[Review Paper]

Liquid Fuel Production Using Microalgae

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Recently, biomass has attracted much attention as a renewable energy resource. Microalgae are particularly promising biomass species because of the high growth rate and high CO_2 fixation ability compared to plants. Effective liquid fuel production from microalgae was studied using *Botryococcus braunii* and *Dunaliella tertiolecta*, which accumulated terpenoid hydrocarbon and glycerol, respectively. *B. braunii* could remove nitrogen and phosphorus from secondarily treated sewage (STS) in a batch system and a continuous bioreactor system with hydrocarbon production. The intracellular glycerol content could be controlled by post-translational modifications in *D. tertiolecta*. *B. braunii* is more profitable for liquid fuel production than *D. tertiolecta* based on calculating the energy balance.

Keywords
Biomass, *Botryococcus braunii*, *Dunaliella tertiolecta*, Energy balance, Liquid fuel production, Microalgae

1. Introduction

Biomass is the organic materials derived from the reaction between carbon dioxide, water, sunlight, and other nutrients via photosynthesis. Biomass is one of the most potentially energetic organic resources, because it is renewable and neutral with regard to carbon dioxide emissions. Energy from biomass would contribute a stable energy supply and local society due to commercial activities. The use of biomass to produce energy must be increased, if we are to reduce the impacts of global warming. The solar energy absorbed by photosynthesis is converted into the chemical bonds of the structural components of biomass. If biomass is processed efficiently, it can provide high-energy outputs to replace conventional fossil fuels. Biomass has always been a major source of energy for mankind and is presently estimated to contribute of the 13% (55 EJ, 1990) of the world's energy supply and a much greater percentage in developing countries[1]. In the past 10 years, there has been renewed interest in biomass as an energy source. Since the biological routes for biomass conversion are efficient in terms of nutrients and organic matter recycling, this study focused on obtaining fundamental data on energy conversion systems utilizing the metabolism of microorganisms. Here, the outlines of liquid fuel production using microalgae will be reviewed to clarify the possibility for complementation of fossil fuel. Our data for the utilization of microalgae for energy production will also be discussed.

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2. Energy Conversion from Biomass

Biomass can be converted biochemically into useful forms in a number of ways. Energy conversion reactions of biomass can be classified into biochemical conversion and thermochemical conversion (**Fig. 1**). Thermochemical conversion, such as gasification, pyrolysis and liquefaction, is the thermal decomposition of organic components in biomass to yield fuel products. Biochemical conversion can be further subdivided into fermentation, anaerobic digestion, bioelectrochemical fuel cells, and other fuel producing processes utilizing the metabolism of organisms. The biological routes for biomass conversion are generally more efficient in terms of nutrient and organic matter recycling.

3. Bioconversion by Microalgae

Microalgae can grow rapidly, so are one of promising photosynthetic biomass sources. In addition, many reagions suffer low productivity due to poor soils or the shortage of sweet water, so farming of microalgae that can be grown in sea or brackish water and marginal land may be almost the only way to increase productivity. Photobiological production of fuels and chemicals from

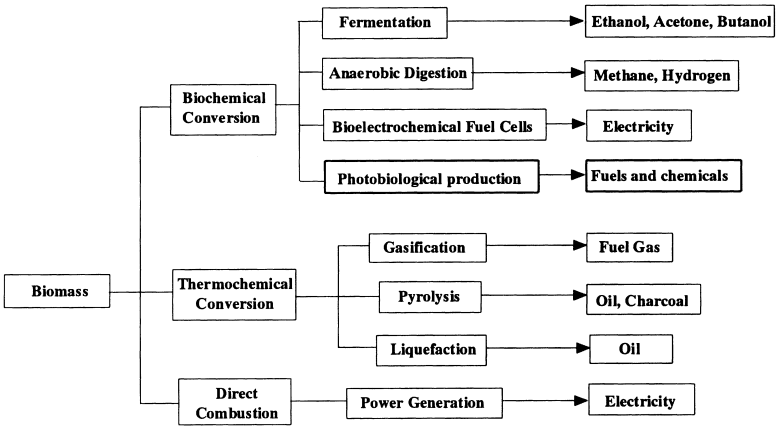


Fig. 1 Energy Conversion Processes from Biomass

microalgae is probably one of the most important avenues towards establishing a significant source of renewable energy supply. The concept of bioconversion by microalgae is the utilization of the photosynthetic process for the production of biomass to be used as a source of energy and chemicals. A microalgal production yield of 15-25 t dry-weight/ha/year has been achieved over a relatively long term and some microalgae are well known natural producers of fatty acids and hydrocarbons[2]. Much research has been conducted to produce useful organic compounds including fuel using microalgae. We have studied effective liquid fuel production from microalgae using *Botryococcus braunii* and *Dunaliella tertiolecta* which accumulated terpenoid hydrocarbon and glycerol, respectively.

Although microalgae are considered to be very efficient for harvesting solar energy for the production of organic compounds via the photosynthetic process, the photosynthetic efficiency of microalgae for the conversion of solar energy seldom exceeds 1%. Therefore, it is important to optimize the overall photosynthetic efficiency of microalgae by physiological, biochemical and genetic approaches.

3. 1. Growth of Hydrocarbon-rich Microalga *B. braunii* in Secondarily Treated Sludge

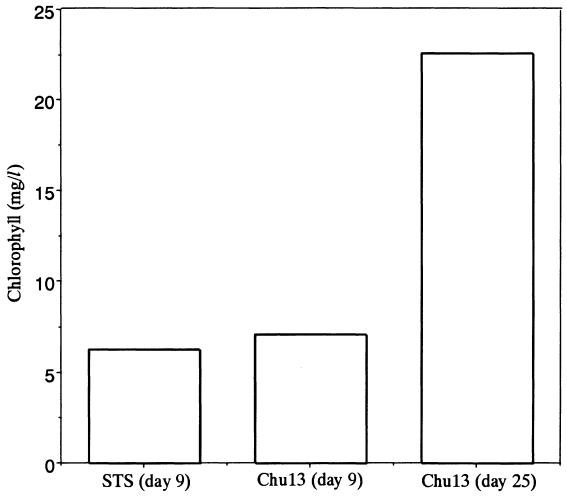
The colonial green microalga, *B. braunii*, has been actively studied because of the high levels of hydrocarbon production. This species produces and accumulates hydrocarbons (hydrococcens C30-C36) at 30-70% of its dry weight[3)~5)]. Commercial production of hydrocarbons by *B. braunii* has not been achieved mainly because of economic and technical barriers. Feeding of *B. braunii* with secondarily treated sewage (STS) could contribute to these problems by reduction of inorganic materials, mainly nitrogen and phosphorus, in the STS. Reduction of inorganic nutrients in STS by microalgal cultivation has been studied[6),7)]. The advantage of using *B. braunii* is the production of valuable hydrocarbons that can be harvested from the algal cells. To achieve cultivation of *B. braunii* in STS as a tertiary waste-water treatment, continuous culture and high cell density should be maintained by a membrane filtration system because the inorganic nutrients in STS are at relatively low concentrations. Growth and hydrocarbon productivity of *B. braunii* fed with STS in a batch system and in a continuous system, and related consumption of inorganic nutrients, have been studied.[8),9)]

Table 1 Hydrocarbon Content of *B. braunii* Grown with STS and Chu 13 Medium

Medium	Hydrocarbon content [%]
STS 1[a)]	53
STS 2[b)]	49
Chu 13[c)]	58

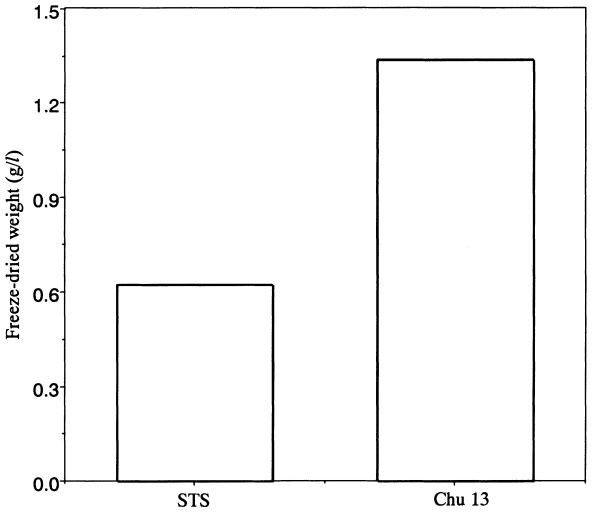
a) Tested cells were cultured on the 9th day in a batch system.
b) Tested cells were cultured on the 32nd day in continuous culture.
c) Tested cells were cultured on the 25th day in a batch system.

The hydrocarbon-rich green microalga, *B. braunii*, can grow well in STS without any modification in a batch system and in a continuous bioreactor system. The chlorophyll density increased 2.8-fold in a batch system. The growth rate in a batch system with STS was 0.35 g/*l* per week. The algal biomass increased at a sustained rate of 196 mg dry weight/*l* per week in the continuous system with STS and the algal concentration in the reactor could be maintained to a fixed value (approximately 400 mg/*l*) after 11 days feeding. The growth in STS in both batch and continuous bioreactor systems was as good as in the artificial medium of Chu 13[10),11)]. Hydrocarbon contents of algae grown in STS were high enough both in these systems compared with the case of the Chu 13 medium on the basis of freeze-dried weight (**Table 1**). *B. braunii* grew well and produced hydrocarbons in STS over a 1-month period in the continuously operated system. The membrane



Tested cells in STS were cultivated on the 9th operational day in a batch system. Tested cells in Chu 13 medium were cultivated on the 9th and 25th day in a batch system.

Fig. 2 Chlorophyll Concentration of *B. braunii* in STS and Chu 13 Medium



Tested cells in STS were cultivated on the 25th operational day in a continuous reactor system. Tested cells in Chu 13 medium were cultivated on the 25th day in a batch system.

Fig. 3 Freeze-dried Weight of *B. braunii* in STS and Chu 13 Medium

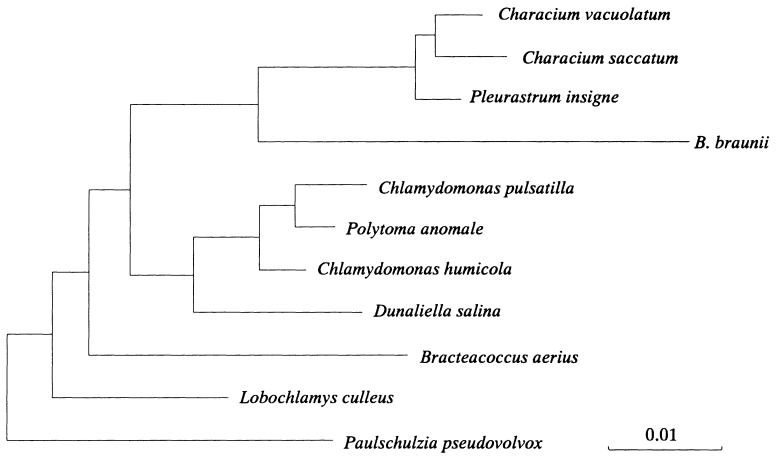
filtration system functioned well in continuous culture to decouple the recovery rate of biomass and dilution rate of the medium.

Nitrate is a main nitrogen component produced by aerobic waste-water treatment, and *B. braunii* utilized nitrate from 7.67 mg/*l* (day 0) to a level below detection of < 0.01 mg/*l* (day 9) in STS, and phosphate, even at an extremely low concentration, was also consumed from 0.02 mg/*l* (day 0) to a level below detection of < 0.01 mg/*l* (day 1) by *B. braunii* in a batch system. Comparing growth in the Chu 13 medium with that in STS, the growth rate after 7 days cultivation is about the same (**Fig. 2**). The low nitrate ion concentration in STS may have limited growth in the batch system. The concentrations of nitrate and phosphate ions in STS decreased from 5.5 to less than 4.0 mg-N/*l* and 0.08 to 0.03 mg-P/*l*, respectively, by algal consumption in the continuous bioreactor system within 3 days.

The growth rate of STS in the continuous culture was limited compared with Chu 13 medium, although the total nitrogen (TN) of the algal cells fed on STS and the artificial medium was the same for both cultures (**Fig. 3**). Since total phosphorus (TP) of the cells fed on STS was one-fifth that of the cells fed on Chu 13 medium, the low phosphate ion concentration in STS could cause a shortage of phosphate in the algal cells and may have limited growth. After consumption of the nitrate, nitrite was consumed in the batch system. However, ammonium was not utilized by growth of *B. braunii* in the batch system. The concentrations of nitrite and ammonium ions were stable and less than 0.02 mg N/*l* throughout a 1-month period and negligible compared with that of nitrate ions in the continuous system.

The total organic carbon (TOC) concentrations increased with the growth of *B. braunii*, ranging from 5.5 to 28.7 mg/*l* in 9 days using STS in the batch system. Although a small increase in TOC by algal organic excretion in the STS cultures was observed in the batch system, particularly later in their growth (stationary phase), algal organic excretion was relatively low in the optimum growth phase. On the other hand, the concentration of OC ranged from 5.0 to 9.0 mg/*l*, and that of inorganic carbon (IC) decreased slightly, and no increase as occurred in the batch system was found. The pH in the culture was stable within the range of 7.6 to 8.0 in the continuous system.

These results show the possibility of using STS as a medium to grow *B. braunii* and for removal of nitrogen and phosphorus by algal consumption in STS presently discharged into rivers, lakes or the ocean. It was reported that *B. braunii* Berkeley-strain grew at 1.2 g/*l* per week with continuous light (250 μE/m^2/s) and 23-25°C[4]. A higher STS supply rate could cause an increase in the algal growth rate in the continuous system. Although the optimum dilution rates for algal cells and STS should be searched so as to maximize algal biomass production and inorganic nutrient utilization, tertiary waste-water treatment appears possible by a continuous feeding system of *B. braunii* with STS. Different optimum conditions are expected for each STS source, since nitrate and phosphate ion concentrations differ with waste-water characteristics and treatment processes.



The dendrogram is generated by comparison of the known nucleic acid sequences.

Fig. 4 The Neighbor-joining Tree of Small Subunit Ribosomal RNA

3. 2. Phylogenetic Position of *B. braunii* Based on Small Subunit Ribosomal RNA Sequence Data

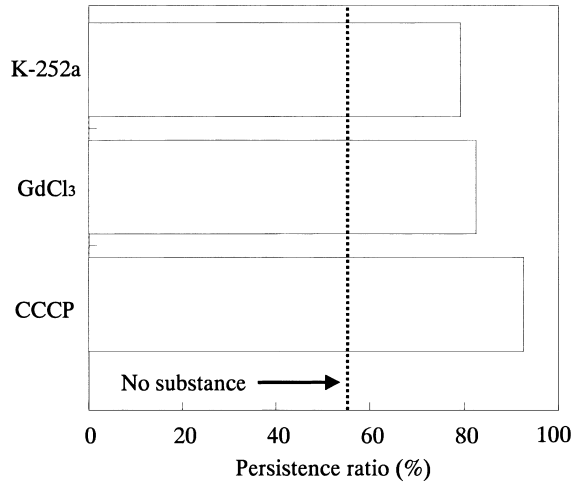
As described so far, a great deal of attention has been directed to the green microalga, *B. braunii*, due to its high hydrocarbon production level. *B. braunii* was first reported as a member of the Chlorophyceae[12], but it was then placed in the Xanthophyteie (Chromophyta) based on the structure of its plastids and starch granules[13]. However, based on ultrastructural studies, *Botryococcus* was retransferred to the Chlorococcales, (Chtorophyceae, Chlorophyta)[14]. Analyses of small subunit ribosomal RNA (rRNA) sequence data have facilitated clarification of phylogenetic relationships among microalgae[15),16]. The phylogenetic position of *B. braunii* has been determined using 16S-like rRNA sequence data[17].

The 16S-like rRNA in the hydrocarbon-rich microalga *B. braunii* was amplified using RNA polymerase chain reaction, and its sequence was determined. The sequence data of *B. braunii* were analized with those of several other algae to determine the phylogenetic relationships among these algae. The phylogenetic tree indicated *B. braunii* to be a member of the Chlorophyta and closely related to *Cha. vacuolatum* (**Fig. 4**). The number of thylakoids per lamella of *B. braunii* and the shape of pyrenoid were reported to coincide with those of the Chlorophyceae from ultrastructural analysis[14]. The sequence analysis supports this phylogenetic classification of the alga. The branching pattern of the tree corresponded to groups of green algae found using ultrastructural data on the flagellar apparatus and rRNA sequence data[18),19]. Zoosporic algae are placed in three groups according to the orientation of the basal bodies, *i.e*. whether they are clockwise (CW), directly opposed (DO), or counterclockwise (CCW), when the cells are viewed "top-down." Although *B. braunii* does not have a flagellate stage, our phylogenetic tree showed *B. braunii* to be closely related to the CW group of *Cha. vacuolatum*.

3. 3. Glycerol Production and Stress Response of *D. tertiolecta*

Higher-performing strains responding favorably to various environmental conditions such as salt stress will be required for successful large-scale cultivation of microalgae. *Dunaliella* can adapt to an extremely wide range of salinities[20]. Glycerol is the major osmoregulatory solute of *Dunaliella*. The unique chemical composition of *Dunaliella* offers benefit for the large-scale cultivation, and the tolerance for salt stress facilitates the maintenance of monoalgal cultures in outdoor ponds. In nature *Dunaliella* cells sometimes have an occasion to cope with rapid dilution of their surrounding medium, *e.g.*, shallow lakes and ponds suddenly flooded with rainwater. If it is possible to prevent a decrease in the intracellular glycerol content in the hypoosmotic stress, *Dunaliella* can produce fuel efficiently. As the osmotic adaptation of *Dunaliella* is not affected by protein synthesis inhibitors[21], post-translational protein modifications may participate in the osmoregulation. Therfore, we examined the mechanism of glycerol dissimilation under hypoosmotic stress in *D. tertiolecta* by a pharmaceutical approach[22),23].

When the external salinity dropped from 1.0 to 0.5 M, the decrease in glycerol content halved after 60 min (**Fig. 5**). When the algae were incubated with the H^+ ionophore CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), glycerol dissimilation was almost completely inhibited (**Fig. 5**). Gd^{3+}, which is known to block stretch-type Ca^{2+} channels localized in the plasma membrane[24], inhibited glycerol dissimilation to a significant extent (**Fig. 5**). Because Gd^{3+} blocked the stretch-activated Ca^{2+} channels localized in the plasma membrane, the influx of Ca^{2+} from the extracellular space *via* the stretch-activated Ca^{2+} channels localized



Effect of CCCP, Ca^{2+} channel blockers and protein kinase inhibitors on glycerol dissimilation under hypoosmotic stress in *D. tertiolecta* was examined.

Fig. 5 Persistence Ratio for Intracellular Glycerol Content before Adding Hypoosmotic Stress

in the plasma membrane would be required for the transduction of osmotic signals in *D. tertiolecta*.

As hypoosmotic shock induces transient phosphorylation of specific proteins in *Dunaliella* cells[25),26)], the effect of protein kinase inhibitors was examined. Indolocarbazole derivatives such as K-252a potently inhibits several types of protein kinase including serine/threonine- and tyrosine-type protein kinases[27),28)]. The cells treated with K-252a showed inhibition of glycerol dissimilation (**Fig. 5**). These results indicate that the influx of extracellular Ca^{2+} and protein phosphorylation are necessary for the osmotic response of *D. tertiolecta*.

3. 4. Microalgal Cultivation in a Solution Recovered from Gasification of Itself

Besides *B. braunii* and *D. tertiolecta*, various algae have been investigated for their growth characteristics, particulary for fast multiplication rate and high protein content. Among these, *Chlorella vulgaris* is the most common species for mass cultivation, and basic information on the physiology of laboratory cultures of *C. vulgaris* is well known. *C. vulgaris* is a microalga artificially cultured for commercial production of food, feed and fine chemicals. However, the potential for fuel production has been neglected. Microalgae have a high moisture content (only 0.5-1 g dry-cell/*l*). If the biomass needs be dried before use for energy production, this drying process would waste energy.

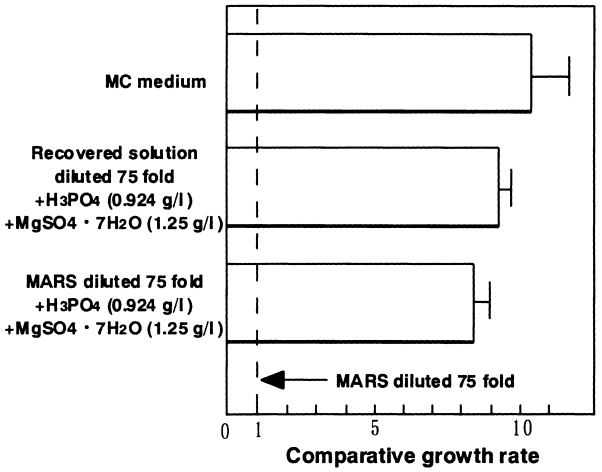
Recently, a low-temperature catalytic method for gasification of biomass with a high moisture content was developed, by which biomass can be gasified directly into methane-rich fuel gas at high temperature (around 400°C) and high pressure (around 20 MPa) using a metal catalyst, and nitrogenous compounds in the biomass are converted to ammonia at the same time[29)]. Therefore, the combination of fuel production from microalgae and gasification of the rest of microalgal biomass will be promising for effective energy production from microalgae. *C. vulgaris* grew in a mixture of the solution recovered from the gasification of *C. vulgaris* and a nutrient solution which contained no nitrogen, that is, nitrogen cycling was occurring[30)]. *C. vulgaris*, however, could not grow well in only the recovered solution, which was considered to be due to a lack of nutrients. The optimal culture conditions for obtaining a high biomass production rate using the recovered solution with supplemented nutrients have been investigated[31)].

It is generally accepted that algae can use a wide variety of nitrogenous compounds, both inorganic and organic, as nitrogen sources for the synthesis of amino acids. *C. vulgaris* could use ammonium as a nitrogen source and grew well in the media containing NH_4Cl at concentrations ranging from 0.22 to 1.11 g/*l*, although NH_4Cl was toxic at a concentration above 16.62 g/*l*. Therefore, the toxicity of ammonia could be disappeared by diluting the recovered solution 30 fold although ammonia derived from the nitrogenous compounds of *C. vulgaris* was found in high quantities (about 9000 mg/*l*) in the recovered solution[30)].

Nickel ions derived from the reduced nickel catalyst were also found in high quantities (240 mg/*l*) in the recovered solution. Although nickel ions inhibit the growth of *C. vulgaris* in a concentration-dependent manner, the toxicity of the nickel ions could be reduced by a 30-fold dilution of the recovered solution. Since *C. vulgaris* is 87.4% moisture[30)], and the density of *C. vulgaris* in culture medium was 0.5-1.0 g dry-cell/*l*, the algal biomass was concentrated 126-252 times before gasification. These results suggest that ammonium and nickel ions in the recovered solution are not toxic to the growth of *C. vulgaris* when a culture solution of the microalga itself is used as the material for gasification.

Phosphate and magnesium ions are considered to be present in insufficient quantities in the recovered solution for the growth of *C. vulgaris*. The growth in the presence of the phosphate and magnesium ions in the 75-fold diluted recovered solution was comparable to that in the MC *Chlorella ellopsoidea* medium (MC) (**Fig. 6**). Phosphorous and magnesium are thus indispensable nutrients to be supplied for the growth of *C. vulgaris* in the recovered solution. Furthermore, *C. vulgaris* grew well in the 75-300-fold diluted recovered solution supplemented with phosphate, magnesium ions and micro-elements, although further studies are necessary to determine the optimum conditions of phosphate, magnesium ions and micro-elements for the growth of *C. vulgaris*.

These results indicate that *C. vulgaris* at a wide



MC medium was used as a control for the growth of *C. vulgaris*.

Fig. 6 Effect of the Addition of Phosphate and Magnesium Ions on the Modified Artificial Recovered Solution (MARS) and the Recovered Solution Supplemented with Micro-element Solution on the Growth of *C. vulgaris* after 14 days of Cultivation

range of densities in the culture solution can be used directly for gasification and the recovered solution obtained can be used for the cultivation of *C. vulgaris* over a wide range of dilution rates if phosphate, magnesium ions and micro-elements as nutrients are added to the medium. In addition, ammonium ions in the recovered solution can be used as a nitrogen source.

4. Possibility of Renewable Energy Production Using Microalgae

The low temperature catalytic gasification of biomass including microalgae and cultivation using the solution recovered from the gasification is a potential source of renewable energy. Liquid fuel production from biomass such as sewage sludge, kitchen garbage, woods and microalgae processed by thermochemical conversion at 250-350°C and 50-200 kg/cm^3 saturated vapour pressure with or without an alkaline catalyst have been actively studied as other themochemical conversion processes[32)~37)]. Low temperature catalytic gasification and thermochemical liquefaction have the advantage of treating wet materials compared with direct combustion and pyrolysis, because it does not require a drying process. The energy balance in the overall process from cultivation to conversion is important to produce renewable energy and mitigate CO_2 emission using biomass. The possibility of energy production and CO_2 mitigation by low temperature catalytic gasification and thermochemicai liquefaction of microalgae has been investigated.

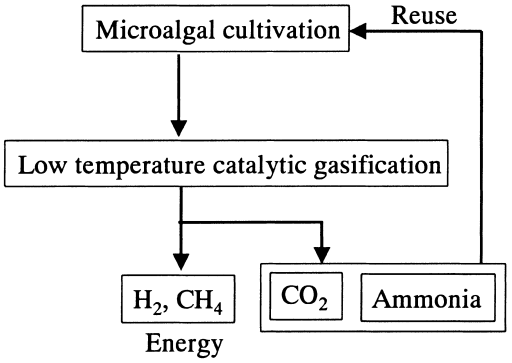


Fig. 7 Flow Diagram of a Microalgal System for Fuel Production by Low Temperature Catalytic Gasification of Biomass

4. 1. Possibility of Renewable Energy Production by Low Temperature Catalytic Gasification

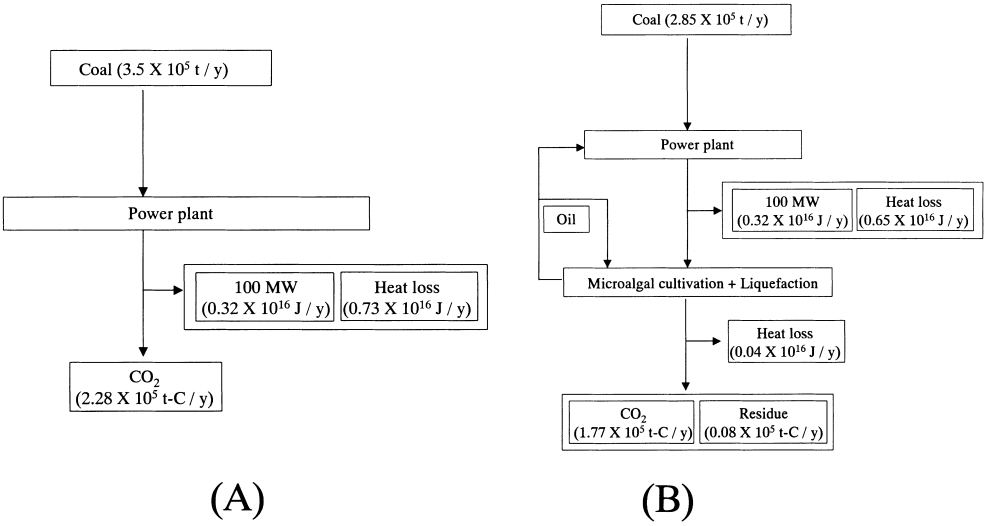
As the energy consumption ratio (energy for gasification/energy of produced gas) for *C. vulgaris* is 0.33[30)], low temperature catalytic gasification is suitable for energy recovery from microalgae. A novel energy production system using microalgae with nitrogen cycling combined with low-temperature catalytic gasification of the microalgae has been proposed (**Fig. 7**)[30)]. Compared with incineration, the increase in the obtained energy (6.68 MJ/kg dry-cell) is higher than the heating energy for gasification (5.95 MJ/kg dry-cell)[30)].

Energy input is necessary into processes such as fertilizers, cultivation and harvesting before the low temperature catalytic gasification process. As ammonium ions in the recovered solution can be used as a nitrogen source, the energy required for nutrients can be reduced. The energy input for nitrogen as fertilizer is proportional to the nitrogen content in the dry algal cells on the assumption that the specific energy unit for nitrogen, the organic content in the dry matter, the gas yield, and the efficiency of algal nitrogen consumption is constant. Effective cell harvesting of *D. tertiolecta* by pH control could reduce the energy required for harvesting. Therefore, *D. tertiolecta* is a suitable microalga for the low temperature catalytic gasification of biomass.

These results suggest that the low temperature catalytic gasification of microalgae which produce useful organic compounds including fuel is promising for renewable energy production and reduction of CO_2 emissions.

4. 2. Possibility of Renewable Energy Production and CO_2 Mitigation by Themochemical Liquefaction of Microalgae

Oil recovery and energy consumption ratio (energy for liquefaction/energy of produced oil) from *B. braunii* were higher than *D. tertiolecta* (**Table 2**)[38)]. Oil recovered by liquefaction from *B. braunii* has 1.6-fold



Assumptions: energy efficiency of the plant is 30%, heating value of coal is 28 MJ/kg, carbon content of coal is 65 wt%, 30% of the CO_2 in the flue gas can be used by *B. braunii*, the heating energy for liquefaction can be supplied by the waste heat from the power plant, microalgal yield is 15 t dry weight/ha/year, and microalgal concentration of culture is 0.5 kg dry weight biomass/m^3.

Fig. 8 Energy and Carbon Flow in a Power Generation Plant Using Coal (A) and Liquid Fuel from *B. braunii* and Coal (B)

Table 2 Oil Recovery and Energy Consumption Ratio in Oil Production from Microalgae Using Thermochemical Liquefaction

	Oil yield [%]	Energy for liquefaction / Energy of produced oil
Botryococcus braunii[a)]	64	0.15
Dunaliella tertiolecta[b)]	42	0.34

a) Ref. 33).
b) Ref. 35)

the heating value (45.9 MJ/kg) of coal (28 MJ/kg). Thermochemical liquefaction is suitable for oil recovery from *B. braunii*. Although energy input at the feeding stage of microalgae before the energy conversion process is required, the energy consumption ratio including fertilizer, cultivation, and harvesting processes {(fertilizer + cultivation + harvesting)/produced oil} indicate that the liquefaction process using *B. braunii* produces net renewable energy, but not that using *D. tertiolecta*. This result is caused by the difference of energy requirements between *B. braunii* and *D. tertiolecta*. Cells of *B. braunii* contain smaller amounts of nitrogen and phosphate on an organic basis than *D. tertiolecta*; therefore, the energy input for fertilizers in *B. braunii* is smaller than that in *D. tertiolecta*.

B. braunii can grow on STS. However, the concentration of nutrients in STS is not sufficiently high to sustain maximum algal growth. Total biomass of *B. braunii* fed with the STS from 1 million people (assumptions, volume of sewage = 0.25 m^3/person/day, nitrate content = 5.0 g-N/m^3) and liquid fuel produced by thermochemical liquefaction are calculated to be roughly 1.3×10^4 t dry-weight/year and 0.8×10^4 t/year, respectively, so fertilizers would be necessary to produce large amounts of liquid fuel from microalgae. Therefore, microalgae that require relatively small amounts of nutrients are suitable for energy production. *B. braunii* is more suitable for liquid fuel production compared with *D. tertiolecta*, although the halotolerant microalga, *D. tertiolecta*, is easier to cultivate on a large scale than *B. braunii*, and the assumed yield of *B. braunii* might be too high for the present technology. Since 80% of the nitrogen was distributed in the aqueous phase resulting from the thermochemical liquefaction of albumin and roughly half of the nitrogen in the aqueous phase was ammonium[39)], there is a possibility of reusing inorganic nutrients in the aqueous phase produced by liquefaction as a part of the growth medium for microalgae, as well cultivation of microalgae in the recovered solution from low temperature catalytic gasification.

Since separating processes for microalgae such as sedimentation and centrifugation (1.0 MJ/kg-biomass)[40)] consume energy, sedimentation and centrifugation were considered for harvesting of microalgae. *B. braunii* forms colonies with diameters of about 100 μm, so it could be harvested with an energy saving method. Energy production from colony forming microalgae may be useful to avoid energy waste in the harvesting

process. As 80% of the total energy requirement was consumed in microalgal biomass (*Spirulina*) production[40], tubular reactors including a biocoil system could be used in more efficient microalgal culture.

A 100 MW thermal plant using coal consumes 3.5×10^5 t/year of coal (2.28×10^5 t-C/year) and emits 8.3×10^5 t/year of CO_2 (2.28×10^5 t-C/year) (**Fig. 8(A)**). If a 100 MW thermal plant using coal is replaced by liquid fuel produced from *B. braunii*, the quantity of CO_2 mitigation would be 1.5×10^5 t/year and 8.4×10^3 ha of microalgal cultivation area could be necessary (**Fig. 8(B)**). In this calculation, 30.1% of produced liquid fuel was consumed for cultivation of *B. braunii*. Coal of 2.85×10^5 t/year should be supplied to compensate for carbon losses at the consumption by the microalgae and the liquefaction process as gas and tar-like fractions. The necessity for a large cultivation area is one of the most important problems in the case of CO_2 mitigation on the site of a power generation plant. The pH control of the culture is also important to use flue gas CO_2 for microalgae. There is a possibility of CO_2 mitigation using microalgal oil for organic materials replacing of fossil fuels.

Bioconversion by microalgae are useful methods of biochemical conversion for energy production from biomass. Further investigation in larger scale plants and economic surveys are needed to put these methods into practical use.

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要　　旨

微細藻類を利用した液体燃料生産

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近年，再生可能で環境調和型のエネルギー資源であるバイオマスが注目されるようになってきている。なかでも，植物プランクトン（微細藻類）は植物よりも増殖が早く，高いCO_2固定能を有しているため，有望なバイオマス種として注目されている。微細藻類からの効率的な液体燃料生産を行うため，テルペノイド系炭化水素を蓄積する*Botryococcus braunii*とグリセリンを細胞内に蓄積する*Dunaliella tertiolecta*を用いて検討を行ったところ，*B. braunii*は，培養に下水処理水を使用することにより，液体燃料生産と同時に処理水中の窒素やリンを除去することが可能であること，*D. tertiolecta*は，細胞内に蓄積するグリセリンを情報伝達系で調節していることが分かった。液体燃料生産のエネルギー収支を計算すると，炭化水素含有率がより高い*B. braunii*の方が*D. tertiolecta*より有利であることが分かった。