental conditions, the following reaction mechanism was derived. In the initial decomposition reactions, long-chain unsaturated hydrocarbons decompose into small unsaturated hydrocarbons, and these low molecular weight hydrocarbons continue to react over time and thus decompose to even smaller molecules. In addition, some of these unsaturated hydrocarbons transform through side reactions to generate different hydrocarbon-type compounds such as cyclic saturated, aromatic, and isomeric unsaturated and saturated hydrocarbons. The increasing contact between the oil and the catalyst increases the extent to which these side reactions occur. Therefore, it is concluded that the converted oil contains saturated, unsaturated, mono-, and poly-aromatic hydrocarbons. However, it is quite difficult to identify all components of the converted oil and to then understand the reaction mechanism of each of the components. To understand the difference between the chemical properties of the different hydrocarbon types, kinetic simulations of some representative well-known species of each hydrocarbon type were performed, although these species were not actually contained in the converted oil. Simulations were performed using the CHEMKIN-PROTM (Reaction design) software with the reaction mechanism obtained by the Model Fuel ConsortiumTM. The following representative compounds were evaluated: n-heptane as an alkane, 2-methyl-2-butene (bc5h10) as an alkene, cyclohexane (CHx) and methylcyclohexane (MCH) as cycloalkanes, and toluene as an aromatic. Calculations were performed under constant pressure and temperature conditions as follows: two temperatures of 800 and 900 K were used, and the pressure range was from 1 to 6 MPa. The PCN of a fuel was determined by comparison of its ignition delay to that of standard

fuels. **Figure 10** shows the relationship between pressure and the PCNs of each composition. Moreover, the sensitivity of the PCN to the pressure for bc5h10 as a representative alkene was quite high compared to other compounds, and the value of PCN decreased with higher pressures. This evaluation suggested that the residual species involving double-bond structures caused the low ignitability of the converted fuel under the engine-like high-pressure conditions. Therefore, it was suggested that the decrease in the amount of the species with double-bond structures in the converted oil led to the improvement in the CN of the oil.

These results show that the present converted oil is not suitable as a drop-in fuel for automobiles. Therefore, further conversion technology development is required to produce a drop-in fuel by using a simple on-site process.

Fig. 10 Effect of pressure on PCN due to chemical kinetic simulation.

4. Conclusion

Experimental analysis was performed for the catalytic conversion of hydrocarbons produced by the Bot-22 strain of B. braunii under mild conditions. The Bot-oil conversion reaction proceeded from approximately 200°C, and the suitable temperature for both the reaction efficiency and fuel properties was determined to be 260°C with a yield of 76%. The physical properties of the converted oil were demonstrated to be similar to those required by the JIS No. 2 diesel fuel specification. However, the CN of the converted oil was less than 30, which was below the specified minimum value for the JIS No. 2 diesel fuel. Therefore, the present converted oil was not suitable for use as a drop-in automobile fuel. It was concluded that the decrease in the amount of residual species containing double-bond structures is a possible approach to improve the CN. It was evident that further development of reaction controls is thus required to achieve the production of a drop-in fuel using a simple on-site conversion process.

References

- Tran, N. H., Bartlett, J. R., Kannangara, G. S. K., Milev, A. S., Volk, H., Wilson, M. A., "Catalytic Upgrading of Biorefinery Oil from Micro-algae", *Fuel*, Vol. 89, No. 2 (2010), pp. 265-274.
- (2) Zhang, Y., "Hydrothermal Liquefaction to Convert Biomass into Crude Oil", *Biofuels Agric. Wastes Byprod.*, Chapter 10 (2010), pp. 201-232., Blackwell Publishing.
- (3) Chisti, Y., "Biodiesel from Microalgae", *Biotechnol. Adv.*, Vol. 25, No. 3 (2007), pp. 294-306.
- Schenk, P. M. et al., "Second Generation Biofuels: High Efficiency Microalgae for Biodiesel Production", *BioEnergy Res*, Vol. 1, No. 1 (2008), pp. 20-43.
- (5) Banerjee, A. et al., "Botryococcus Braunii: A Renewable Source of Hydrocarbons and Other Chemicals", Crit. Rev. Biotechnol., Vol. 22, No. 3 (2002), pp. 245-279.
- (6) Metzger, P. and Largeau, C., "Botryococcus Braunii: A Rich Source for Hydrocarbons and Related Ether Lipids", Appl. Microbiol. Biotechnol., Vol. 66, No. 5 (2005), pp. 486-496.
- (7) Nagano, S. et al., "Physical Properties of Hydrocarbon Oils Produced by *Botryococcus Braunii*: Density, Kinematic Viscosity, Surface Tension, and Distillation Properties", *Procedia Environ. Sci.*, Vol. 15 (2012), pp. 73-79.
- (8) Hillen, L. W. et al., "Hydrocarbon Fuels from Plants", *Fuel*, Vol. 59, No. 6 (1980), pp. 446-447.

- (9) Hillen, L. W. et al., "Hydrocracking of the Oils of *Botryococcus Braunii* to Transport Fuels", *Biotechnol. Bioeng.*, Vol. 24, No. 1 (1982), pp. 193-205.
- (10) Kitazato, H. et al., "Catalytic Cracking of Hydrocarbons from Microalgae", *J. Jpn. Pet. Inst.* (in Japanese), Vol. 32, No. 1 (1989), pp. 28-34.
- (11) Ishimatsu, A. et al., "Biosynthesis of Isoprene Units in the C₃₄ Botryococcene Molecule Produced by *Botryococcus Braunii* Strain Bot-22" *Procedia Environ. Sci.*, Vol. 15 (2012), pp. 56-65.
- (12) Inagaki, S. et al., "Synthesis of Highly Ordered Mesoporous Materials from a Layered Polysilicate", *J. Chem. Soc., Chem. Commun.*, No. 8 (1993), pp. 680-682.
- (13) Allard, L. N. et al., "Diesel Fuel Ignition Quality as Determined in the Ignition Quality Tester (IQTTM)-Part 4", *SAE Tech. Paper Ser.*, No. 2001-01-3527 (2001).
- (14) Kaneko, T., "Research Report on Biofuels Applications in Diesel Vehicles", *JATOP Conference*, <www.pecj.or.jp/english/jcap/jatop/pdf/003.pdf>, (accessed 2017-9-10).
- (15) Takeda, A. et al., "Analysis Result of Marine Residual Fuel Oil by Constant Volume Combustion Chamber Method (IP541)", *Journal of the JIME* (in Japanese), Vol. 44, No. 2 (2009), pp. 126-130.
- (16) Fuyuto, T. et al., "Combustion Visualization Using Newly-developed Optically Accessible Single-cylinder Engines", *R&D Review of Toyota CRDL*, Vol. 44, No.2 (2013), pp. 43-53.
- (17) Yamamoto, S. et al., "Catalytic Conversion of *Botryococcus Braunii* Oil to Diesel Fuel Under Mild Reaction Conditions", *J. Appl. Phycol.*, Vol. 26, No.1 (2014), pp. 55-64

Figs. 1-4, 6 and Table 2

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Fig. 5

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