千葉工業大学

博士学位論文

A study on culture conditions of lipid production for biofuel using *Nannochloropsis oceanica* IMET1

（バイオ燃料の生産のための微細藻類
Nannochloropsis oceanica IMET1
による脂質生産条件に関する研究）

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Abstract

Biofuel produced by microalgae has been proposed as an alternative to fossil fuels. However, the price of biofuel produced by microalgae is higher than that of existing fuels. Therefore, the process of producing biofuels using microalgae has not been realized practically. For improving the practical issue of high cost biofuel production, a search for a new microalgae, a optimization of cultivation condition, and a clarification of biofuel production mechanism is needed.

The purpose of this study is to reduce the cost of biofuel production using microalgae mainly focused on two ways: microalgae selection and the investigation of culture conditions. In this research, the effect of photosynthetic factors on the growth performance of *Nannochloropsis* sp. were initially investigated, and the optimum culture conditions was investigated. The effect of nitrogen and carbon sources on biomass and lipid production were also researched for the first time.

N. oceanica IMET1 shows the best growth performance and highest growth rate in five different microalgaes. *N. gaditana* CCMP526, 1894 also shows good growth performance and growth rate. As there were studies reported the high lipid content about *N. gaditana* CCMP526, so *N. gaditana* CCMP526 is selected as a comparison of *N. oceanica* IMET1.

We investigate the growth performance of *N. oceanica* IMET1 compared with that of *N. gaditana* CCMP526 in various cultivation conditions. The growth performance of *N. oceanica* IMET1 is better than that of *N. gaditana* CCMP526. In particular, the growth rate of *N. oceanica* IMET1 is higher than that of *N. gaditana* CCMP526, regardless of culture medium under CO_2 concentrations > 10 vol%. The optimum growing conditions for *N. oceanica* IMET1 is a CO_2 concentration of 2 vol%, light intensity of 53μmol·m^{-2}·s^{-1}, and 30°C when MBG11 is used. The higher growth rate of *N. oceanica* IMET1 is evident in the flask cultures. The yield of lipid

products obtained by *N. oceanica* IMET1 can produce the high yield of lipid products with an comparison of *N. gaditana* CCMP526. *N. oceanica* IMET1 seems to contain a higher amount of C16:0 fatty acid than *N. gaditana* CCMP526.

The effect of nitrogen source (nitrate and urea) on growth and lipid production of *N. oceanica* IMET1 is investigated. Nitrate increased dry cell weight, biomass yield, and lipid weight in comparison with urea. Replacing nitrate with urea had a negative effect on dry cell weight and lipid yield. Fatty acid composition was affected by nitrogen source. Nitrate cultures produced mainly C16:0 (6.3%) and C18:2 (14.9%) and urea cultures produced mainly C16:0 (6.8%), C18:3 (8.6%), and C22:0 (33.1%) fatty acids. Fatty acid composition differed when different nitrogen sources were used. The result of the investigation of glucose on growth perfomance, shows a decrease by the effect of streptomycin for inhibiting bacterial growth. However, the lipid content was increased.

This study found that the growth prefomance of *N. oceanica* IMET1 is higher than that of other *Nannochloropsis* spp regardless of culture medium under CO_2 concentrations higher than 10%, can adapt to a harsh environment which undergoes a great degree of change in light, temperature, and the CO_2 content, and the lipid yield of *N. oceanica* IMET1 is high. Therefore *N. oceanica* IMET1 can be a possible candidate to produce high efficiently biofuel, would truly be a renewable option of great potential for global energy needs.

We also estimate the cost of biofuel production from *N.oceanica* IMET1, with a value of 220 ¥/L,which is lower than that reported by other researchers, when BG11 is used as medium. We found that *N. oceaninca* IMET1 is a possible candidate to produce biofuel in low cost.

Future issue include the clarification of produced biofuel mechanism for the improvement of biofuel yield, and to study the production technology of biofuel for pilot plant production.

Contents

Introduction

Microalgae is unicellular algae and has chlorophyll in the cell. Microalgae spices are 100,000 spices in marine or freshwater. Microalgae fix carbon dioxide to produce oxygen by photosynthesis, and then produce oil and grease in the cell.

Nannochloropsis oceanica IMET1 was brought to the Institute of Marine and Environmental Technology (IMET) from the National Center for Mariculture (NCM), Eilat, Israel by Odi Zmora in 2001. This strain has been maintained at IMET for more than 10 years, and is mainly used to feed rotifers in aquaculture. Zmora obtained this *Nannochloropsis* strain from a fish hatchery at Zadar, Croatia (former Yugoslavia) in 1987, but could not trace its origin further back. This strain was recently named *N. oceanica* IMET1 to establish a clear strain identity for publication. According to Zmora, IMET1 has been widely used and distributed to different laboratories and industries in the past 25 years. Several earlier publications used the same strain without strain identity. It has been reported that IMET1 was able to produce 69% lipid (dry weight) in a 23-day batch culture. Proteomic and metabolic profiles of IMET1 under nitrogen- deficiency stress have been reported.

Biofuel produced by microalgae has been expected instead of fossil fuel. However, the price of biofuel produced by microalgae is higher than that of existing fuel. Therefore, the process of produced biofuel using microalgae has not been practically. For improvement of practical issue of production biofuel as high cost, a search of new microalgae, a optimization of cultivation condition, clarify of produced biofuel mechanism is required. Among these requirements, the main study focuse on the search of new microalgae because of lipid product content and kinds of lipid are depend on species. For example, *Botryococcus braunii* has 25-75% oil content and *Chlorella* sp. has 28-32% oil content.

In this paper, the purpose of this study is to reduce the cost of biofuel production using microalga mainly focused on the two ways: microalga selection and the investigation of culture conditions. In this research, the effect of photosynthetic factors on the growth performance of *Nannochloropsis* sp. are investigated firstly, and the optimum culture conditions are investigated. And the effect of nitrogen and carbon sources on biomass and lipid production are researched for the first time.

1. Current status of biofuel production using microalgae and the purpose of this study

1.1 *Nannochloropsis oceanica* IMET1

1.1.1 Microalga

Microalgae is a generally generic name of the microscopic size (about 0.5 ~ 100 μm), photosynthetic organisms that live in the water, excluding multicellular algae in marine plants. As a primary producers, microalgae produces the organic matter and release O_2, making contributions in the ecosystems. The microalgae group is not classified in taxonomy because microalgae contains algae evolved from various genealogical families.

Microalgae generally produces organic compounds except cellulose. Therefore, the products synthesized by microalgae is expected to widely application for food, health, medicine, plastic, fuel and so on.

1.1.2 *Nannochloropsis* spp.

Nannochloropsis is a genus in the Eustigmatophyceae lineage, ranging in size of 2–5 μm and widely distributed in marine, fresh and brackish waters[1-1)]. (**Fig. 1-1**)

6 species of *Nannochloropsis* spp.[1-2)] are known, such as *N. gaditana*, *N. granulata*, *N. limnetica*, *N. oculata*, *N. salina* (**Fig. 1-2**)[1-3)], and *N. oceanica*.

1.1.3 *N.oceanica* IMET1

N. oceanica IMET1 was brought to the Institute of Marine and Environmental Technology (IMET) from the National Center for Mariculture (NCM), Eilat, Israel by Odi Zmora in 2001. This strain has been maintained at IMET for more than 10 years, and is mainly used to feed rotifers in the aquaculture.

Zmora obtained this *Nannochloropsis* strain from a fish hatchery at Zadar, Croatia (former Yugoslavia) in 1987, but could not trace its origin further back. This strain was recently named *N. oceanica* IMET1 to establish a clear strain identity for publication [1-4)]. According to Zmora, IMET1 has been widely used and distributed to different laboratories and industries in the past 25 years.

Several earlier publications used the same strain without strain identity[1-5~1-11)]. It

has been reported that *Nannochloropsis* strain was able to produce 69% lipid (dry weight) in a 23-day batch culture [1-12)]. Proteomic and metabolic profiles of IMET1 under nitrogen- deficiency stress have been reported[1-12, 1-13)].

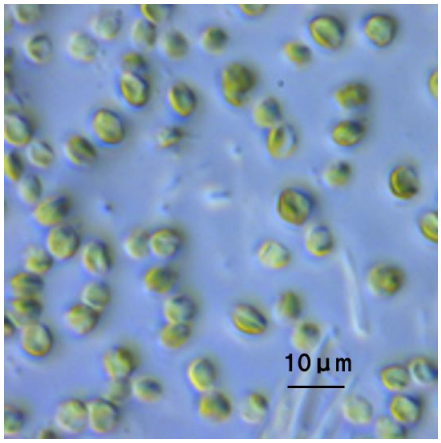

Fig.1-1 *Nannochloropsis*[1-2)]

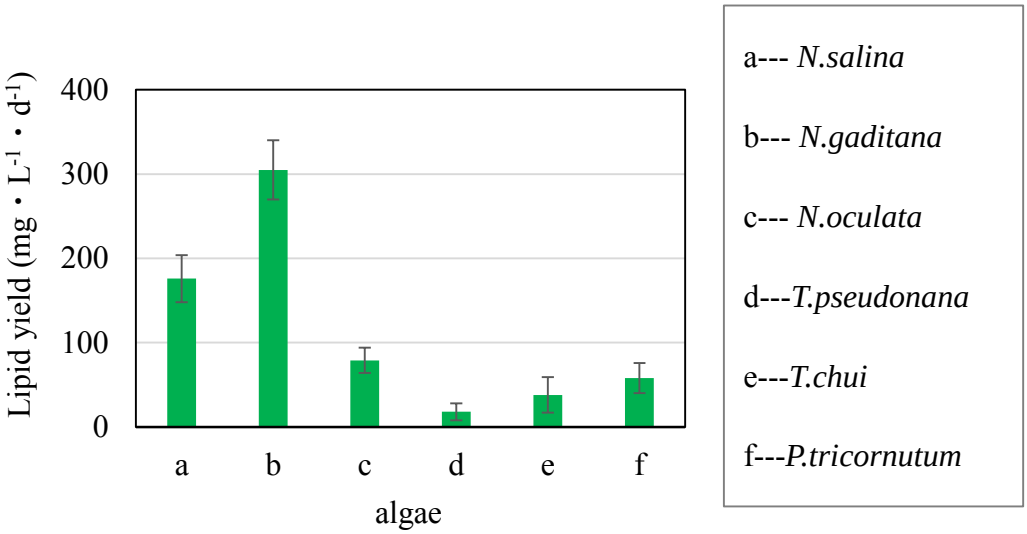

Fig. 1-2 Lipid yield of six microalga[1-3)]

1.2 Production of biofuel using microalgae

1.2.1 Biofuel

Lipid products from microalgae are expected to have wide applications as shown in **Fig. 1-3**.[1-14] Biofuel is produced from plant and animals as raw materials. These biomasses are converted to biofuel, such as gases (hydrogen, methane, etc.), liquid (ethanol, fatty acid, etc.) and solid (char coal, etc.), by thermal conversion, chemical conversion and biological conversion.

Fossil fuel are petroleum, coal and natural gases. Those fuels are mined in the earth. Petroleum consists of mainly hydrocarbon of widely molecular weight and other organic compounds.

Biofuels consist mainly of ethanol, triacylglycerol and fatty acid (neutral lipid, glycolipid, phospholipid), in contrast to petroleum.

1.2.2 The advantages of biofuel production using microalgae

The advantages of microalgae over higher plants as a source of transportation biofuels are numerous. (1) oil yield per area of microalgae cultures could greatly exceed the yield of the best oilseed crops (**Table 1-1**) [1-15]; (2) microalgae grows in an aquatic medium, but need less water than terrestrial crops; (3) microalgae can be cultivated in seawater or brackish water on non-arable land, and do not compete for resources with conventional agriculture; (4) microalgae biomass production may be combined with direct bio-fixation of waste CO_2 (1 kg of dry algal biomass requiring about 1.8 kg of CO_2); (5) algae cultivation does not need herbicides or pesticides; (6) the residual algal biomass after oil extraction may be used for feed or fertilizer, or fermented to produce ethanol or methane; (7) the biochemical composition of the algal biomass can be modulated by varying growth conditions.

Since of these advantages of biofuel, in this study, we focus on the production of lipid products as biofuel, using microalgae *N. oceanica* IMET1.

1.2.3 The essential of biofuel production from microalgae

Biofuel is a non toxic and biodegradable alternative fuel that is obtained from renewable sources. The recent research has proven that oil production from algae is clearly superior to that of terrestrial plants such as palm, rapeseed, soybean, and has the potential to completely displace fossil fuel, because of the high oil yield

Table 1-1[1-15].

On the other hand, there are some rigorous problems in the world. The amount of petroleum in the earth is gradually decreasing, and with the production of oil (**Fig.1-4**) leading to the increasing of oil price (**Fig. 1-5**). Rising demand for energy from developing nations threatens the availability of sustainable energy for future generations, so it is essential to research a renewable energy instead of petroleum.

Another problem is that the increasing amount of CO_2 released into the air around the world, and the environment situation has become a severe problem in all the countries of the world. The microalgae could accumulate oil when got some stress during growth in the medium, and at the same time, could consume CO_2 during photosynthesis, which could potential to solve the problem of increasing CO_2 emissions in the world. Compared with the other materials, microalgae has some advantages that are introduced in chapter 1.2.2, so we think the production of biofuels from microalgae is a necessary developing trend in the future. It is important to screen for a kind of microalgae can adapt to a harsh environment which undergoes a great degree of change in light, temperature, and the CO_2 content. Furthermore, two thirds of the surface of earth is covered with water providing a large potential growing region, and an algae which had strong tolerance and adaptation to such environment, would truly be a renewable option of great potential for global energy needs.

1.2.4 Production of biofuel using microalgae

Microalgae stores oil as hydrocarbon and triacylglycerol (TAG) in their cells. TAG is first extracted from microalgae, and then methylated under a catalyst to form fatty acid methyl esters (FAMEs). FAMEs are used for biodiesel fuel. Hydrocarbon is directly used as fuel. (**Fig. 1-6**) Oil content varies and depends on microalgae species as shown in **Table 1-2**[1-15]. Moreover, the content of hydrocarbon or TAG are also depending on microalgae species.

Botryococcus spp. produces a high content of hydrocarbon generally, not grow fast, so that is not widely applied. On the other hand, *Nannochloropsis* is also a rich source of high quality protein[1-18], and an omega-3 fatty acid with numerous health benefits[1-19,1-20]. Therefore oil from *Nannochloropsis* is expected to be widely applied.

1.2.5 Issue of production of biofuel using microalgae

The cost of biofuel production from microalgae is higher than petrodiesel. Global demand for alternative biodiesel feedstock and decreasing cost of biomass production, along with lower theoretically high biomass productivities and achievable lipid productivities of algal cultures, are motivating much research efforts toward the development of a cost-effective processes[1-21)].

The productivities of algal biomass and the amount of lipid in algal cells are the two key factors that influence the cost of algal biofuel production. If we could raise the biomass productivity, the cost of the biodiesel feedstock could be reduced. One solution is to search for a new microalga of high biofuel production. The biomass productivity, lipid content and lipid productivity of microalgae are affected by environmental conditions such as light intensity, nutrient (mainly nitrogen) limitation, salinity, temperature, pH and CO_2 concentration[1-21~1-28)]. Therefore, it is important to understand how the growth performance of microalgae is affected by environmental conditions.

1.3 Aim of this study

Microalga, as the third generation feedstock of biofuel production, have some advantages as compared to terrestrial plants, such as high growth rate, lipid content and carbon neutrality[1-1,1-15)]. Microalga can produce lipids for sustainable biodiesel production, however, relatively low lipid productivity have been one of the major obstacles impending their commercial production. In lieu of that, we select the oleaginous algae strain, and make clear which factors would promote the growth of the algae and accelerate the oil synthesis in the cells of algae.

In this study we investigate methods to reduce the cost of biofuel production using microalgae mainly focuse on the two ways: microalgae selection and the investigation of culture conditions. In this research, the effect of photosynthetic factors on the growth performance of *Nannochloropsis* sp. are investigated first, and then the optimum culture conditions is investigated. The effect of nitrogen and carbon sources on biomass and lipid production are researched for the first time.

1.4 Outline of this research

We study the effects on CO_2 concentration of five strains of *Nannochloropsis* sp., from these results, we select two oleaginous strains: CCMP526 and IMET1. We research the effects on temperature, CO_2 concentration and light of the two strains and mainly analyze the oil concentration and the fatty acid composition of the two strains under different nitrogen sources. (**Fig.1-7**)

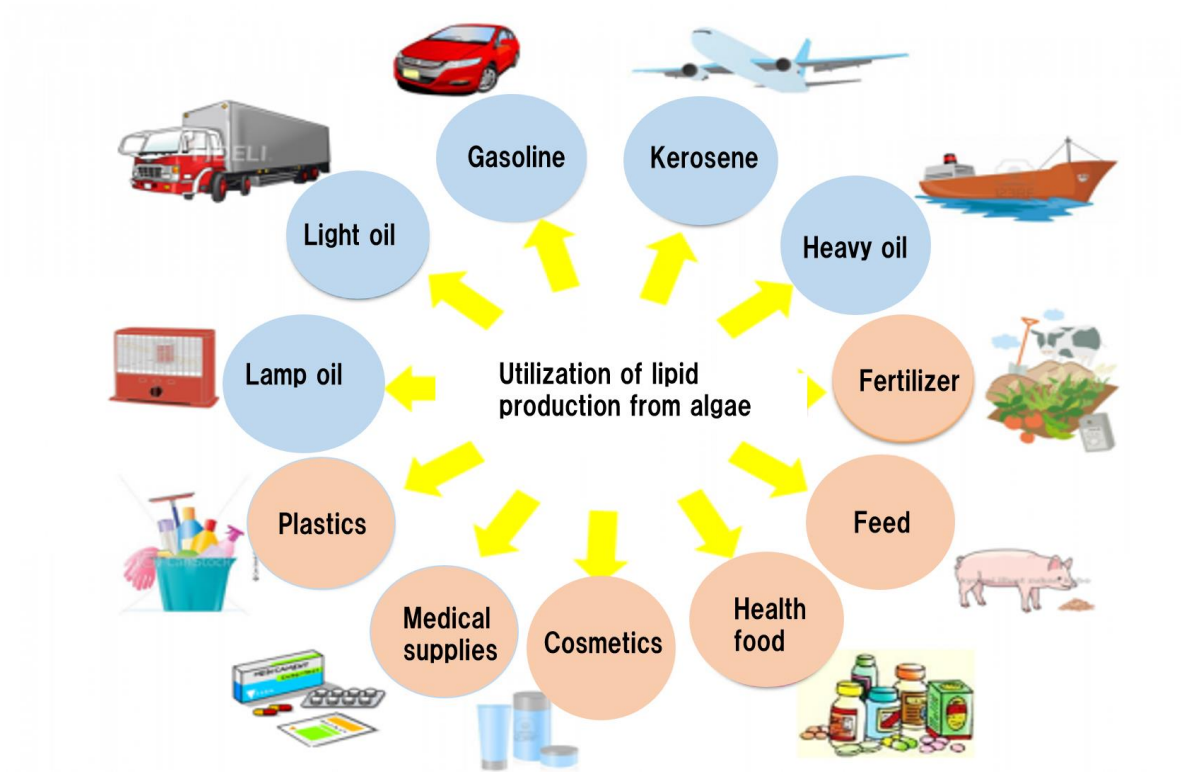

Fig. 1-3 Utilization of lipid production from algae [1-14)]

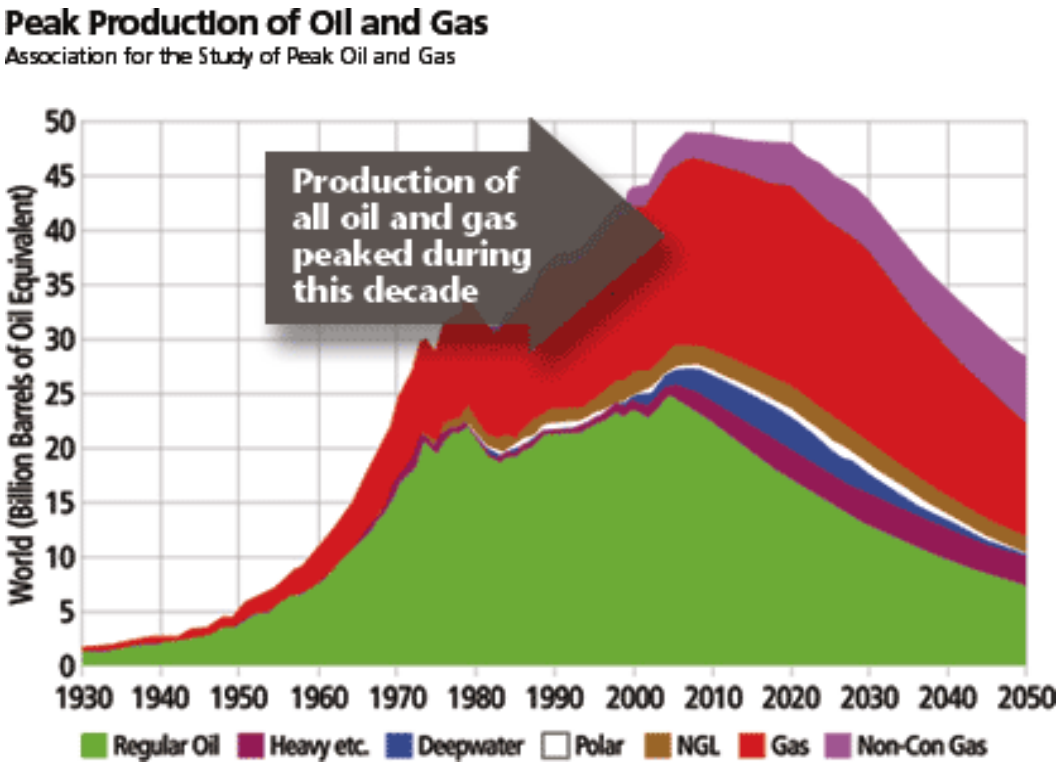

Fig. 1-4 Peak production of oil and gas[1-16)]

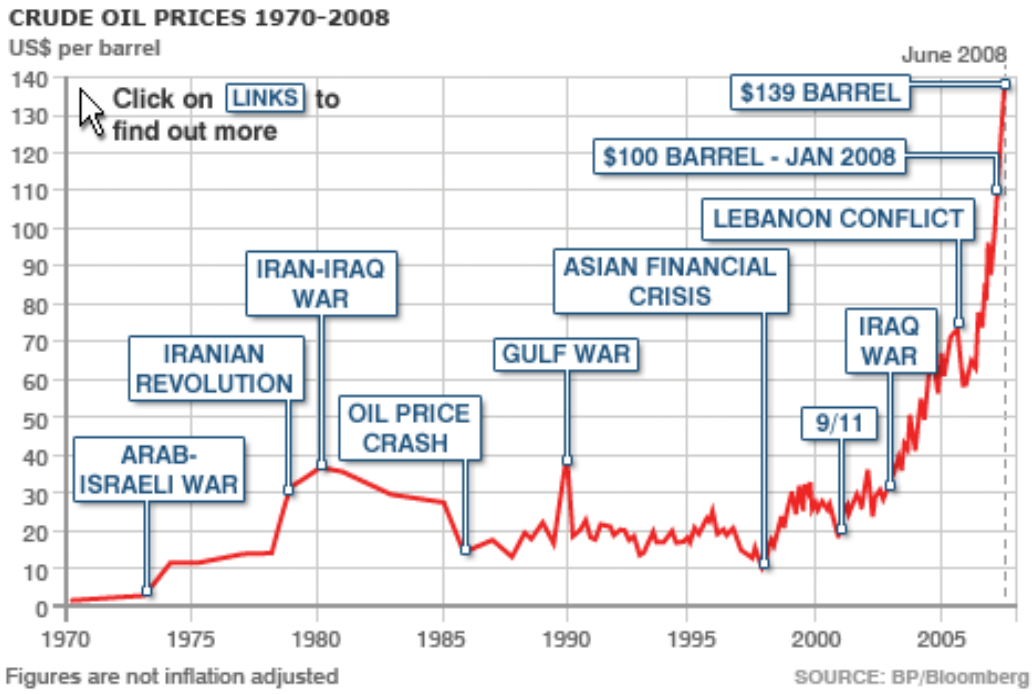

Fig. 1-5 Increase of oil price [1-17)]

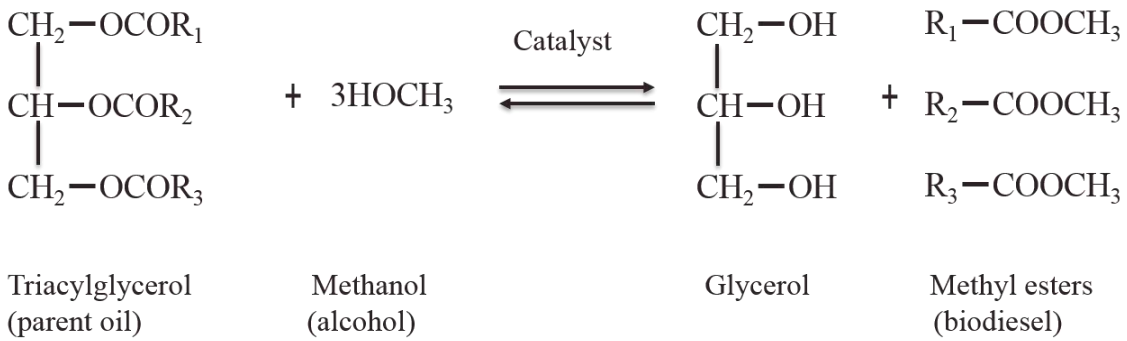

Fig.1-6 Biofuel production[1-15)]

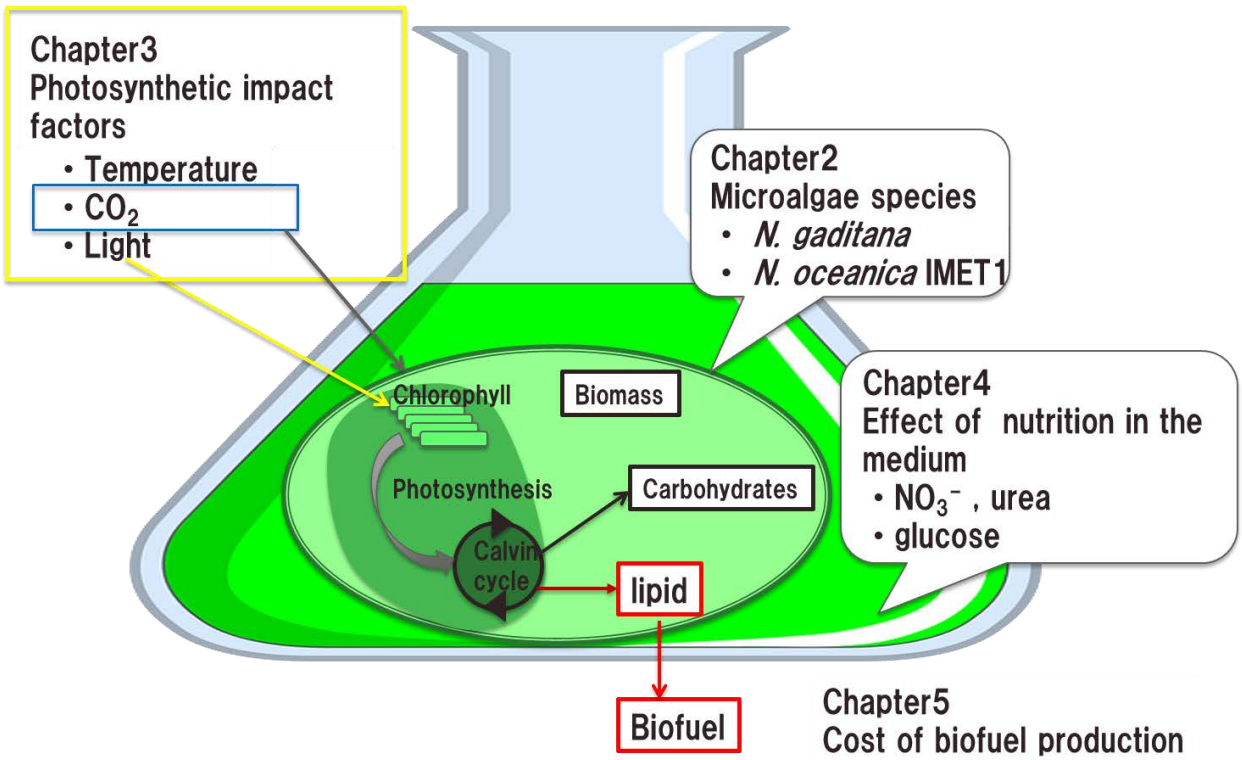

Fig.1-7 Outline of the biofuel production using microalgae in this study

Table 1-1 Comparison of some sources of biofuel [1-15)]

Crop	Oil yield(L/ha)	Land area needed(M ha)[a]	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

a For meeting 50 % of all transport fuel needs of the United States

b 70 % oil (by wt) in biomass

c 30 % oil (by wt) in biomass

Table 1-2 Oil content of some microalga [1-15)]

Microalga	Oil content(% dry wt)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella prmolecta	23
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23

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2. Comparison of the growth performance of *Nannochloropsis oceanica* IMET1 with that of *N. gaditana*

2.1 Materials and Methods

2.1.1 Algal Strains and Culture Conditions

N. oceanica IMET1 and *N. gaditana* CCMP526, 527, 1775, 1894 are used in this study. *N. oceanica* IMET1 was obtained from the IMET, Maryland University Center for Environmental Science. A modified BG11 (MBG11) medium was used to grow both strains. BG11[2-1)] is a common culture medium for algae. MBG11 medium is BG11 with salinity adjusted to 30 ppm with NaCl.

N. oceanica IMET1, *N. gaditana* CCMP526, 527, 1775, 1894 are routinely cultured in flasks containing MBG11. The log-phase cultures were harvested, and the pellets were washed (6,000 × g, 10 min) with the respective medium to remove wastes. The algal pellets were re-suspended in MBG11 medium. One ml of each culture was added to one well of a 48-well Costar plate (Corning Glass, Corning, NY, USA), and six replicates were performed for each culture. The microplates containing the algal cultures were placed inside a GasPak^{TM} bag (Becton Dickinson, Parsippany, NJ, USA).

2.1.2 Specific Growth Rate

Cell density (O.D.$_{600}$) was determined at an absorbance of 600 nm using a multi-mode microplate reader (Spectra Max M5; Molecular Devices, Sunnyvale, CA, USA). The microplate was taken from the GasPak^{TM} bag to read absorbance and was then placed back into the GasPak^{TM} bag.

Cell density was set to an O.D.$_{600}$ of 0.4. Cell density was measured every 2 days at the media change time. All O.D.$_{600}$ data indicated the average value of n = 6 with the standard deviation.

Specific growth rate μ was calculated from the $O.D._{600}$ value on 0 day of culture ($O.D._{.0}$) and the $O.D._{600}$ value on 21th day of culture ($O.D._{.21}$) using the following equation.

$$\mu = \ln(O.D._{.21}/O.D._{.0})/21$$

The specific growth rate was the average value of n = 6 with the standard deviation.

2.1.3 Growth Conditions

The effect of CO_2 concentration on growth rate was investigated under the following conditions. The CO_2 concentration (%) was set by blending pure CO_2 and air using a gas mixture device with two gas flow meters[2-2)]. The microplates containing the algal cultures were placed in a GasPak™ bag and charged with 0.03, 2, 10 and 20 % CO_2 mixture. The algal cultures were incubated at 23ºC with a light intensity of 53 μmol・m^{-2}・s^{-1} .

2.2 Results and Discussion

Fig. 2-1, 2-2, 2-3, 2-4 shows the growth performance of five microalgae in different CO_2 concentration. *N. oceanica* IMET1 shows the highest $O.D._{600}$ in five microalgae and the increase of $O.D._{600}$ during the cultivation time. Especially, *N. oceanica* IMET1 also grows well at CO_2 concentration of 20 %.
We found that *N. oceanica* IMET1 shows the better growth performance than that of *N. gaditana* CCMP526, 527, 1775, 1894. In *N. gaditana*, *N. gaditana* CCMP526, 1894 showed good growth performance.

Fig. 2-5 shows the comparison of $O.D._{600}$ of *N. oceanica* IMET1 and *N. gaditana* on 10th day under the various CO_2 concentrations. The $O.D._{600}$ of IMET1 is higher than that of *N. gaditana* irrespective of the CO_2 concentration. It found that *N. oceanica* IMET1 shows better growth performance than that of *N.*

gaditana under various CO_2 concentrations.

Fig. 2-6 shows the growth rate of each microalgae in different CO_2 concentration. *N. oceanica* IMET1 indicated the highest growth rate irrespective of CO_2 concentration. It suggest that *N. oceanica* IMET1 is expected as a possible candidate for lipid production.

N. gaditana CCMP526, 1894 also show good growth rate. Because of some study were reported about *N. gaditana* CCMP526 has high lipid content, therefore, *N. gaditana* CCMP526 is selected as a comparison of *N. oceanica* IMET1 in this study.

2.3 Conclusion

N. oceanica IMET1 shows high growth performance and growth rate under a wide range of CO_2 contents, and *N. gaditana* CCMP526, 1894 also shows good growth performance and growth rate. There is a few study about *N. gaditana* CCMP526, and reported that *N. gaditana* CCMP526 has high lipid content, so it is selected as a comparison with *N. oceanica* IMET1.

We selected *N. oceanica* IMET1 and *N. gaditana* CCMP526 as the study of microalgae.

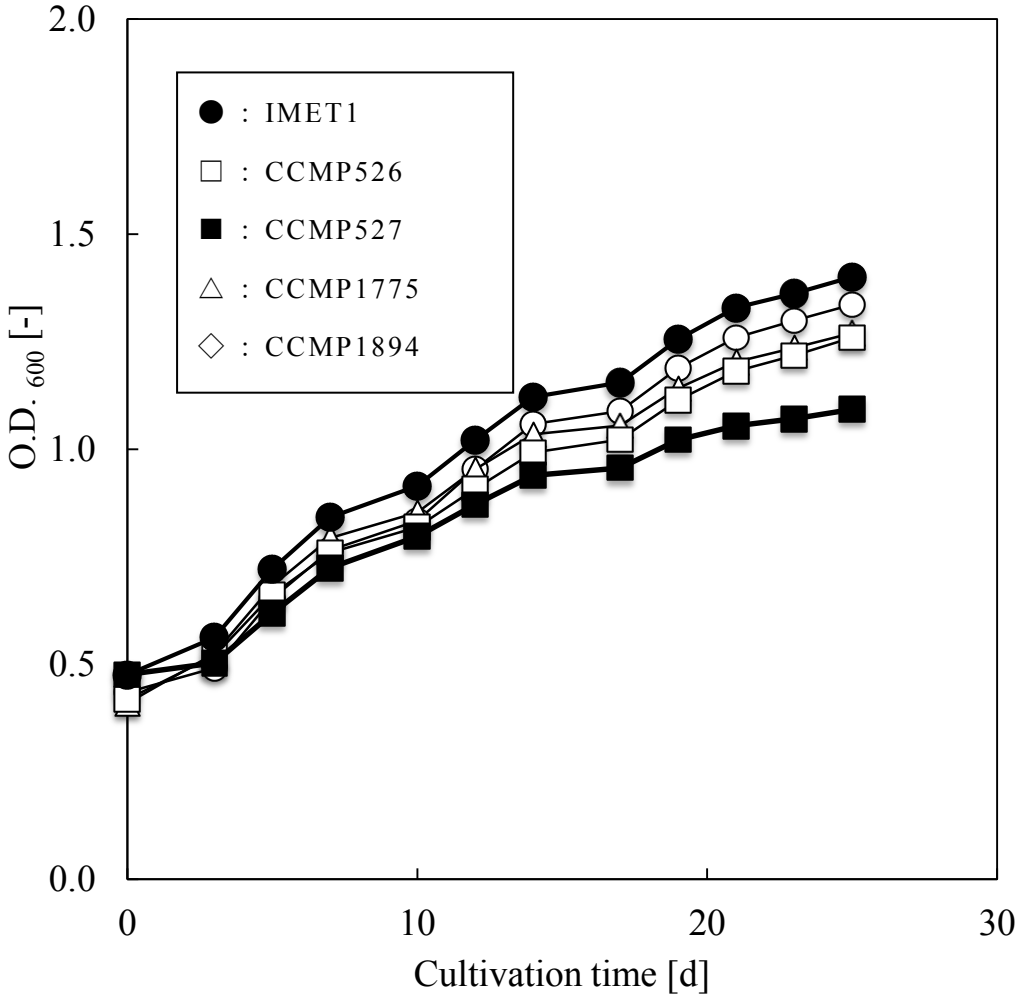

Fig. 2-1 Growth curve of *N. oceanica* IMET 1 and *N .gaditana* under 0.03 % CO_2 concentration

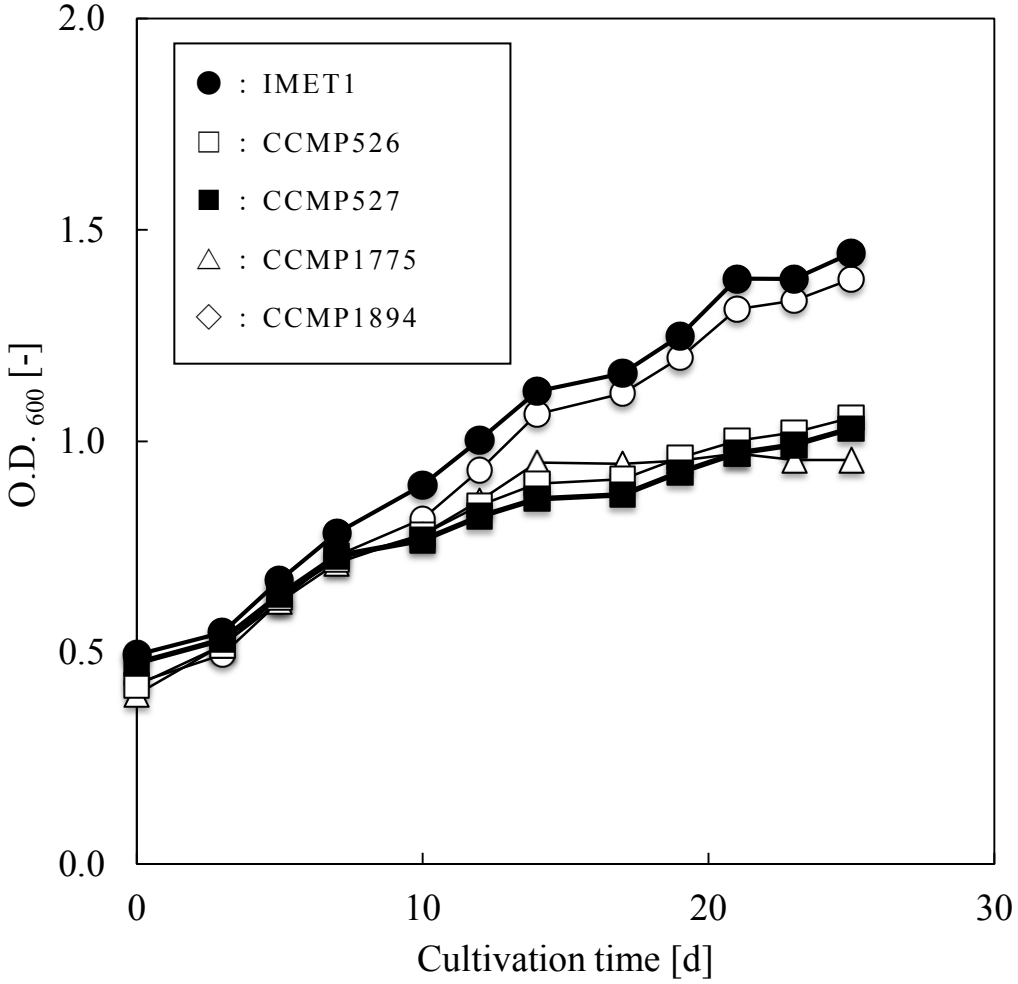

Fig. 2-2 Growth curve of *N.oceanica* IMET 1 and *N.gaditana* under 2 % CO_2 concentration

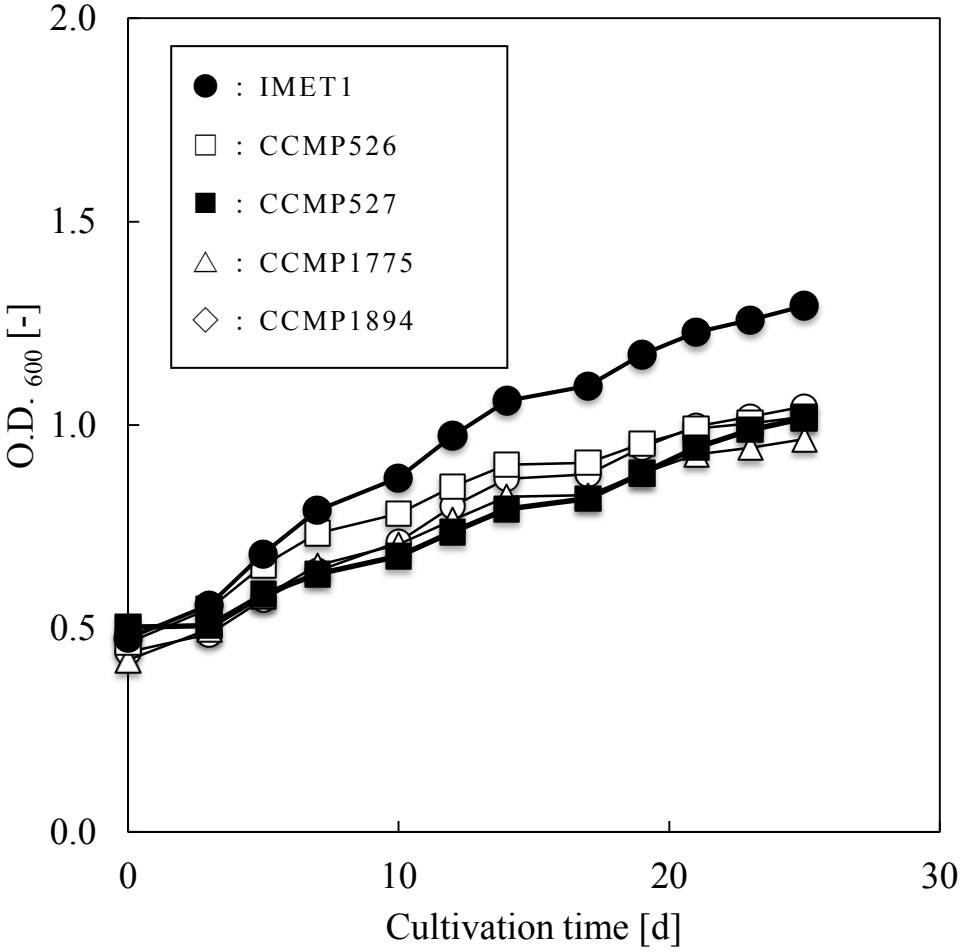

Fig. 2-3 Growth curve of *N. oceanica* IMET 1 and *N. gaditana* under 10 % CO_2 concentration

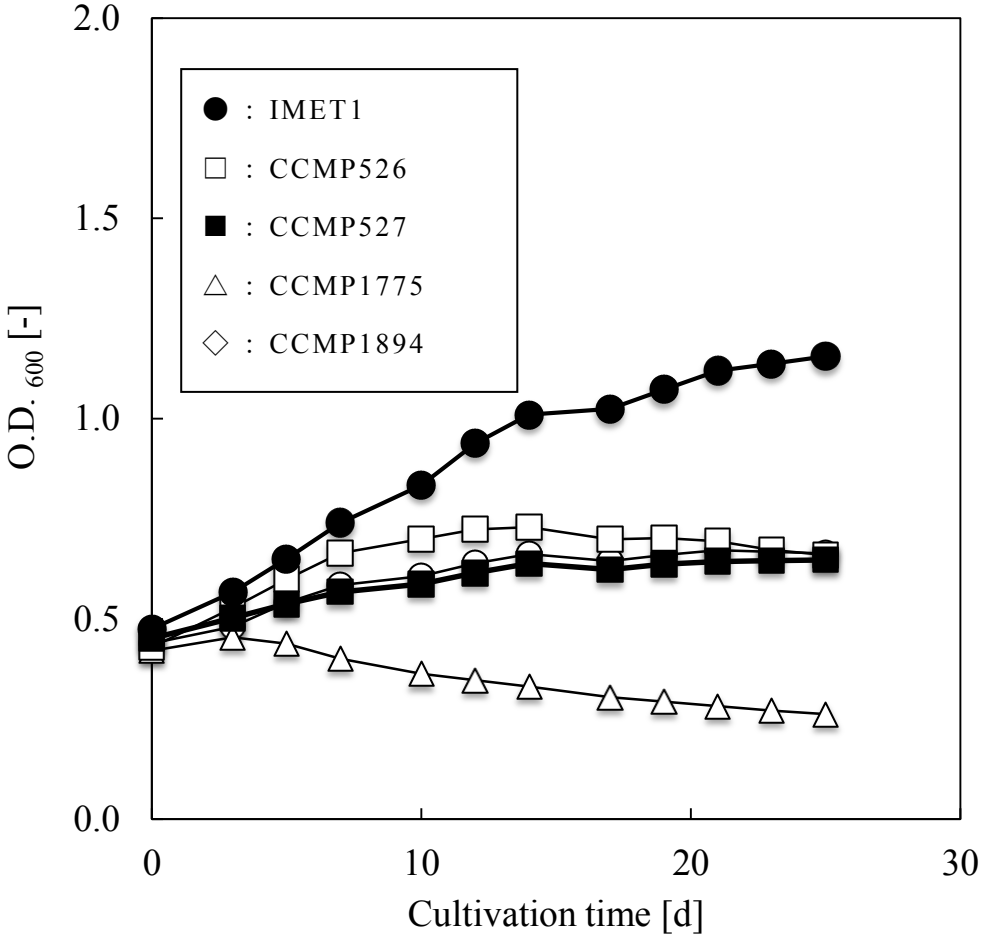

Fig. 2-4 Growth curve of *N.oceanica* IMET 1 and *N.gaditana* under 20 % CO_2 concentration

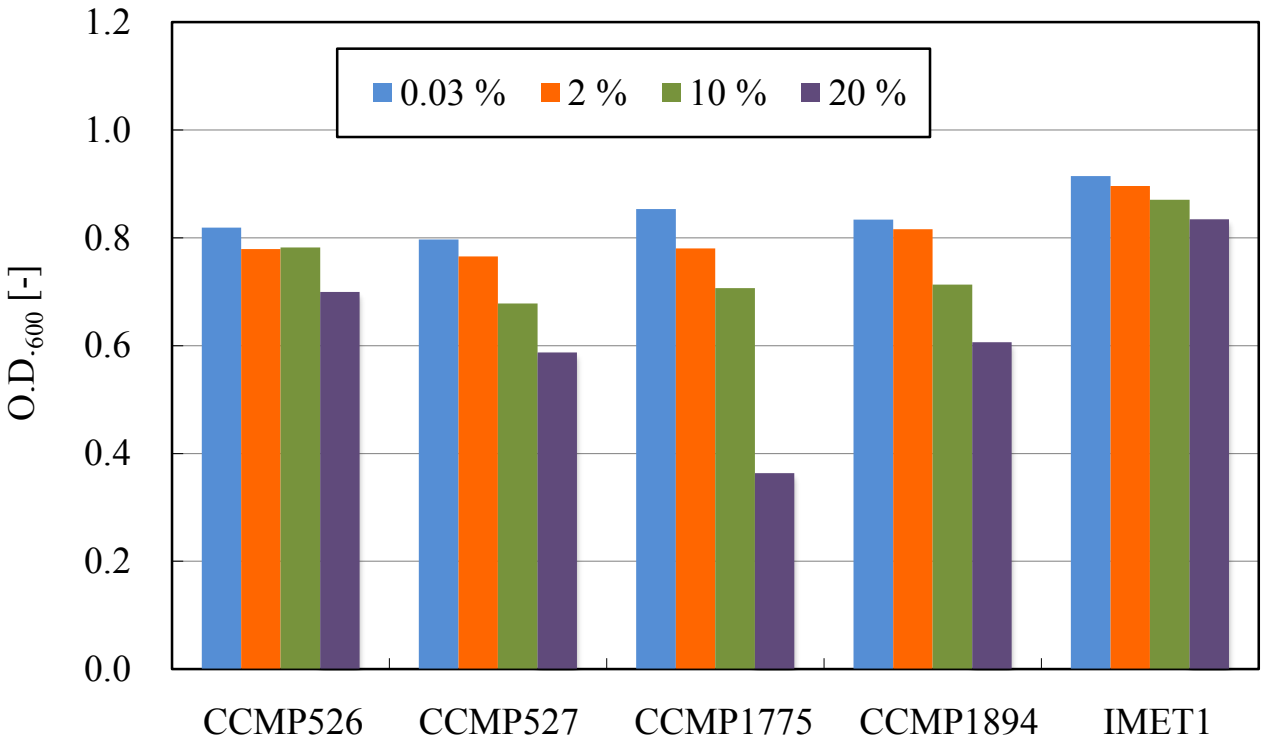

Fig. 2-5 O.D.$_{600}$ of *N. oceanica* IMET 1 and *N. gaditana* on 10th day under various CO_2 concentrations

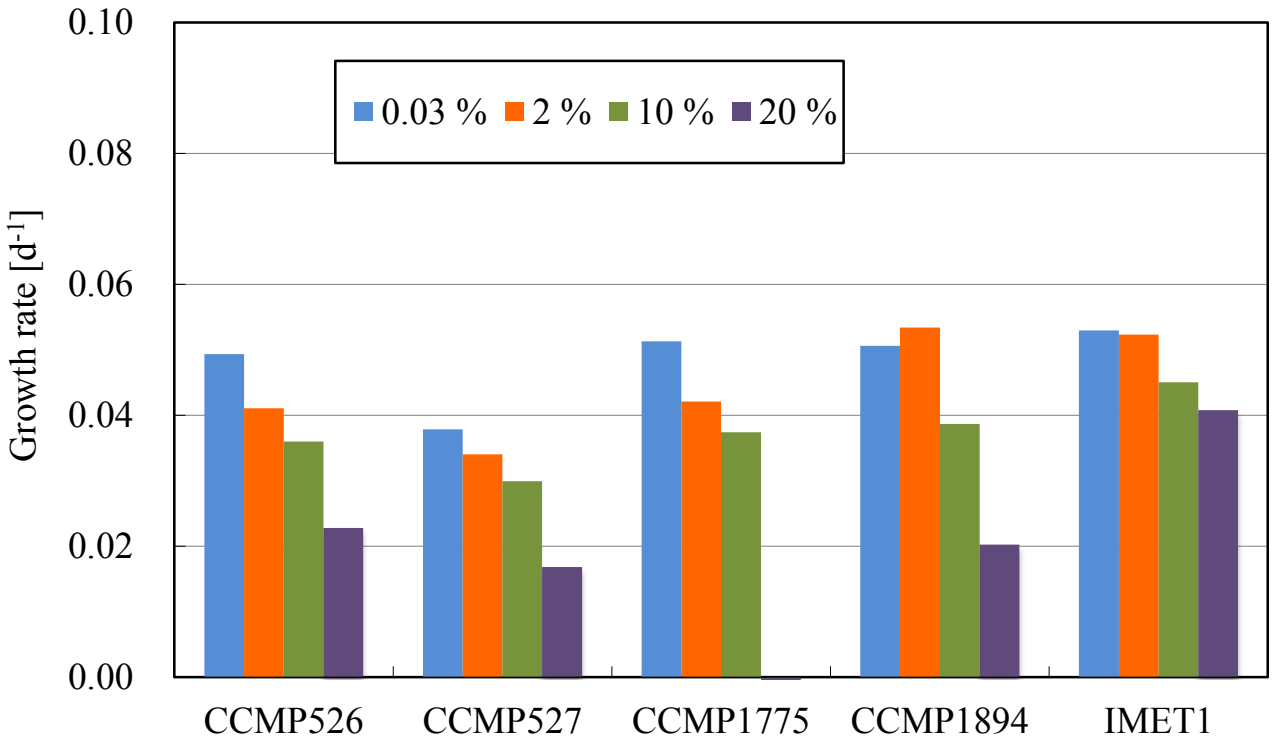

Fig. 2-6 Growth rate of *N. oceanica* IMET1 and *N. gaditana* on 10th day under the various CO_2 concentrations

References

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3. Effect of photosynthetic impact factors (temperature, CO_2, light) on the growth performance for biofuel production

Nannocholopsis spp., which live in marine, fresh, and brackish water, are a potential resource for biofuel production[1-5,1-13,1-19,3-1,3-2]. In addition, *Nannocholopsis* spp. are a good source of the high quality protein[1-18], eicosapentaenoic acid[1-19,1-20].

N. oceanica IMET1 was collected from Eilat, Israel has been maintained at the Institute of Marine and Environmental Technology (IMET) for > 10 years. *N. oceanica* IMET1 has also been distributed to and studied in other laboratories.

Wang *et al.* investigated the bacterial communities isolated from groundwater at different temperatures on biofuel production using *N.oceanica* IMET1[1-4]. Xiao *et al*. evaluated the effects of nitrogen deficiency stress on liquid content, fatty acid distribution, and metabolic profiles under CO_2 concentrations of 1.5–2 % at 20°C[1-12]. Dong *et al*. reported that long-term nitrate depletion changes the *N. oceanica* IMET1 proteome using a two-dimensional gel electrophoresis proteomic approach[1-13]. Ma *et al*. applied heavy-ion irradiation to improve *N. oceanica* IMET1 characteristics for biofuel production[3-3]. Li *et al*. clarified the triacylglycerol-producing mechanism in *N. oceanica* IMET1 under N-repleted and N-depleted conditions[3-4].

The growth performance and characteristics of IMET1 under various culture conditions such as different temperatures, CO_2 concentrations, light intensities, and media have not been reported. Understanding growth performance under different conditions is important to determine the optimum conditions for producing biofuel, eicosapentaenoic acid. Therefore, we investigate the effects of CO_2 concentration, temperature, light intensity, and culture media on the growth performance of *N. oceanica* IMET1 and *N. gaditana* CCMP526.

3.1 Materials and Methods

3.1.1 Algal Strains and Culture Conditions

N. oceanica IMET1 and *N. gaditana* CCMP526 are used in this study. *N.*

oceanica IMET1 was obtained from the IMET, Maryland University Center for Environmental Science. We used *N. gaditana* CCMP526 based on the good growth performance. *N. gaditana* CCMP526 was purchased from the National Center for Marine Microalgae and Microbiota.

A modified BG11 (MBG11) medium and artificial seawater (ASW) medium are used to grow both strains.

BG11[2-1)] is a common culture medium for freshwater algae and contains more nutrients than those in ASW medium. MBG11 medium is BG11 with salinity adjusted to 30 ppm with NaCl. ASW medium is prepared according to the ingredients described by Radakovits *et al*.[1-3)].

Both *N. oceanica* IMET1 and *N. gaditana* CCMP526 were routinely cultured in flasks containing ASW. The log-phase cultures were harvested, and the pellets were washed (6,000 × g, 10 min) with the respective medium to remove wastes. The algal pellets were re-suspended in both ASW and MBG11 medium. One ml of each culture was added to one well of a 48-well Costar plate (Corning Glass, Corning, NY, USA), and six replicates were performed for each culture. The microplates containing the algal cultures were placed inside a GasPak™ bag (Becton Dickinson, Parsippany, NJ, USA).

3.1.2 Specific Growth Rate

Cell density ($O.D._{600}$) was determined at an absorbance of 600 nm using a multi-mode microplate reader (Spectra Max M5; Molecular Devices, Sunnyvale, CA, USA). The microplate was taken from the GasPak™ bag to read absorbance and was then placed back into the GasPak™ bag.

Cell density on day 0 was set to an $O.D._{600}$ of 0.35. Cell density was measured every 2 days at the media change time. All $O.D._{600}$ data indicated the average value of n = 6 with the standard deviation.

Specific growth rate μ was calculated from the $O.D._{600}$ value after 2 days of culture ($O.D._2$) and the $O.D._{600}$ value after 8 days of culture ($O.D._8$) using the following equation

$$\mu = \ln(O.D._8/O.D._2)/6$$

The specific growth rate was the average value of n = 6 with the standard

deviation.

3.1.3 Growth Conditions

The effect of temperature on growth rate was investigated under the following conditions. Temperature was set to 10, 23, and 30°C, respectively. The algal cultures in the microplates were incubated under a 2 % CO_2 concentration, light intensity of 53 μmol·m^{-2}·s^{-1}, and a 12 h/12 h light-dark cycle. Each treatment contained six replicates.

The effect of CO_2 concentration on growth rate was investigated under the following conditions. The CO_2 concentration (%) was set by blending pure CO_2 and air using a gas mixture device with two gas flow meters[2-2)]. The microplates containing the algal cultures were placed in a GasPak™ bag and charged with 0.03, 2, 10, 15, and 20 % of CO_2 concentration. The algal cultures were incubated at 23ºC with a light intensity of 53 μmol·m^{-2}·s^{-1} and a 12 h/12 h light-dark cycle.

The effect of light intensity on growth rate was investigated under the following conditions. Light intensities were set to 13, 53, 82, and 132 μmol·m^{-2}·s^{-1}, respectively. The algal cultures in the microplates were incubated under a CO_2 concentration of 2 % at 23°C with a 12 h/12 h light-dark cycle. Each treatment contained six replicates.

3.2 Results and Discussion

3.2.1 Growth Performance under Different CO_2 Concentrations

Fig. 3-1, 3-2 shows the growth performance under different CO_2 concentrations. *N. oceanica* IMET1 grows with increasing cultivation time irrespective of CO_2 conditions. *N. gaditana* CCMP526 grows well at CO_2 concentration of 0.03 and 2%, but however hardly grows at high CO_2 concentration.

The growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 were the highest at a CO_2 concentration of 2 %. Moreover, the growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 were higher in MBG11 medium than those in ASW medium. Growth of both strains was inhibited at higher CO_2 concentrations (**Fig. 3-3**).

N. oceanica IMET1 maintained relatively higher growth rates compared with those of *N. gaditana* CCMP526 at CO_2 concentrations of 10, 15, and 20 %. The growth rate of *N. oceanica* IMET1 outperformed the growth rate of *N. gaditana* CCMP526 in both ASW and MBG11 culture media at the higher CO_2 concentrations. At a CO_2 concentration of 20 %, in MBG11 medium, *N. gaditana* CCMP526 nearly ceased growing (μ = 0.002 d^{-1}); however, the growth of *N. oceanica* IMET1 only slowed down at ~35% compared to the highest growth rate at the 2 % CO_2 concentration. Both strains seemed to grow better in MBG11 medium than in ASW medium, particularly under the higher CO_2 concentrations. The growth rate of *N. oceanica* IMET1 outperformed that of *N. gaditana* CCMP526 at all tested CO_2 concentrations in ASW medium.

Hu and Gao reported that biomass yield while supplying air with 2,800 μl CO_2 l^{-1} was higher than that supplying air with 350 μl CO_2 l^{-1}, when *Nannochloropsis* sp. (PP983) was used[3-5]. Chiu *et al.* reported that biomass concentration and growth rate at a CO_2 concentration of 2 % were higher than those at other CO_2 concentrations because *N. oculata* utilizes CO_2 in response to adding CO_2[3-6]. We also found the highest growth rate at a CO_2 concentration of 2 %. Chiu *et al.* also reported that biomass concentration and growth rate decreased when the CO_2 concentration was > 5 %, which correlates with our result. Although growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 decreased when CO_2 concentration was > 10 %, the growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526. The growth performance of *Nannochloropsis* spp. was not clear; therefore, it is difficult to understand the reasons for the difference in growth rate over CO_2 concentration of 10 % between *N. oceanica* IMET1 and *N. gaditana* CCMP526. This may resulted from the different genetic backgrounds between *N. oceanica* IMET1 and *N. gaditana* CCMP526, which are not closely related[1-3]. In addition, *N. oceanica* IMET1 has different genes in the Kennedy pathway from those of *C. reinhardtii*[3-4].

3.2.2 Growth Performance under Different Temperatures

Fig. 3-4, 3-5 show the growth performance under different temperatures. *N. oceanica* IMET1 grows with increasing cultivation time irrespective of

temperature. The growth of *N. oceanica* IMET1 in MBG11 at 30ºC is higher than that of 23ºC within 10 days cultivation time, however the growth of *N. oceanica* IMET1 in MBG11 at 23ºC well grows over 12 days. When ASW is used, *N. oceanica* IMET1 grows well with raising temperature, however growth at 23 and 30 ºC is not so different at 16 days.

Fig. 3-6 shows the comparison of the growth rates between *N. oceanica* IMET1 and *N. gaditana* CCMP526 at 10, 23, and 30°C. The growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 increased with a rise in temperature. The growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526 at all temperatures, regardless of the medium. These results indicate that the growth rate of *N. oceanica* IMET1 at 30°C was the best in MBG11 medium (μ = 0.1 d^{-1}). Growth of *N. oceanica* IMET1 was inhibited at 10°C in a CO_2 concentration of ~25 %. Although the growth rates of *N. gaditana* CCMP526 at 23 and 30°C were acceptable, the growth rate of *N. gaditana* CCMP526 at 10°C was inhibited (μ = 0.03 d^{-1}). *N. oceanica* IMET1 and *N. gaditana* CCMP526 grew better in MBG11 medium than that in ASW medium. In general, both strains maintained rapid growth at 23 and 30°C; however, their growth rates significantly decreased when temperature dropped to 10°C. Wu *et al.* reported that the maximum growth rate of *Monoraphidium* spp. occurred at 30°C[3-7]. Their result correlated with our result. These results suggest that *N. oceanica* IMET1 had higher growth rates than those of *N. gaditana* CCMP526, regardless of temperature or culture conditions. These results may be because of the activities of five putative genes related to carbon fixation such as carbonic anhydrate mediated carbon-concentrating metabolism and a C4 cycle mechanism in *N. oceanica* IMET1[3-4].

In this study, the growth performance of *N. oceanica* IMET1 and *N. gaditana* CCMP526 was compared in 48-well microplates. The microplate culture system is different from other culture systems such as flasks or bioreactors. Therefore, it may be difficult to compare our results with those obtained from large cultivation systems with air bubbling. For example, growth of *N. oculata* NCTU-3 was completely inhibited when cultured at CO_2 concentrations of 5 vol%, 10 vol%, and 15 vol% in flasks[3-6]. In our experiments, different concentrations of CO_2 were

contained in GasPak™ bags. Nevertheless, the growth rates of these two *Nannochloropsis* strains were compared in parallel in this study and they differed greatly in terms of their capability of handling different environmental stressors.

3.2.3 Growth Performance under Different Light Intensities

Fig. 3-7 shows the comparison of growth rates between *N. oceanica* IMET1 and *N. gaditana* CCMP526 irradiated under light intensities of 13, 53, 82, and 132 μmol·m^{-2}·s^{-1}. The growth rate of *N. oceanica* IMET1 in MBG11 was maximum at a light intensity of 53 μmol·m^{-2}·s^{-1} and was higher than that of *N. gaditana* CCMP526 regardless of light intensity. The growth rate of *N. oceanica* IMET1 in ASW was nearly the same as the growth rate of *N. gaditana* CCMP526 in ASW at the various light intensities. The growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 in MBG11 medium were higher than those in ASW. These results indicate that light intensity affects the growth rate of *Nannochloropsis* in MBG11. In addition, the growth rate of *N. oceanica* IMET1 in MBG11 is the highest at a light intensity of 53 μmol·m^{-2}·s^{-1}.

Optimal growth of both strains occurred at 53 μmol·m^{-2}·s^{-1}. Because the experiments were conducted in 48-well microplates, the optimal light intensity was generally lower than the optimal light intensity of a flask-based culture system. Growth of both *N. oceanica* IMET1 and *N. gaditana* CCMP526 was photoinhibited to some extent at a light intensity of 132 μmol·m^{-2}·s^{-1}. *N. oceanica* IMET1 and *N. gaditana* CCMP526 again preferred MBG11 medium to ASW medium. Dipasmita also reported the same result in a light intensity experiment in which the growth rate of *Nannochloropsis* spp. declined under high light intensity in 13 g/L NaCl [3-7].

3.3 Conclusion

We investigated the growth performance of *N. oceanica* IMET1 compared with that of *N. gaditana* CCMP526. The growth performance of *N. oceanica* IMET1 was better than that of *N. gaditana* CCMP526. In particular, the growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526, regardless of culture medium under CO_2 concentrations > 10 %. In addition, growth of *N.*

oceanica IMET1 was maintained at a high level under a wide range of light intensities and temperatures in both culture media. The optimum growing conditions for *N. oceanica* IMET1 was a CO_2 concentration of 2 %, light intensity of 53 μmol·m^{-2}·s^{-1}, and 30°C when MBG11 was used.

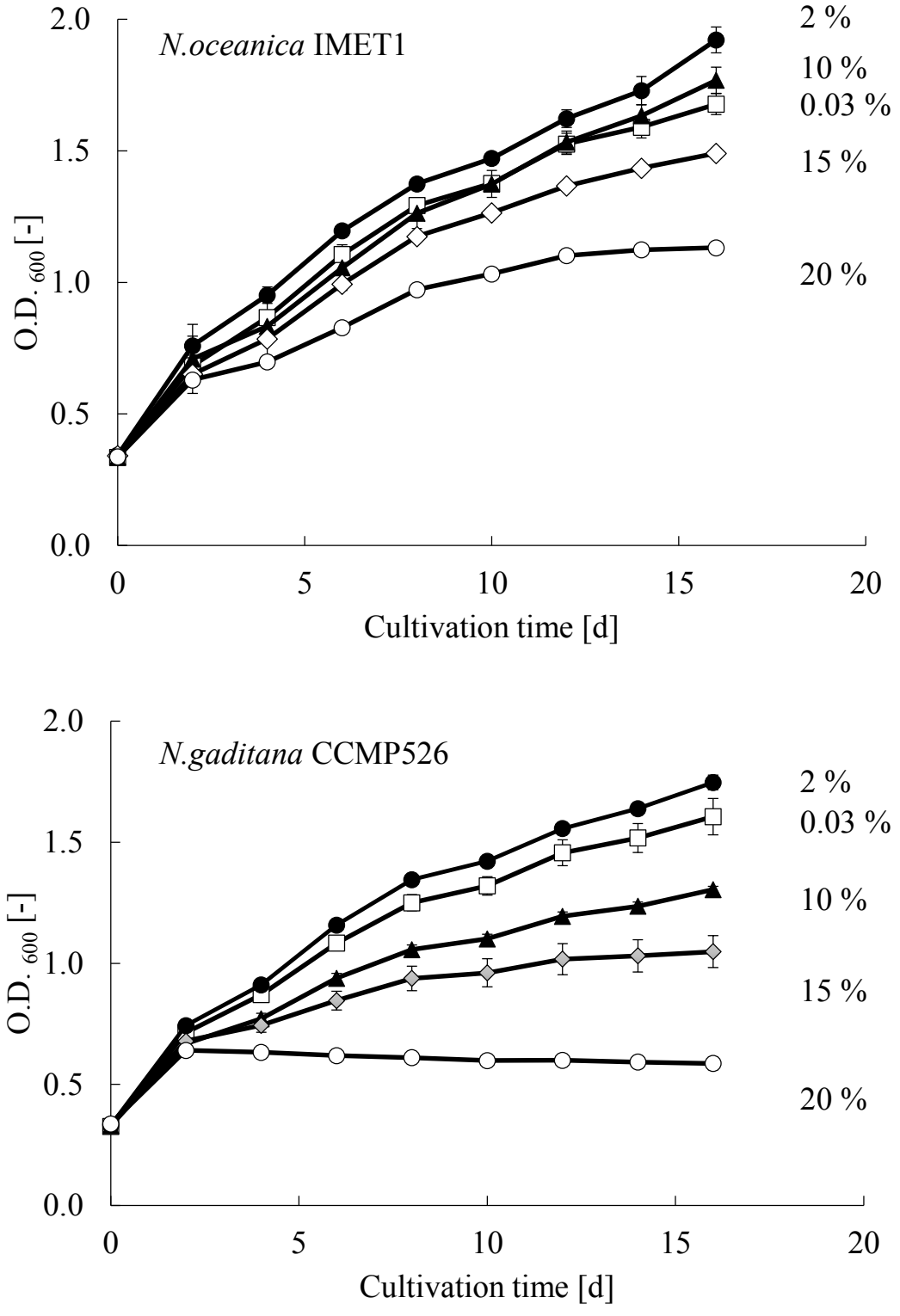

Fig. 3-1 Growth curve of *N.oceanica* IMET1 and *N.gaditana* CCMP526 in MBG11 under different CO_2 concentrations

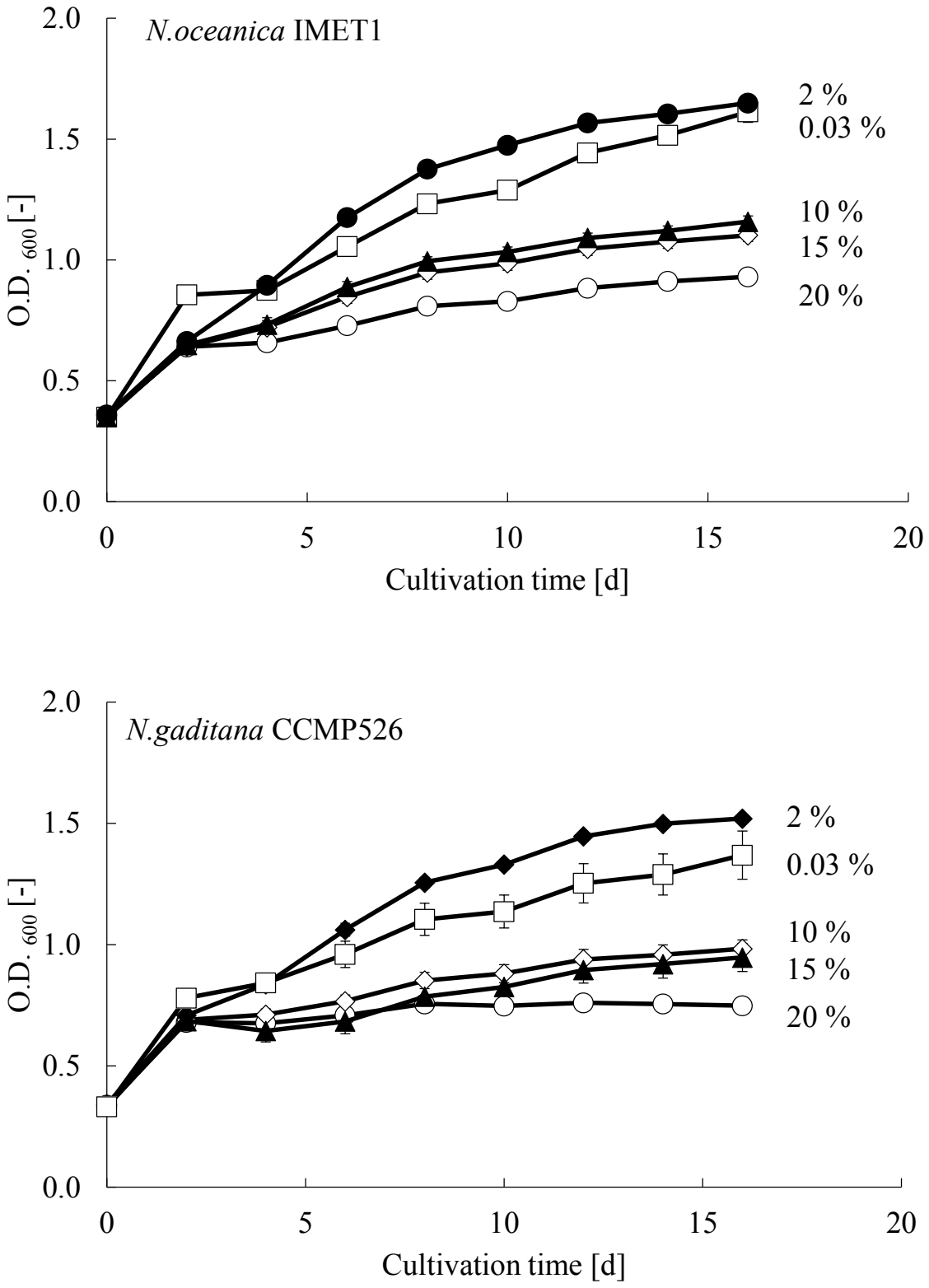

Fig. 3-2 Growth curve of *N.oceanica* IMET1 and *N.gaditana* CCMP526 in ASW under different CO_2 concentrations

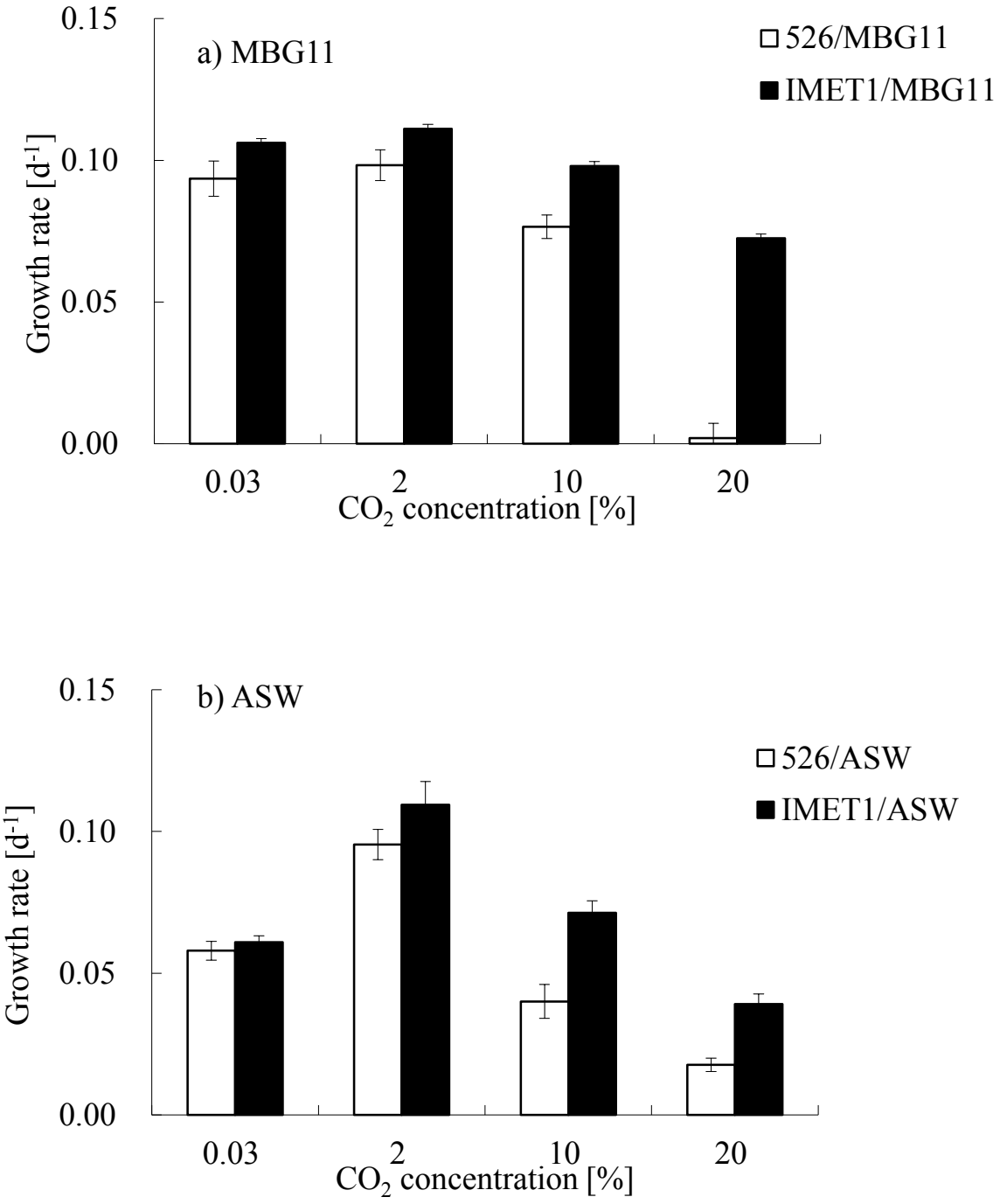

Fig. 3-3 Growth rate under different CO_2 concentrations

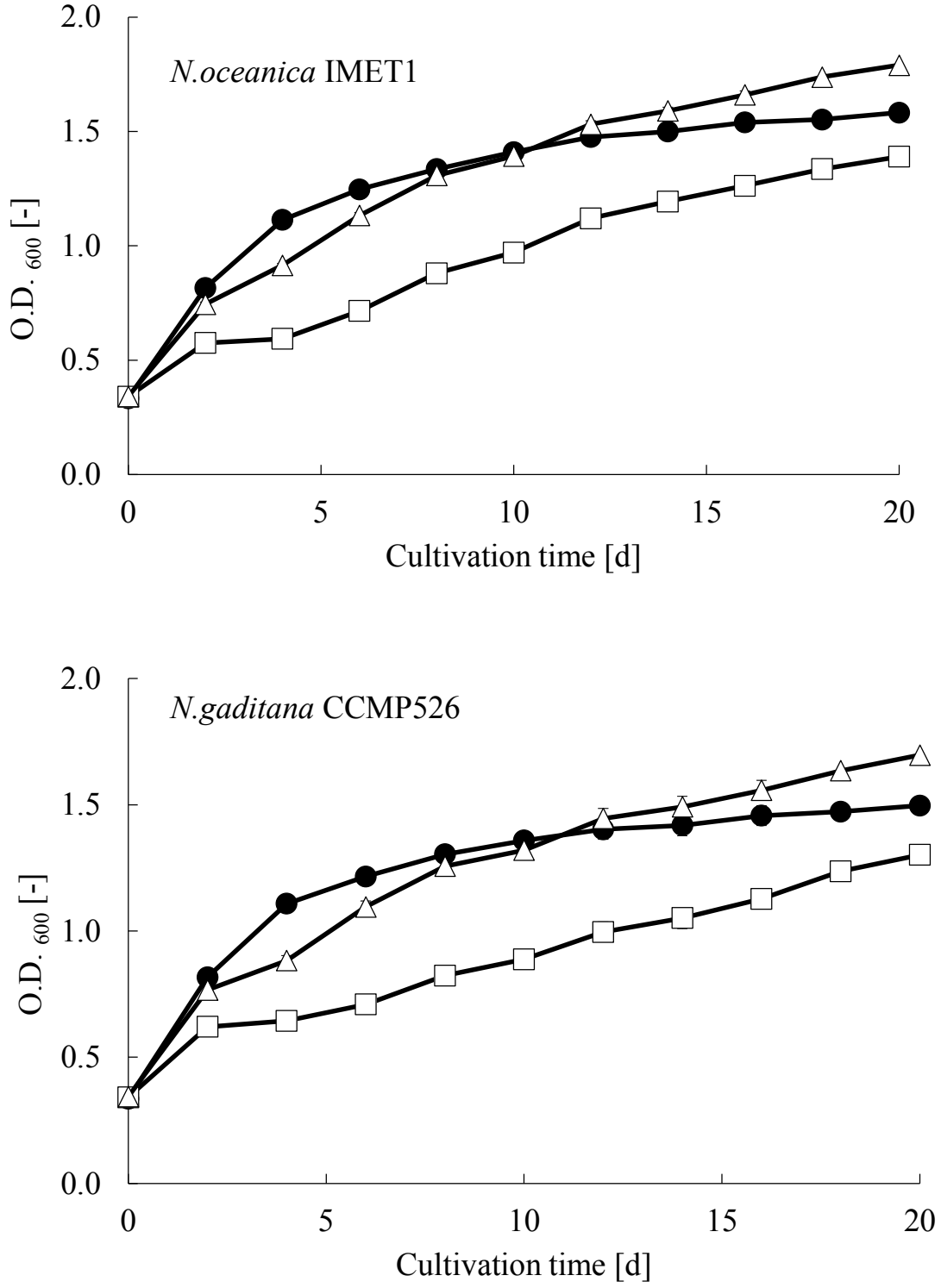

Fig. 3-4 Growth performance of *N.oceanica* IMET1 and *N.gaditana* CCMP526 in MBG11 under different temperatures (□: 10℃, △: 23℃, ●: 30℃)

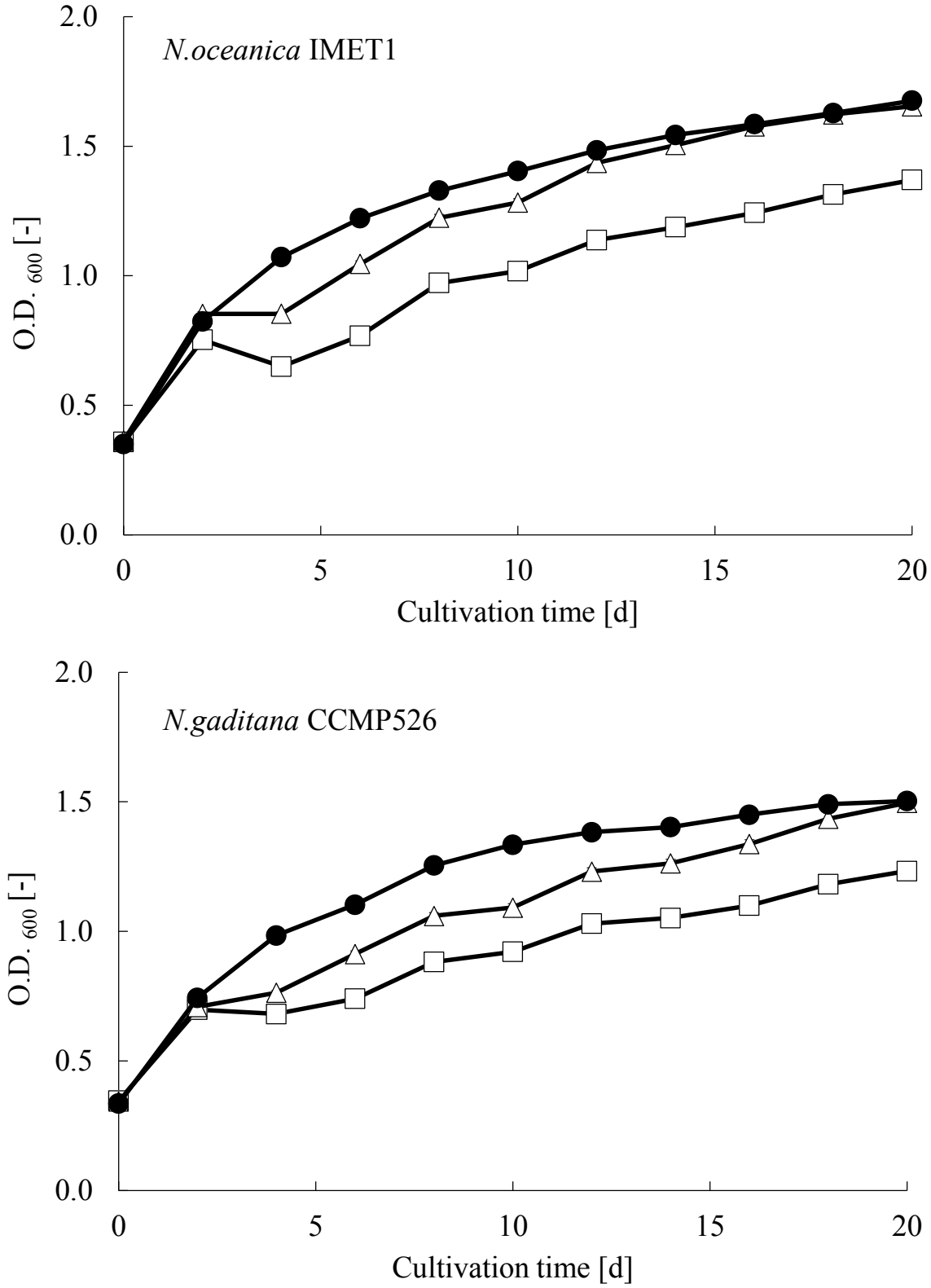

Fig. 3-5 Growth performance of *N.oceanica* IMET1 and *N.gaditana* CCMP526 in ASW under different temperatures (□: 10℃, △: 23℃, ●: 30℃)

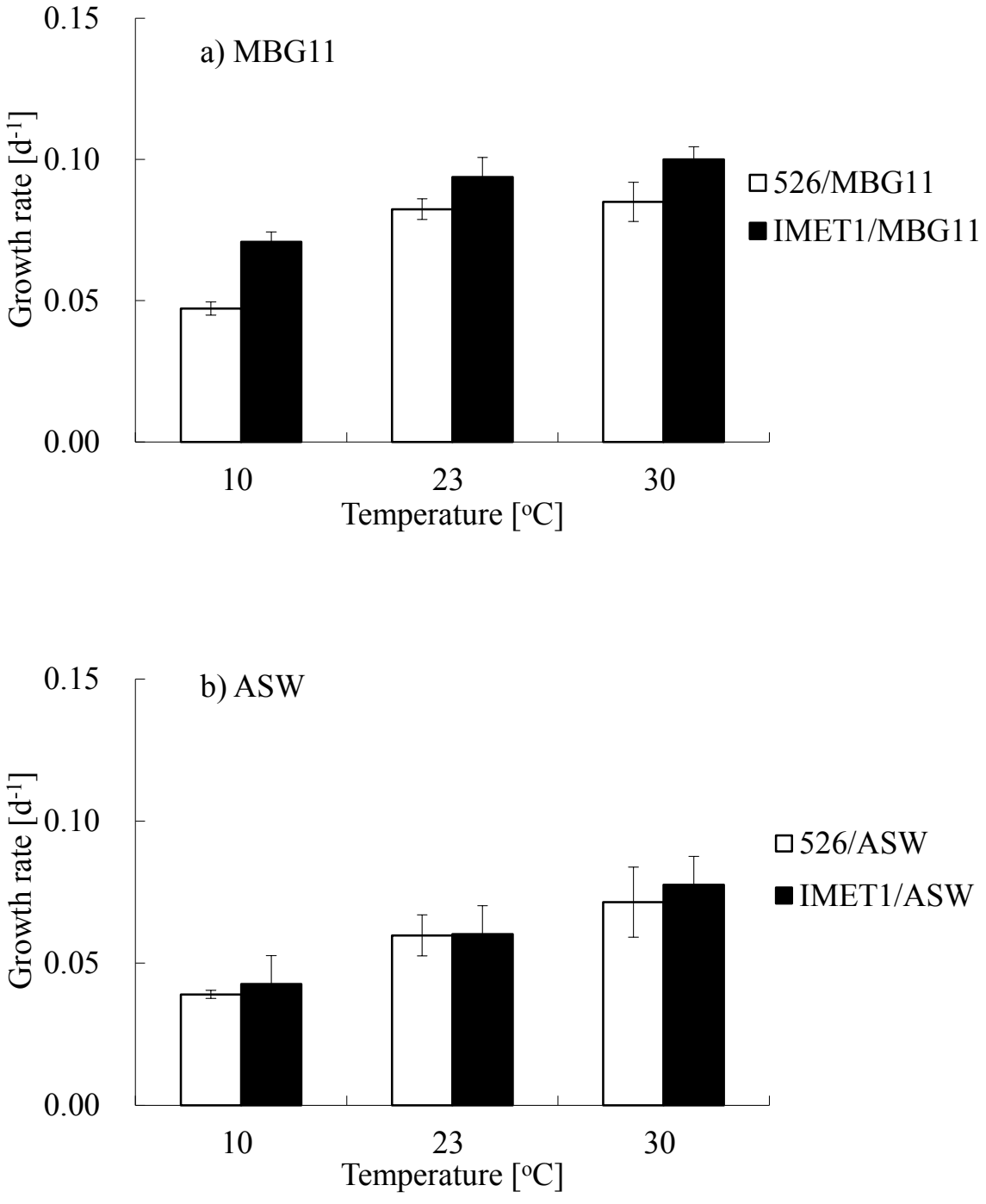

Fig. 3-6 Growth rate under different temperatures

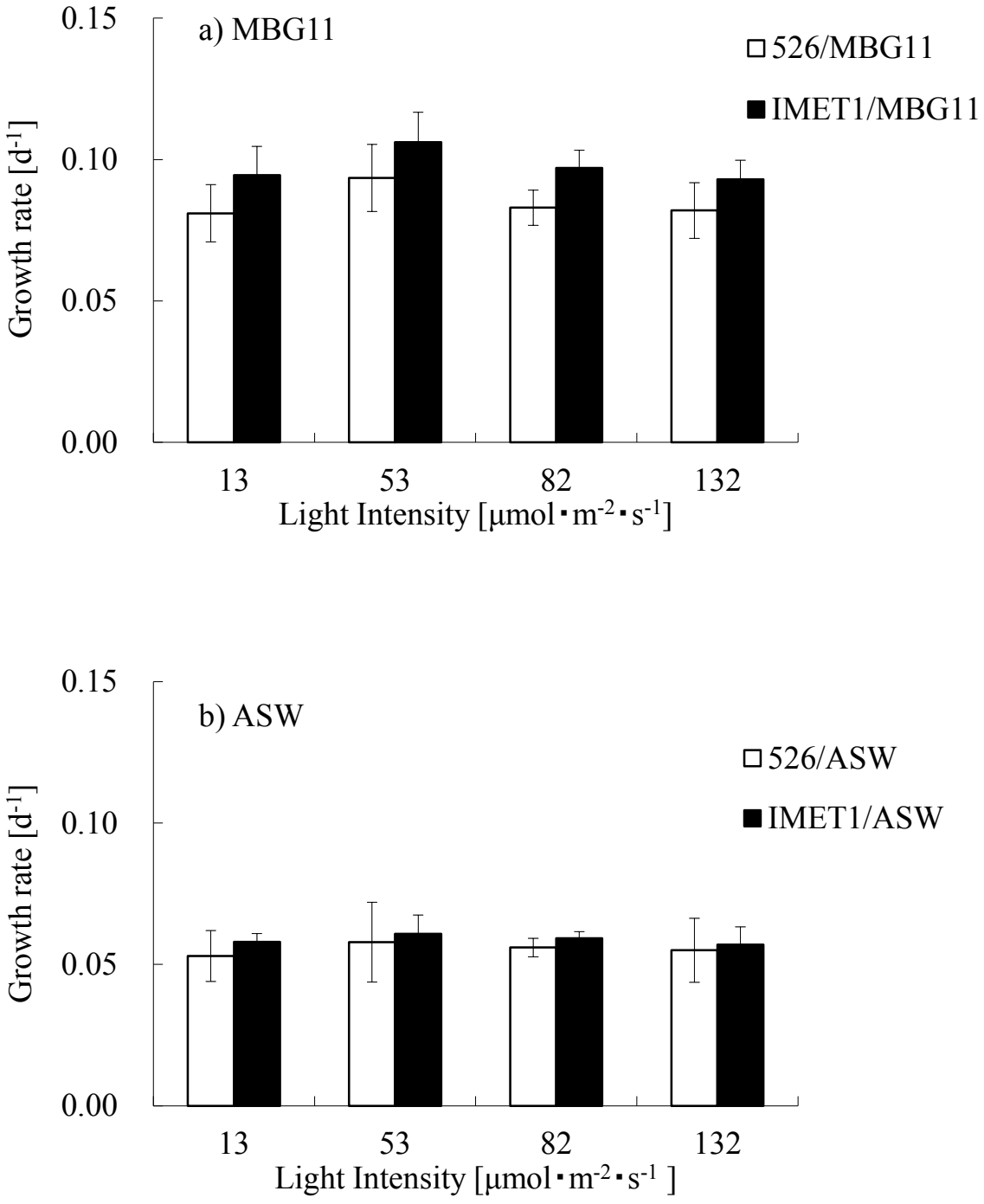

Fig. 3-7 Growth rate under different light intensities

Table 3-1 Composition of BG-11 medium[2-1)]

$NaNO_3$	1.5 g/L
$MgSO_4$	75 mg/L
K_2HPO_4	39 mg/L
$CaCl_2 \cdot 2H_2O$	38 mg/L
Na_2CO_3	20 mg/L
Citric acid $\cdot H_2O$	6 mg/L
Ferric ammonium citrate	6 mg/L
Na_2EDTA	1 mg/L
A6 (microcomponent)	1 mL/L

Table 3-2 Composition of ASW medium[1-3)]

NaCl	15 g /L
$MgSO_4 \cdot 7H_2O$	6.6 g /L
$MgCl_2 \cdot 6H_2O$	5.6 g /L
$CaCl_2 \cdot 2H_2O$	0.5 g /L
KNO_3	1.45 g /L
KH_2PO_4	0.12 g /L
$NaHCO_3$	40 mg /L
$FeCl_3 \cdot 6H_2O$	10 mg /L
Na_2-EDTA	35 mg /L
$MnCl_2 \cdot 4H_2O$	0.25 ml/L
trace metal mix	0.5 ml/L

References

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4. Effect of nutrition in the medium (nitrogen, carbon sources) on biomass and biofuel production

4.1 Material and Method

4.1.1 Microalgae and culture conditions

Microalgal cells of *N. oceanica* IMET1 and *N. gaditana* CCMP526 were cultured in a BG11 medium. The cells were then incubated at 30°C in Erlenmeyer flasks and illuminated continuously at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with white fluorescent lamps. CO_2 (2 %) composed by blending pure CO_2 and air using a gas mixture device with two gas flow meters was supplied.

4.1.2 Nitrogen source

Two nitrogen sources were used: sodium nitrate ($NaNO_3$) and urea (CH_4N_2O). For investigating the effect of sodium nitrate, BG11 medium was used. For investigating the effect of urea, BG11 medium with urea instead of sodium nitrate was used. The initial cell densities were adjusted to 0.4 by absorbance at 600 nm ($O.D._{600}$). The cultivation ran for 12 days.

4.1.3 Glucose

BG11 medium was modified due to the influence of the effect of glucose on the growth performance and lipid product. The organic carbon sources such as citric acid, ammonium ferric citrate, ethylenediaminetetraacetic acid, sodium carbonate in BG11 were instead of glucose. Five mg of streptomycin as antibiotics was added into BG11 medium to minimize bacteria growth. The initial cell densities were adjusted at 0.4 by absorbance rate of 600 nm ($O.D._{600}$). The cultivation ran for 12 days.

4.1.4 Cell density and Dry cell weight

Cell concentrations were measured by hemacytometer. Dry cell weight was measured using the following procedure: A 50-mL sample was taken from the Erlenmeyer flask and placed in a centrifuge tube. The cells were separated by a

centrifugal separator at 10,000 rpm for 15 min. The supernatant was removed and the precipitate was washed with deionized water and centrifuged at 10,000 rpm for 15 min. This process was repeated twice. The cells were frozen at –20^{o}C and lyophilized using vacuum freeze-drying equipment for 24 h. Dry cell weight was measured using an electric balance, and divided by 50 mL and expressed as a concentration.

4.1.5 Lipid production

The Blight–Dyer method[4-1)] was used for the extraction of lipid from the dried cells. The remained layer mixed with pure hexane and neutral lipids were extracted. The hexane extraction process was repeated three times. The cell extracts were combined in a recovery flask and evaporated under vacuum. The weight of the remaining lipid was expressed as a proportion of the dry cell weight.

The lipid products were separated by TLC to extract only TAG. The TLC spot of TAG was scraped off the plate with a spatula and placed in a glass tube. To methylate the fatty acid production, a fatty acid methylation kit (product number 06482-04) that produced by Nacalai tesque Inc. was used. Fatty acid methyl ester (FAMEs) were analyzed using a gas chromatograph with flame ionization detector (GC-14B, Shimadzu Co.). UD-wax was used as the separation column. Analysis conditions were as follows: the oven temperature was initially set at 100°C, rising to 240°C at 15°C/min, and being held for 10 min. The split ratio was 1:50. A 1-μL sample was injected. Fatty acid composition was expressed as the peak area ratio of each fatty acid.

4.2 Results and discussion

4.2.1 Cultivation in flask system

For the biomass and biofuel production, cultivation was carried out in 500-mL Erlenmeyer flask. The suitable culture condition obtained from chapter 3, were a temperature of 30°C, CO_2 concentration of 2%, light intensity of 53 μmol • m^{-2} • s^{-1}. To analyse the lipid composition, we cultured the microalgae in flask. As the height of flask was higher than micro-plate for making sure the microalgae in the

upper levels of flask can receive the light, we designed the light experiment. When flask reactor was used, the result showed that cultured with 100 μmol・m^{-2}・s^{-1} indicates higher growth performance (**Fig. 4-1**).

Fig. 4-2 shows the growth performance of *N. oceanica* IMET1 comparing with CCMP526. Dry cell weight is increased during cultivated time, and dry cell weight of *N. oceanica* IMET1 is higher than that of *N. gaditana* CCMP526 irrespective of cultivation time. This trend is the same as the result that obtained from the experiment carried out in microplate.

Fig. 4-3 shows the fatty acid composition. The dominant fatty acids (>5 % of the total FA content) for both algal strains were C16:0, C16:1, C18:1, C18:2, C18:3. The C16:0 fatty acid was the most abundant FAs in both *N. oceanica* IMET1 and *N. gaditana* CCMP526, but C16:0 in *N. oceanica* IMET1 was nearly twice as that in *N. gaditana* CCMP526. In addition, *N. oceanica* IMET1 also contained more C18:2, C18:3, C18:0 fatty acids than *N. gaditana* CCMP526. In contrast, IMET1 contained less C14:0, C16:1, C18:1, C20:5 fatty acids compared to *N. gaditana* CCMP526. The fatty acid composition C14:0, C16:0, C16:1, C18:1, C18:2, C20:5 were also reported by Dong *et al*.[1-12)] in the *N. oceanica* IMET1 to long-term nitrogen starvation. But in our experiment, the amount of C16:0 and C18:2 fatty acids were higher than those reported by Dong *et al*.[1-12)] The composition of fatty acids of *N. oceanica* IMET1 is similar to that reported by Xiao *et al*. [1-13)]

4.2.2 Effect of nitrogen source

Fig. 4-4 shows the comparison of cell densities during the culture time. Under both nitrogen sources, cells increased with increasing cultivation time. Urea led to a significantly greater cell increase than nitrate.

Fig. 4-5 shows the comparison of dry cell weights during the culture time. Dry cell weight also increased with increasing cultivation time in both of nitrogen sources. Although dry cell weight was almost the same irrespective of the nitrogen source from 0 to 6 days, and the dry cell weight for nitrate was greater than that for urea after 8 days. These results showed the same trend with the report that cultured *N. salina* (strain 1776) using various nitrogen sources reported by Campos *et al*. [4-2)]. They also reported that urea led to more cells than

nitrate, and nitrate-grown cells had larger diameters than urea-grown cells. Li *et al.* reported the effect of nitrogen source on *Neochloris oleoabundans* UTEX 1185[4-2]. They found that the dry cell weight with nitrate was greater than that with urea. The trend was also the same irrespective of using the different microalgae with ours. Moreover, Goksan[4-3] also reported that the dry cell weight of *Haematococcus pluvialis* cultivated with nitrate was greater than that with urea.

Thus, nitrate increases dry cell weight of *N. oceanica* IMTE1 over urea, the effect of nitrogen source on the growth of *N. oceanica* IMET1 show the same trend with the effect on the other microalgae.

Fig. 4-6 shows the comparison of lipid production under different nitrogen sources and **Fig. 4-7** shows the lipid content cultured for 12 days. The lipid content cultured with nitrate was greater than that with urea. This result shows the same trend as previous studies of the effect of nitrogen source on microalgae[4-4]. Nitrate increased lipid content in *N. oceanica* IMTE1.

Fig. 4-8 shows chromatograms of fatty acid composition. When nitrate was used, C14:0, C16:0, C18:0, C18:1, C18:2, C20:0, C22:0, C22:1, and C24:0 were detected. When urea was used, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, and C22:0 were detected. Other small peaks were detected using both nitrogen sources. From the area of the main peak, the fatty acid component ratio was calculated (**Fig. 4-9**). The main product showed different abundances for nitrate and urea. When nitrate was used, the main fatty acid composition comprised C16:0 (6.3 %) and C18:2 (14.9 %). In contrast, when urea was used, the fatty acid composition comprised C16:0 (6.8 %), C18:3 (8.6 %), and C22:0 (33.1 %). Thus, different nitrogen sources led to differences in fatty acid composition. However, in a previous study, Campos *et al.* reported almost the same lipid FAME fraction irrespective of nitrogen source when *N. salina* was cultivated in f/2 medium[4-4]. This result is different from ours. They reported the C14–C20 fatty acid components, whereas we analyzed the C14–C24 and >C25 components (**Fig.4-8**). The fatty acid composition differed between studies. Xiao *et al.* reported metabolic profiles of *N. oceanica* IMET1 cultured in a modified f/2 medium in 2013[1-13]. The main fatty acids were C16:0 and C16:1, which differed from our results. They also

reported C14–C20 fatty acids, but found no >C22 fatty acids. They use f/2 medium without $NaNO_3$, whereas we used BG11 and BG11 containing urea instead of $NaNO_3$. Thus, the differences in fatty acid composition between their study and ours may be caused by different medium composition.

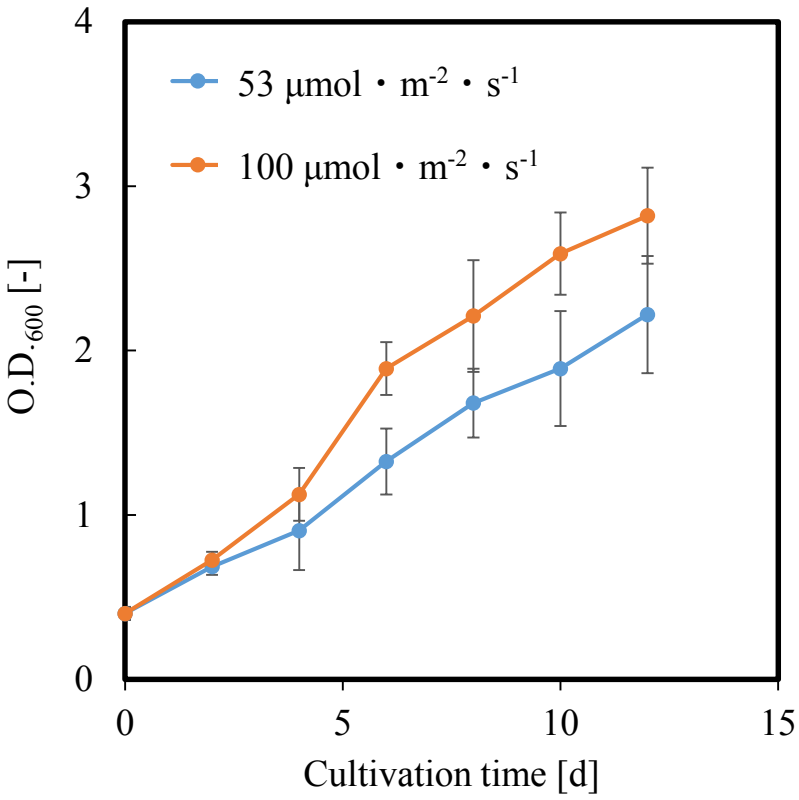


Fig. 4-1 Effect of light intensity on growth curve in 500mL-Erlenmeyer flask. Each data point is the average of three replicates, and error bars represent standard deviations (n=3)

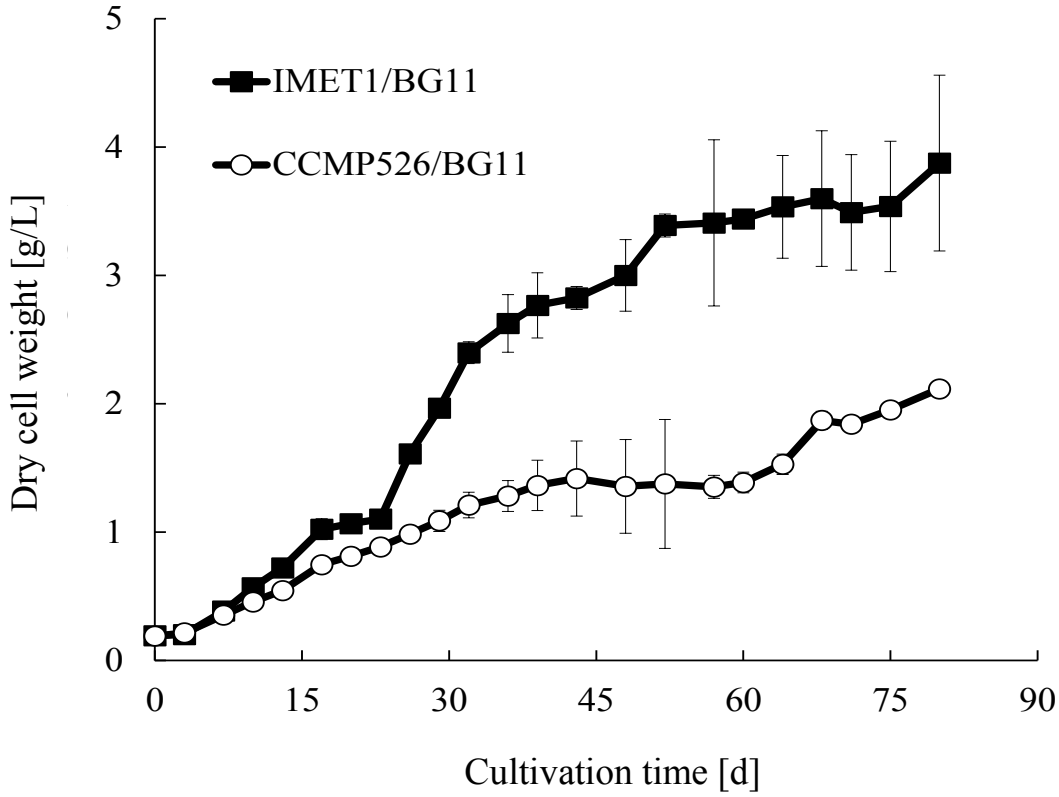


Fig. 4-2 Growth performance cultivated in 500mL-Erlenmeyer flask. Each data point is the average of three replicates, and error bars represent standard deviations (n=3)

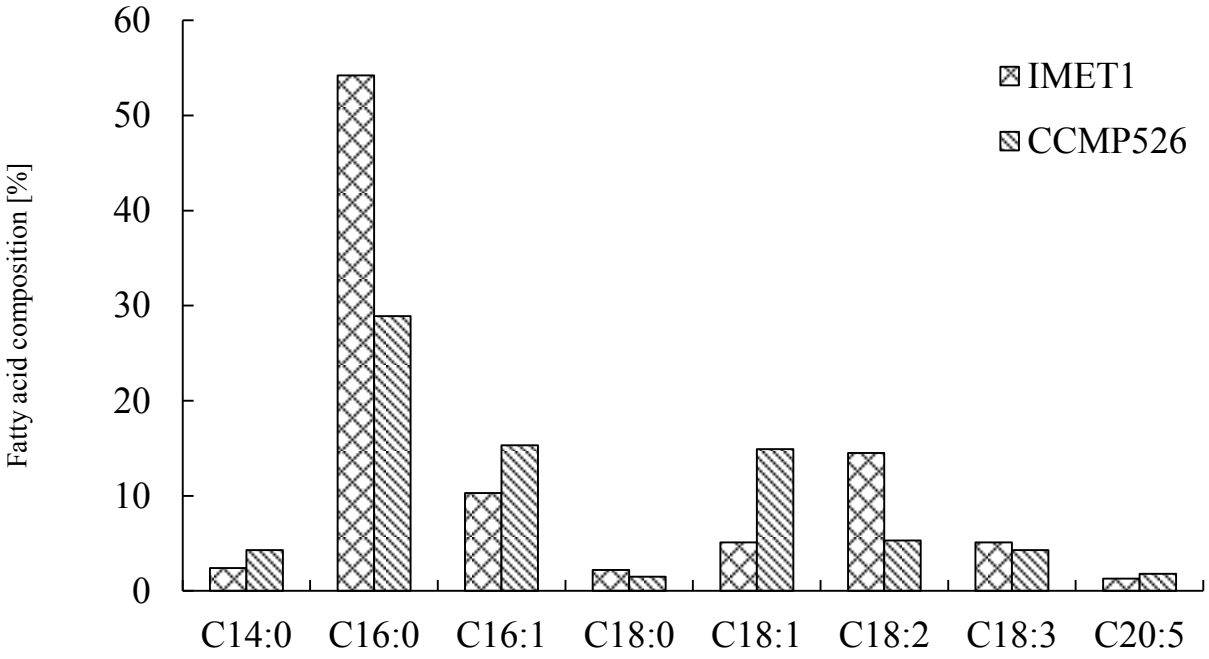


Fig. 4-3 Fatty acid compositions cultivated in Erlenmeyer flask. Each data point is the average of three replicates (n=3)

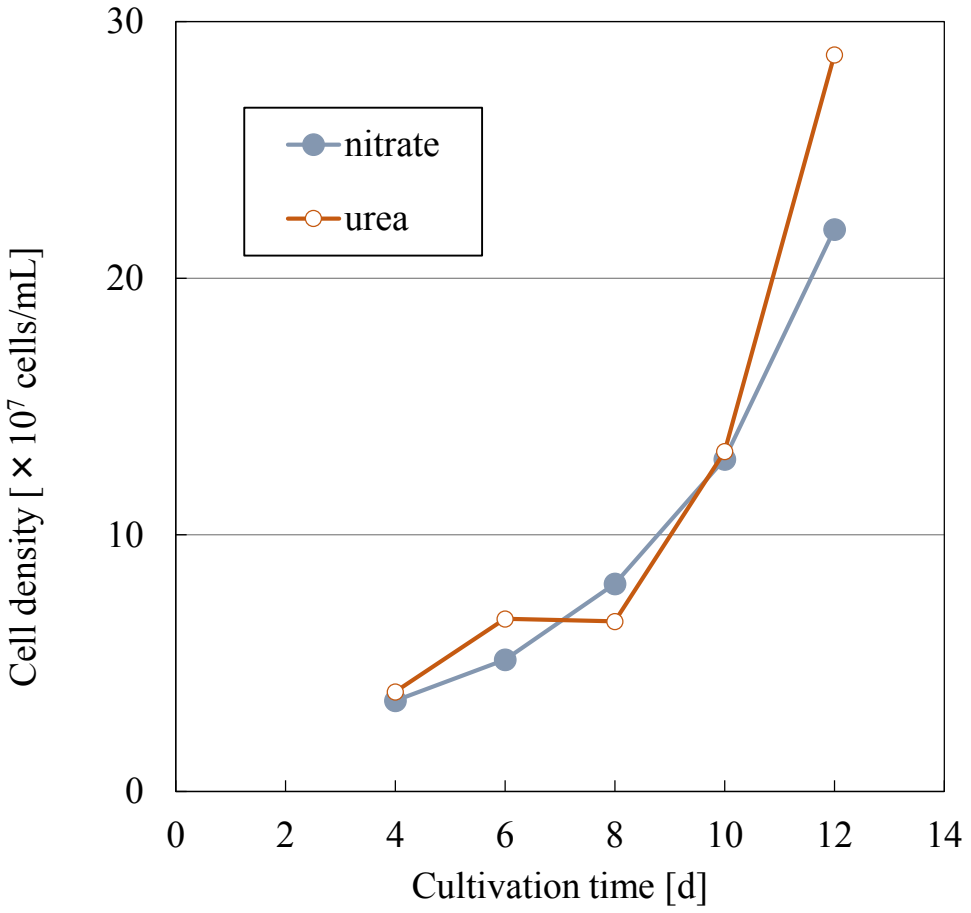


Fig. 4-4 Comparison of cell density under different nitrogen sources. Each data point is the average of three replicates (n=3)

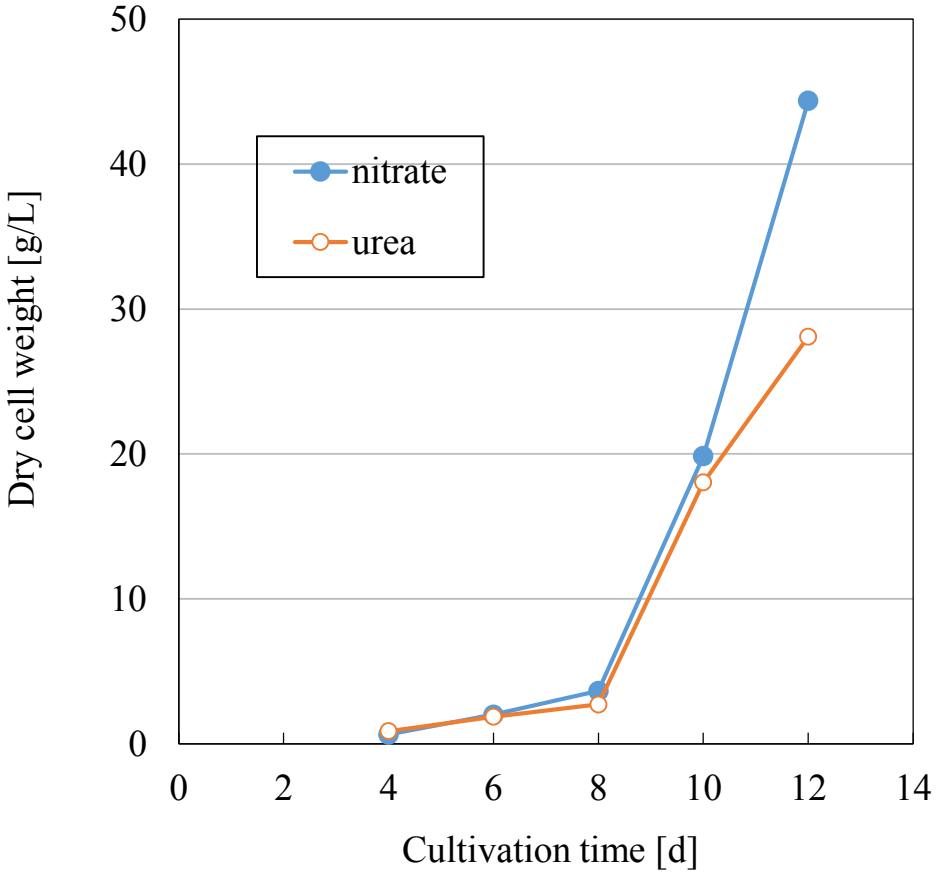


Fig. 4-5 Comparison of dry cell weight under different nitrogen sources. Each data point is the average of three replicates (n=3)

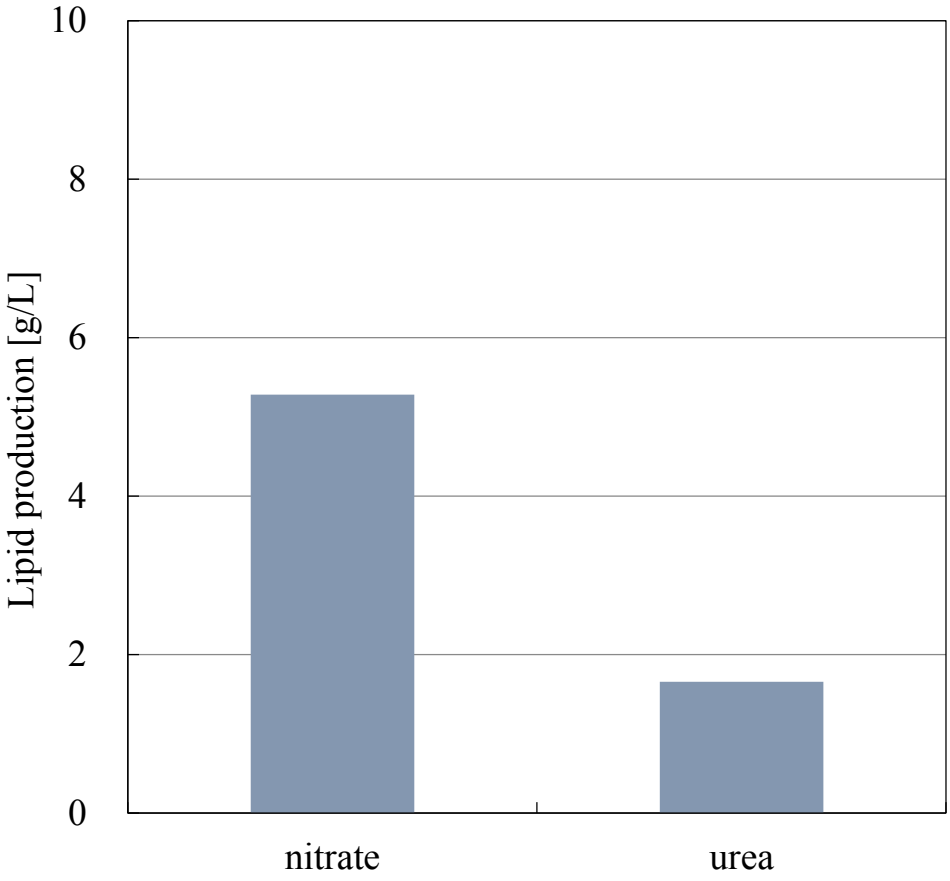


Fig. 4-6 Comparison of lipid production under different nitrogen sources. Each data point is the average of three replicates (n=3)

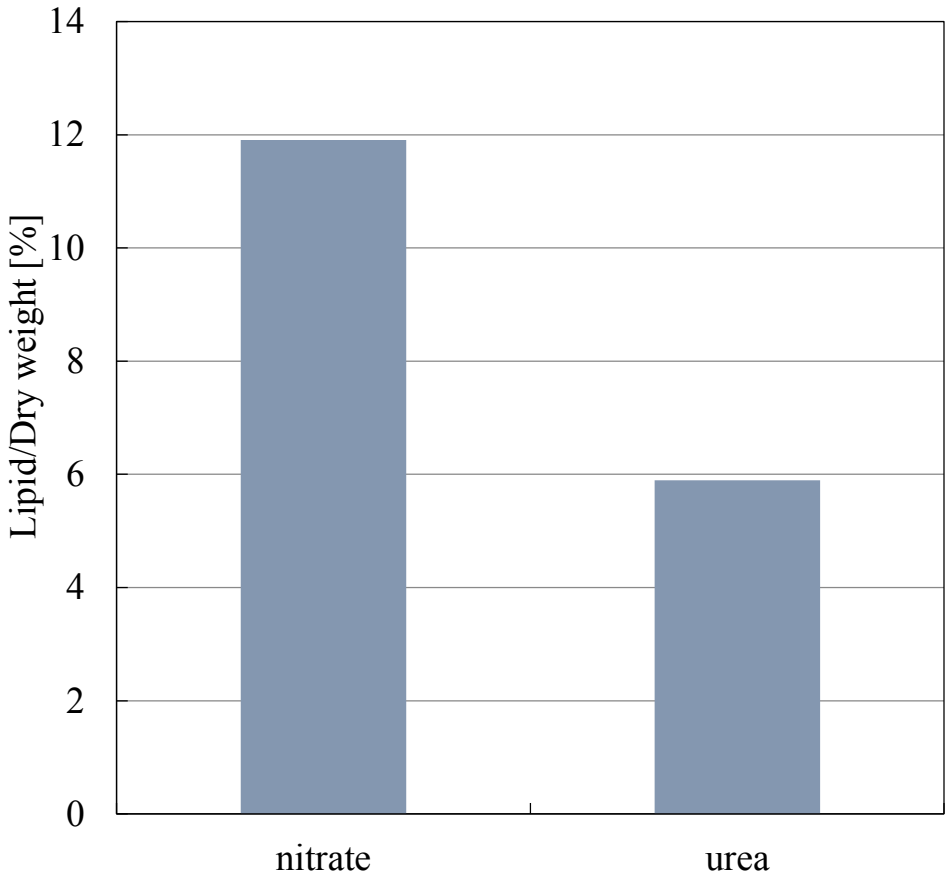


Fig. 4-7 Comparison of lipid content under different nitrogen sources. Each data point is the average of three replicates (n=3)

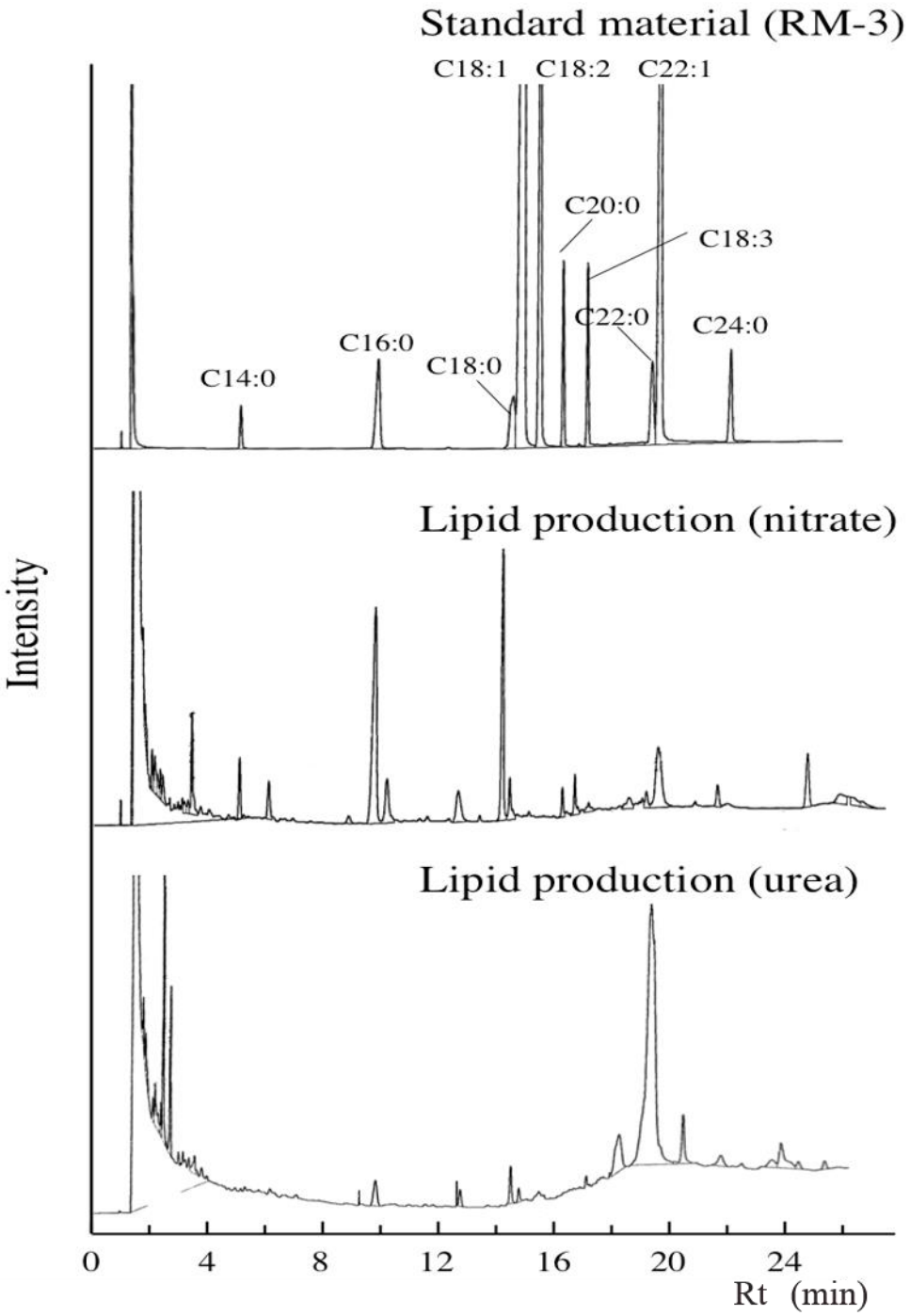


Fig. 4-8 Comparison of gas chromatograms of lipid products under different nitrogen sources.

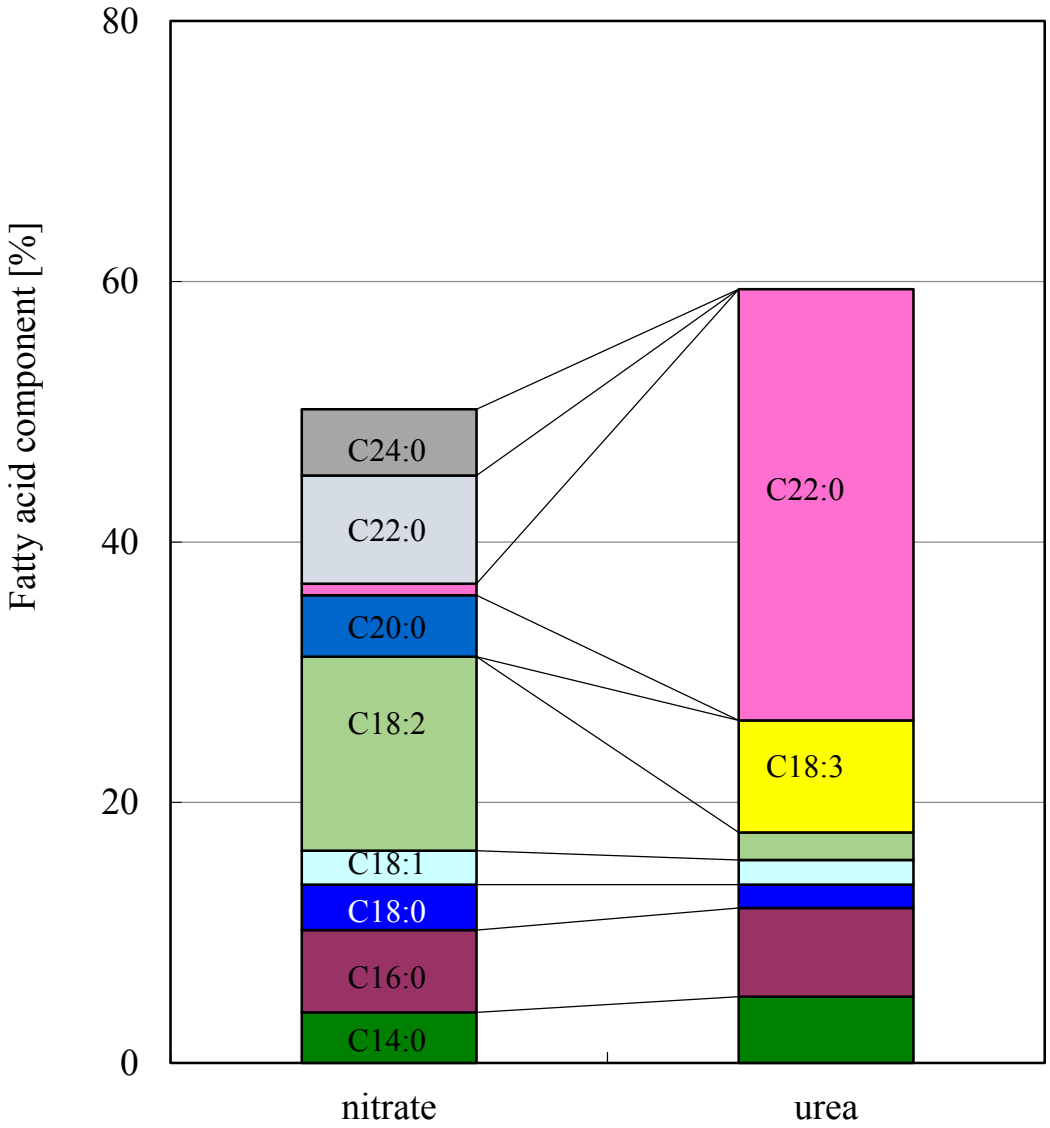
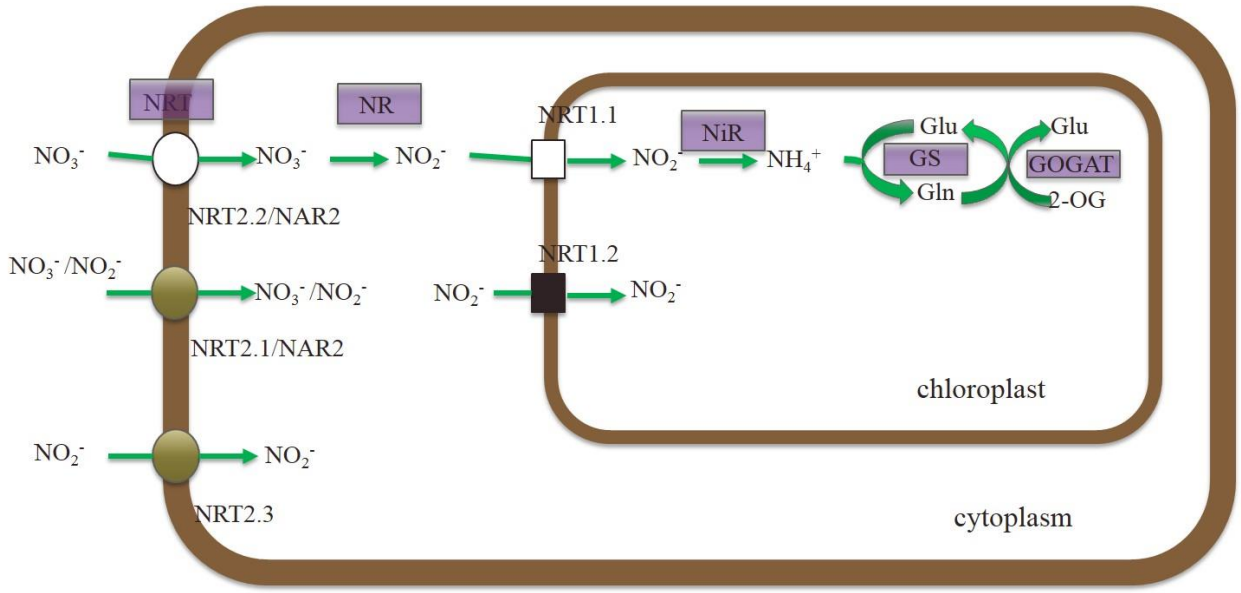


Fig. 4-9 Comparison of the components of lipid products under different nitrogen source



NRT: Nitrate ion transporter, NR: Nitrate reductase, NiR: Nitrous acid reductase, GS: Glutamine synthetase, GOGAT: Glutamine oxoglutarate aminotransferase

Fig. 4-10 The Nitric acid metabolic pathway of *Chlamydomonas* (green algae)

4.2.3 Effect of glucose

Fig.4-11 shows the responsiveness changes in the metabolite levels of *Nannochloropis* spp. treated with different nitrates. As we know, the microalgae utilized the photosynthesis, with CO_2, synthetize the glucose, and was acted on the Acetyl-CoA, synthetized the lipid. Refering this reaction process, we infer that if make more of the reaction substrate glucose, and it will promote the reaction to synthetize the lipid. So we design this glucose experiment.

Fig.4-12 shows the cell density what cultured in BG11 medium with and without glucose. The cell density without glucose increased with increasing cultivation time. On the other hand, the cells with glucose decreased with increasing cultivation time. The cells without glucose are significantly higher than those with glucose. It founds that glucose decreases cell density with increasing cultivation time.

Fig.4-13 shows the dry cell weight when cultured in BG11 medium with and without glucose. The dry cell weight without glucose increases with increasing cultivation time. The dry cell weight with glucose slightly increases with increasing cultivation time. The dry cell weight without glucose is higher than those with glucose irrespective of cultivation time. It suggested that adding glucose decreased the lipid production.

Fig.4-14 shows the lipid production cultured in BG11 medium with and without glucose. Lipid production without glucose was higher than that with glucose irrespective of cultivation time. It suggested that the lipid production not only related to lipid content, but also related to the cell growth rate in BG11 medium.

Fig.4-15 shows the comparison of lipid content when cultured in BG11 medium with and without glucose. Lipid content with glucose was higher than that without glucose irrespective of cultivation time. It suggested that glucose increases lipid production by comparing with lipid production without glucose because of added glucose and streptomycin in BG11.

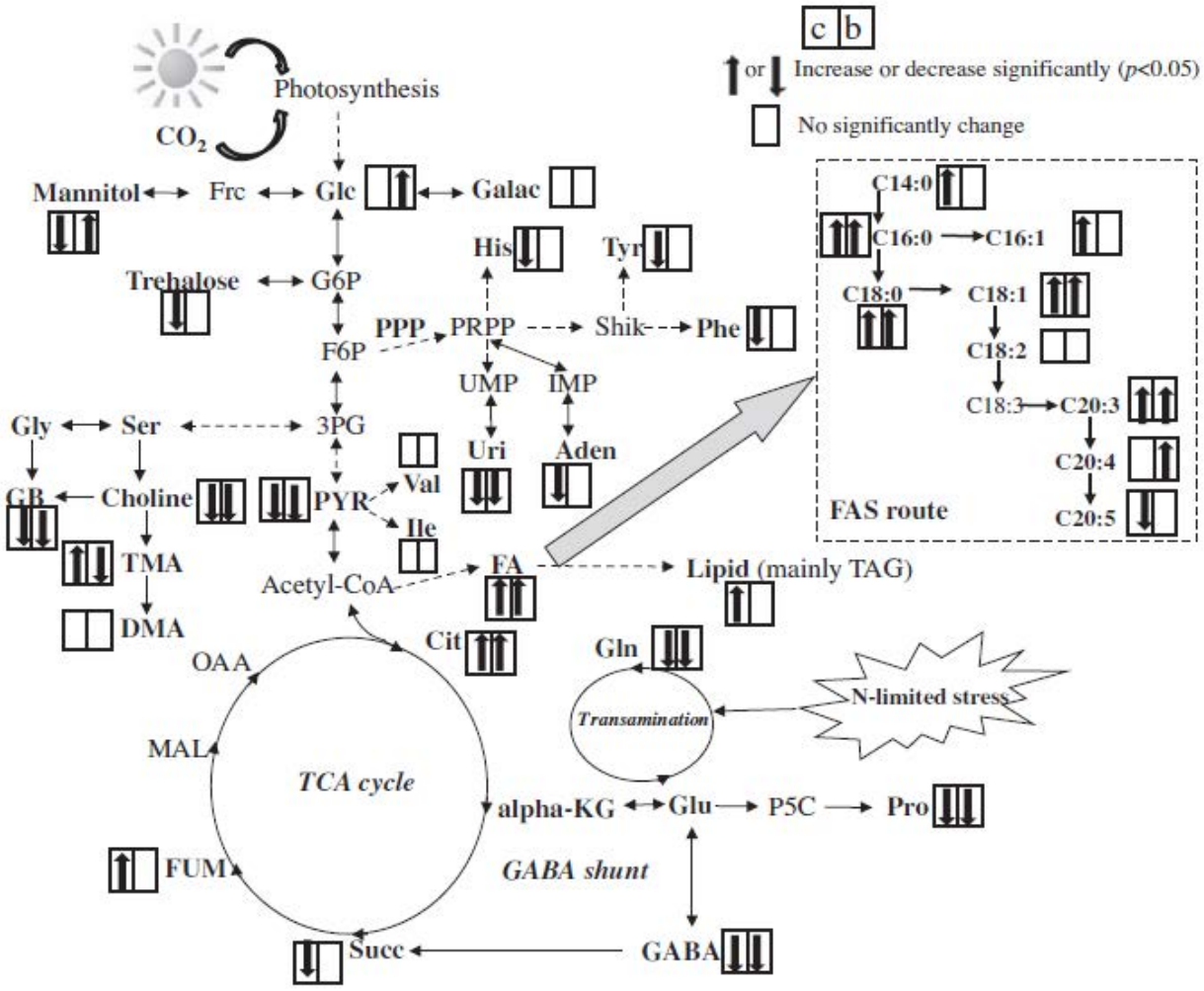


Fig. 4-11 Responsiveness changes in the metabolite levels of *Nannochloropis* spp. treated with different nitrates

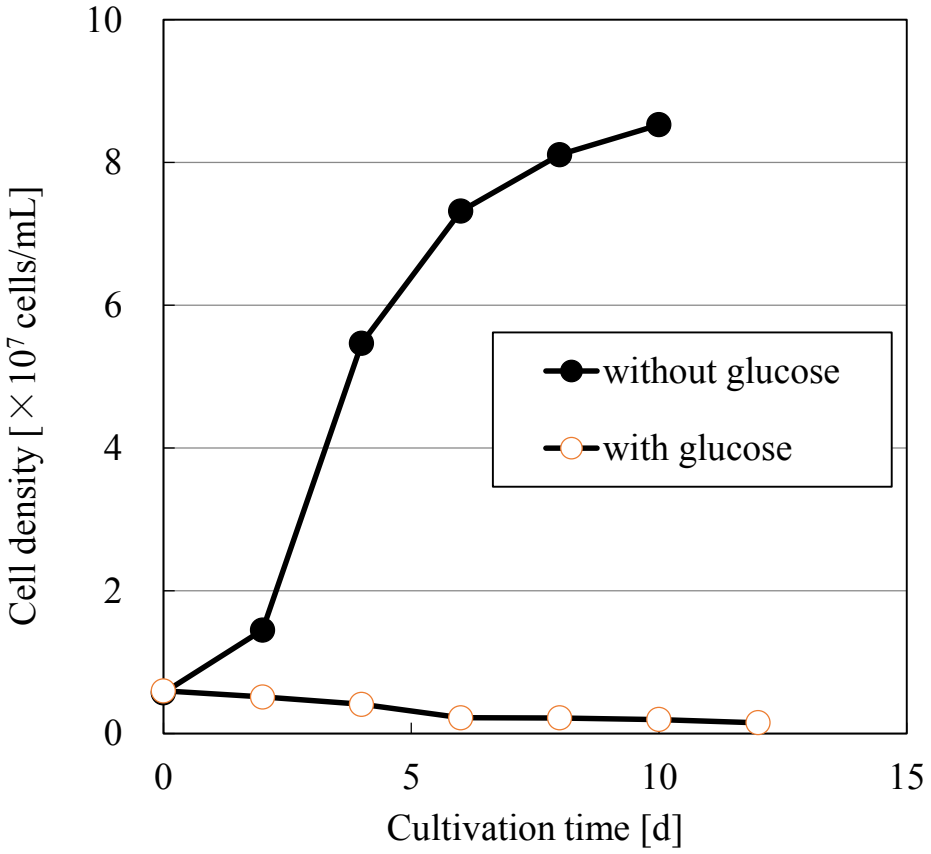


Fig. 4-12 Comparison of cell density in BG11 with/without glucose. Each data point is the average of three replicates (n=3)

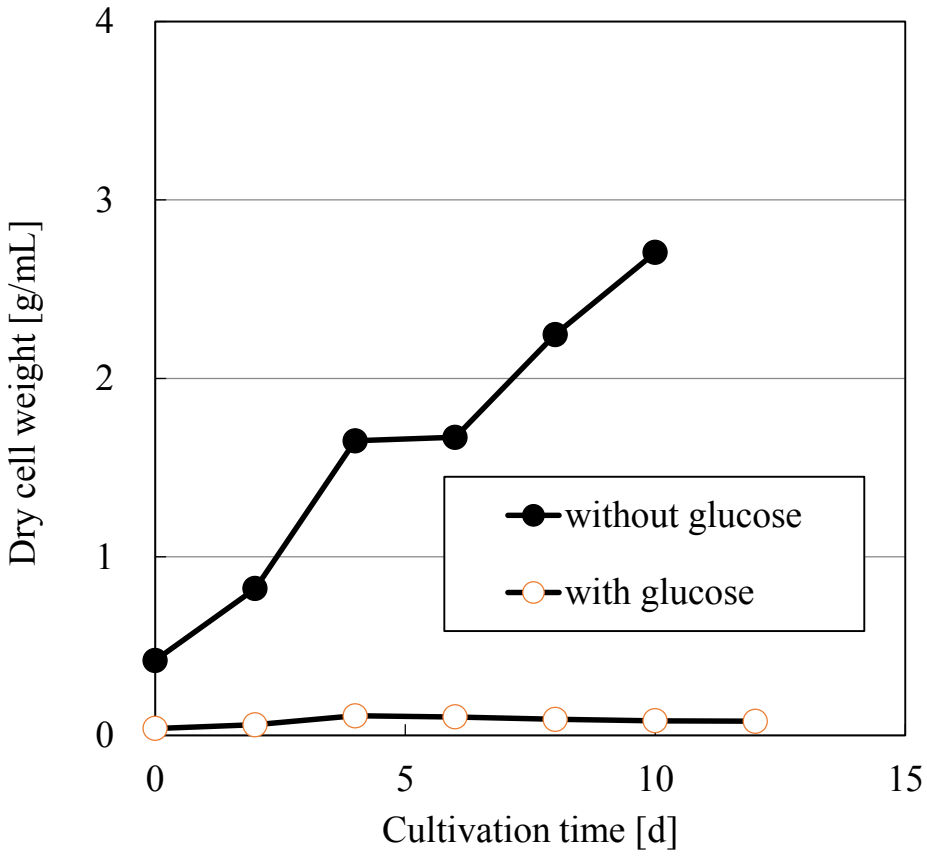


Fig. 4-13 Comparison of dry cell weight in BG11 with/without glucose. Each data point is the average of three replicates (n=3)

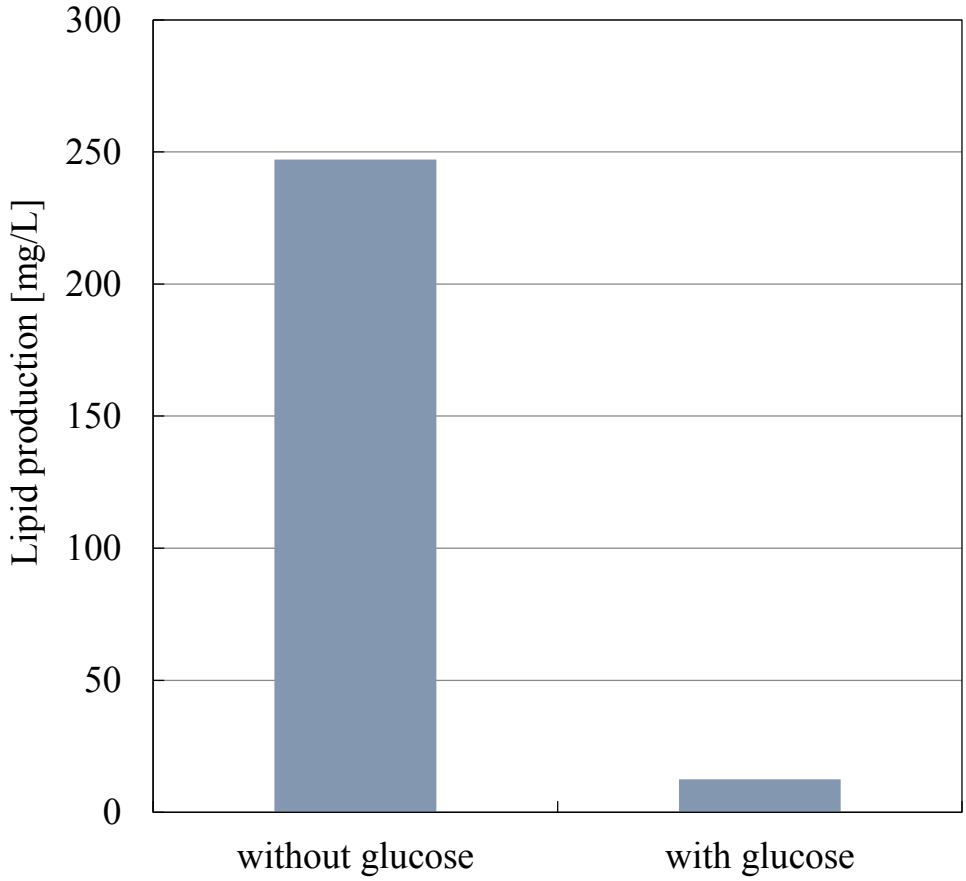


Fig. 4-14 Comparison of lipid production in BG11 with/without glucose. Each data point is the average of three replicates (n=3)

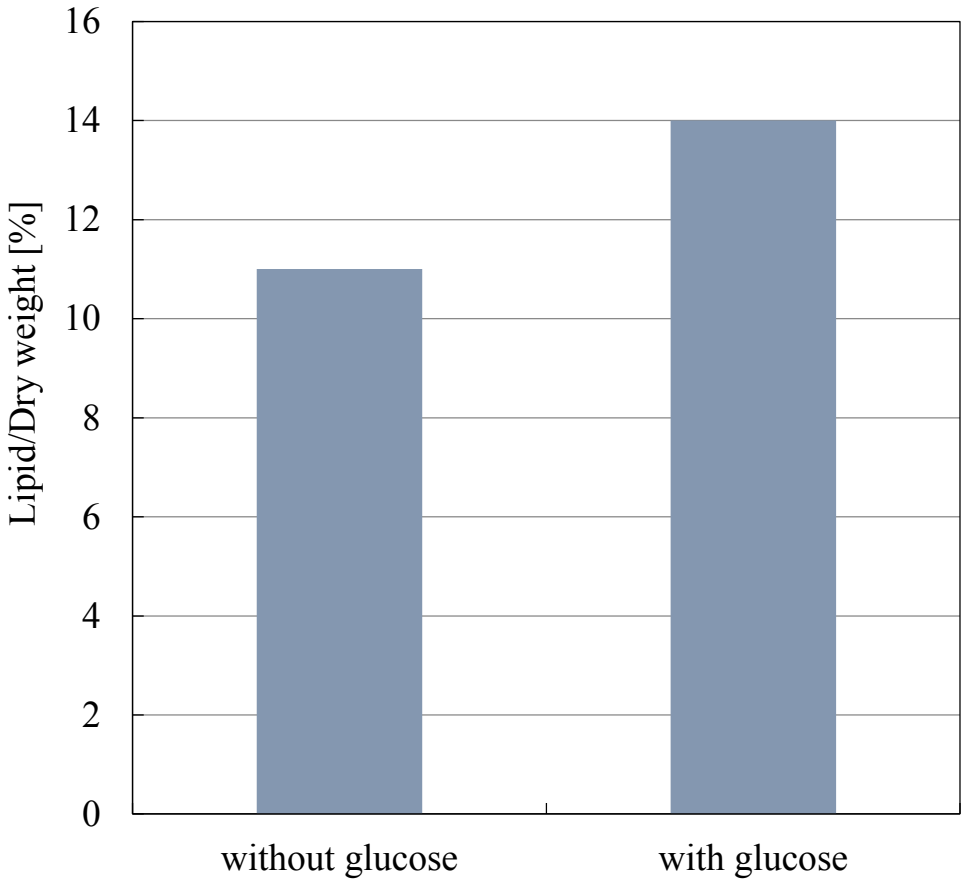


Fig. 4-15 Comparison of lipid content in BG11 with/without glucose. Each data point is the average of three replicates (n=3)

When glucose was added in BG11 as carbon source, a bacterium increased with increasing cultivation time (showed in **Fig.4-16**). For controling bacterium, we used 5 mg/L of streptomycin as antibiotics. It might have effect on the growth performance of *N. oceanica* IMET1.

Fig.4-17 shows the $O.D._{600}$ in BG11 with streptomycin. When streptomycin concentration was 0 mg/L, the $O.D._{600}$ increased with increasing cultivation time. The $O.D._{600}$ with streptomycin concentration of 5, 10 mg/L, however, showed the maximum $O.D._{600}$ at the 4^{th} day, and was lower than that without streptomycin irrespective of cultivation time.

Fig.4-18 shows the pH changed with increasing cultivation time. The pH change is unstable with cultivation time irrespective of cultivation time. It was considered that streptomycin dose not affect pH value.

Fig.4-19 shows the cells change with increasing cultivation time. When streptomycin concentration was 0 mg/L, cells increase with increasing cultivation time, however cells with streptomycin concentration of 5, 10 mg/L increased until cultivation time of 4 days, and although cells decreases over cultivation time of 4 days. Moreover, cells with streptomycin concentration of 5, 10 mg/L was smaller than that with streptomycin concentration of 0 mg/L irrespective of cultivation time.

Fig.4-20 shows the dry cell weight. The trend of dry weight cell with increasing cultivation time was almost the same with cells change. Based on the results, streptomycin can hindrance the growth performance of *N. oceanica* IMET1.

Fig.4-21 shows the lipid content with different streptomycin concentration. The lipid content of streptomycin concentration of 0 and 5 mg/L was almost same value, however the lipid/dry cell weight of streptomycin concentration of 10 mg/L was higher than that. It was found that the lipid/dry cell weight was increased by adding different concentration of streptomycin.

The result showed in **Fig.4-21** that cultured in BG11 medium with streptomycin of 5 mg/L, so it does not affect the lipid content. Therefore, the increase of the lipid content in **Fig.4-21** since added glucose.

Fig. 4-16 Photo of cultivation without streptomycin

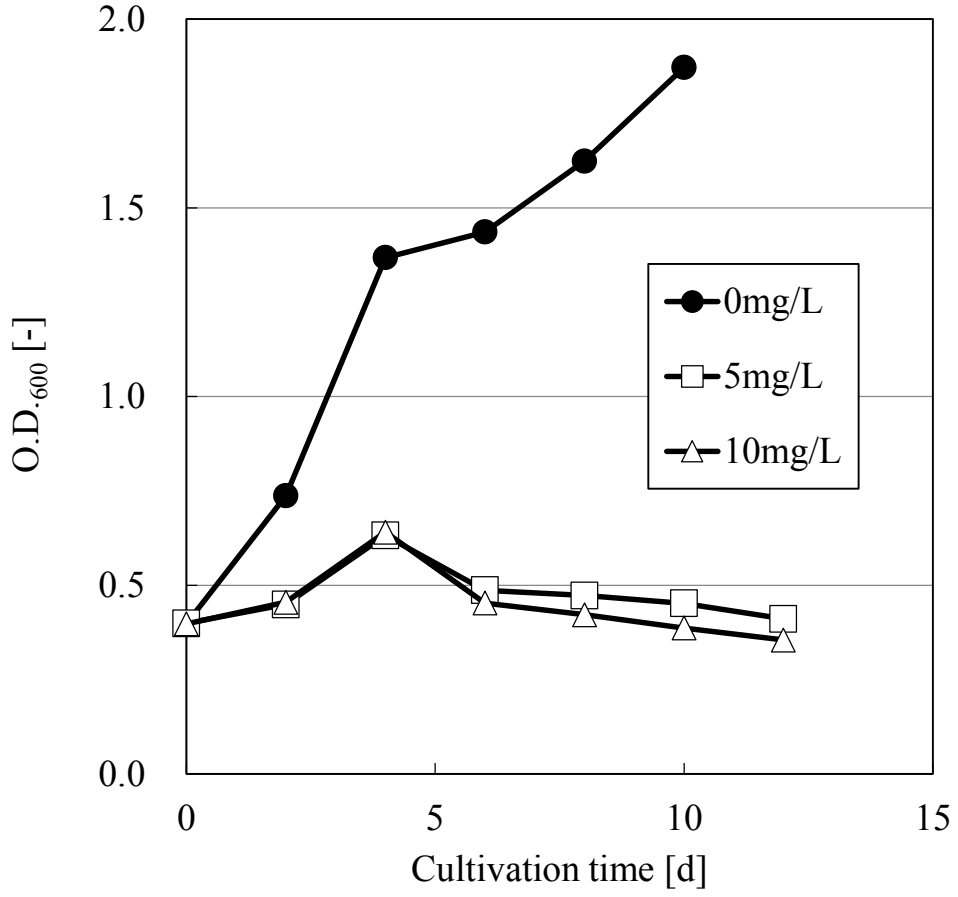


Fig. 4-17 Effect of streptomycin concentration on O.D.$_{600}$. Each data point is the average of three replicates (n=3)

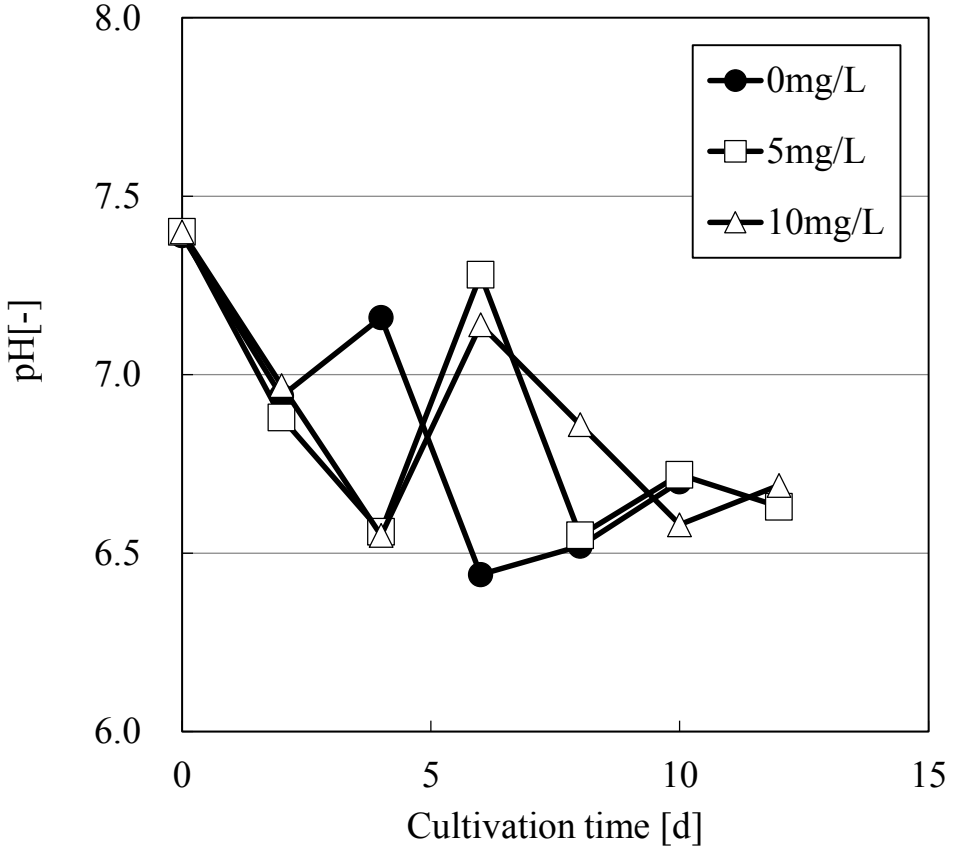


Fig. 4-18 Effect of streptomycin concentration on pH. Each data point is the average of three replicates (n=3)

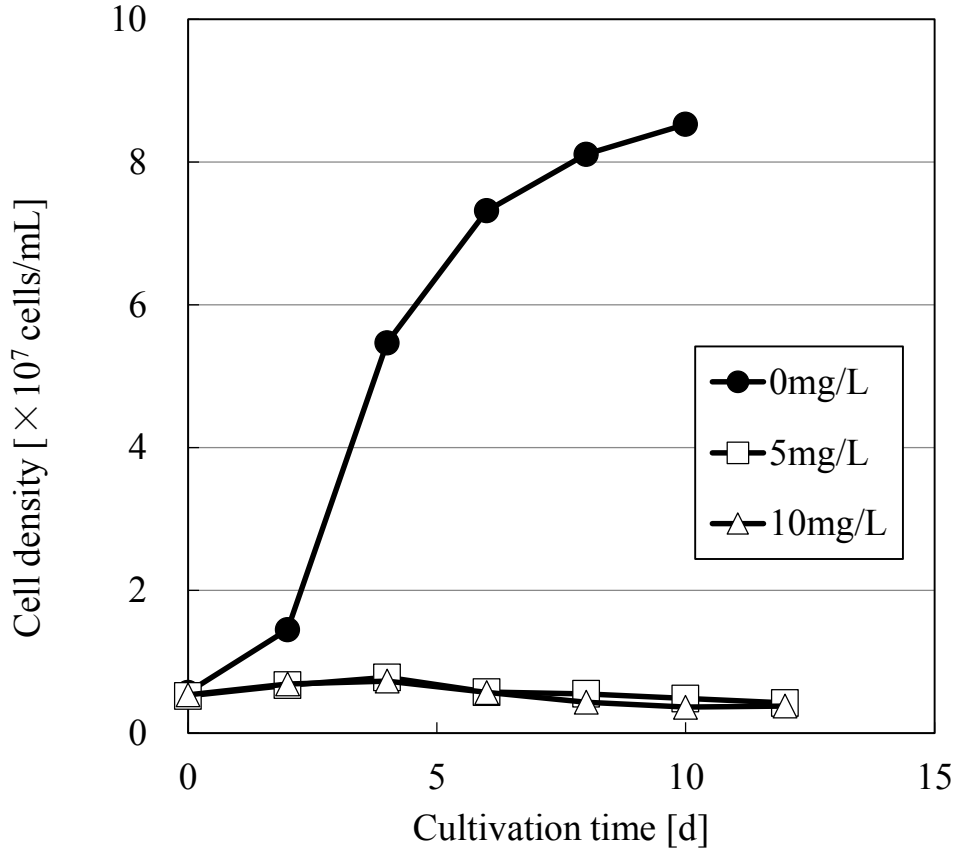


Fig. 4-19 Effect of streptomycin concentration on cell density. Each data point is the average of three replicates (n=3)

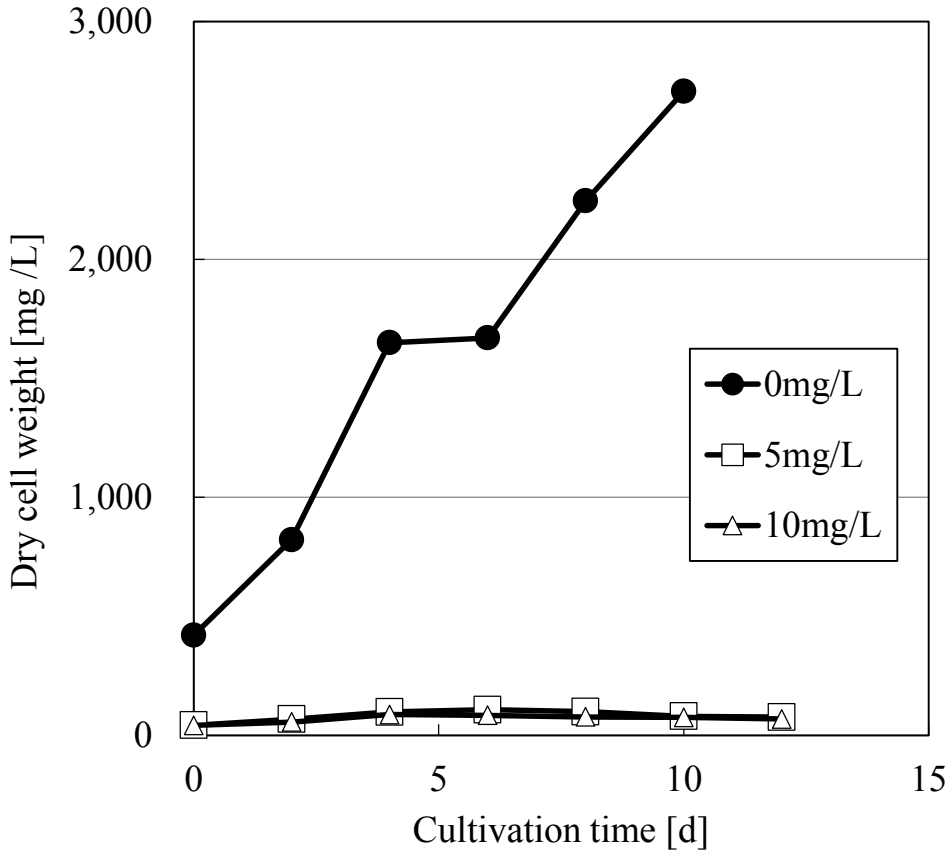


Fig. 4-20 Effect of streptomycin concentration on dry cell weight. Each data point is the average of three replicates (n=3)

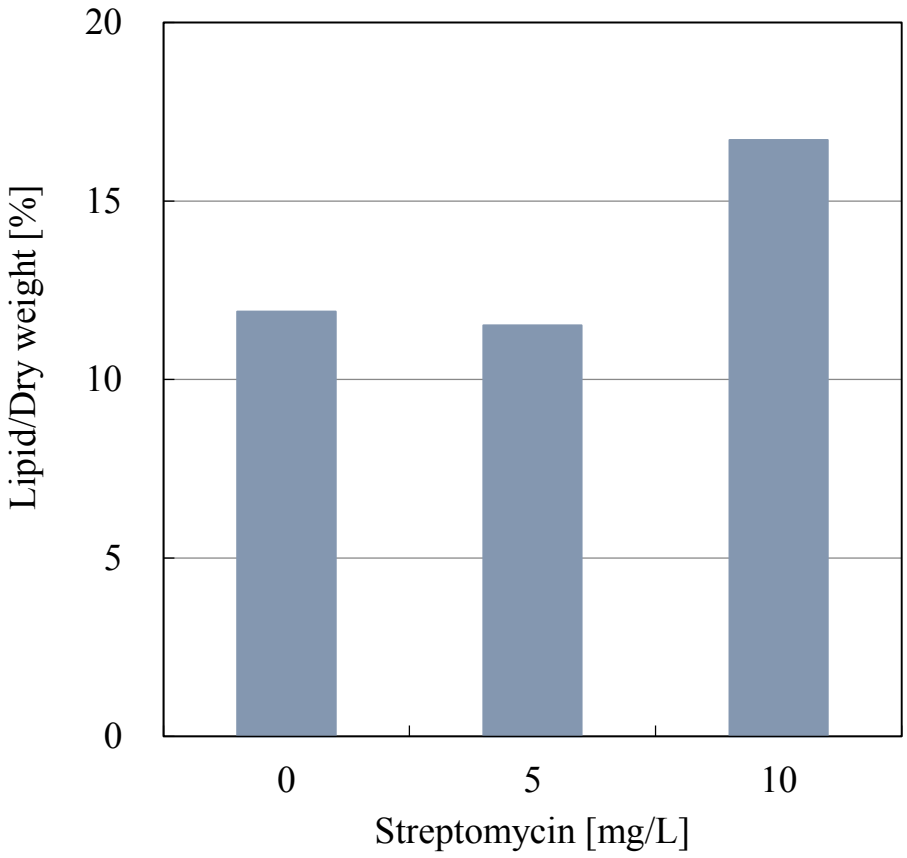


Fig. 4-21 Effect of streptomycin concentration on lipid/dry cell weight. Each data point is the average of three replicates (n=3)

4.3 Conclusion

The effect of nitrogen source on growth and lipid production of *N. oceanica* IMET1 was investigated, and got the results as following: Nitrogen source affected cells, dry cell weight, and biomass productivity. Nitrate increased dry cell weight, biomass yield, and lipid content in comparison with urea. Replacing nitrate with urea had a negative effect on dry cell weight and lipid yield. Fatty acid composition was affected by nitrogen source. Nitrate culture produced mainly C16:0 (6.3 %) and C18:2 (14. 9%) and urea produced culture mainly C16:0 (6.8 %), C18:3 (8.6 %), and C22:0 (33.1 %) fatty acids. Fatty acid composition differed when different nitrogen sources were used. Due to the different energy consumption and the application of different metabolize pathway in the nitrogen metabolic pathway in *N. oceanica* IMET1.

Glucose decreased growth performance, but increased the lipid content. Streptomycin controlled the increasing of bacterium. The streptomycin concentration which is higher than 10 mg/L can increase the lipid/dry cell weight.

References

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4-4) Campos, H., Boeing, J.W. and Barry N. (2014) Dungan, Tanner Schaub, Cultivating the marine microalga *Nannochloropsis salina* under various nitrogen sources: effect on biovolume yields, lipid content and composition, and invasive organisms. *Biomass and Bioenergy*, **66**, 301-307.

5. Cost of biofuel production using *N. oceanica* IMET1

Biofuels have been proposed as an alternative petroleum based fuels, however biofuels have the issue of a high production cost. Petrodiesel price in 2012 in the USA was 0.79USD/L without tax[5-1)]. And approximately 97.3¥/L in Japan without tax [5-2)]. On the other hand, biofuel price was reported by Chisti *et al.* in 2007 to be 2.95USD/L[1-15)]. This particular biodiesel was produced from *Phaeodactylum tricornutum* using photobioreactor. *N. oceanica* IMET1 which has a characteristic of a high growth performance, high biomass productivity and high lipid production, so that the cost of biofuel production from *N. oceanica* IMET1 is calculated for the comparison of the cost of petrodiesel and previous biofuel production.

5.1 Calculation Method of biofuel production

5.1.1 The process for biofuel production

The process for biofuel production has four main processes starting from cultivation, to collection, extraction and finally purification. Process costs considered are electrical, cooling (water), mechanical efficiency and chemical resource. In this study, each process cost was referred the report "The project of development of fuel using microalgae" published by Council on Competitiveness-Nippon at 2011[5-3)].

5.1.2 Premise conditions

The necessary cost of each process as electric, water, component for medium and etc. are shown in **Table 5-1**.

The amount of biofuel production per year is assumed to be 1.73×10^8 L. This is equivalent to the diesel consumption per year in Okinawa-Pref.

The reactor for culture is a 500 mL flask. Cultivation time assumed to be 30 days, and repeated eight times over. The best photosynthetic impact factors are a temperature of 30ºC, a CO_2 concentration of 2 % and a light intensity is 100 $\mu mol \cdot m^{-1} \cdot s^{-1}$. Growth rate, lipid yield is used at this condition from above results.

5.1.3 Method of cost calculation

Cost is calculated by the following equation. Meaning and calculation method of symbol in the equation is also explained.

Cost＝[(A2+A3)+(B1+B2)+C+(D1+D2+D3)]/A1

Meaning and calculation method of symbol is following.

A1: Production scale (L)

A2 :Broth cost one year＝Broth unit-price×Required culture vessel×Incubator volume×1000

A2=a1×a2×a3×1000

a2: (Required culture vessel) = A1 / [{(a4×a5×30) × (a7/30) × (a5/100)}/a6]

A3: Electricity charges for cultivation ＝electric fee per day × Operating days [day/year]

A3=1500/365×10000×a7

B1: Flocculant costs＝Flocculant unit-price×Flocculant amount

B1=b1 ×b2× b3× b4

b3: (amount of produced by one reactor one year) = (a4×a5×30) ×(a7/30) ×(a5/100)

B2: Electricity charges for concentration process＝Concentrated energy × （cultivation liquid amount in one year）

B2=b4× (b5×b6×8)

C: Electricity charges for dry = electric fee (17 ¥/kWh) ×(cultivation liquid amount in one year)/18 ×40.69 kJ/mol（Latent heat of vaporization of water）×1000×(0.278×10^{-6})

C=c4 ×c2× 8× c3/18× c1×1000×0.278×10^{-6}

D1: water rates for extraction process＝water fee (40¥/ton) × 11(ton-water/ton-dry)×The amount of oil produced by one incubator one year ×Required culture vessel / 10^6

D1=d1× d2× d3× d4/10^6

d3 (amount of produced by one reactor one year) = (a4×a5×30) × (a7/30) × (a5/100)

D2: Electricity charges for extraction process＝electric fee (17¥/kWh)×25(kWh/ton-dry)×The amount of oil produced by one incubator one year ×Required culture vessel /10^6

D2=d5 ×d6 ×d4 /10^6

D3 Hexane cost＝1200(¥/L)×2(L/ton-dry)×The amount of oil produced by one incubator one year ×Required culture vessel /10^6

D3=d7× d8× d3× d4/10^6

5.2 Result and discussions

Table 5-2, 5-3, 5-4 shows the effect of CO_2 concentration, temperature, light intensity on the biofuel production cost.

Fig.5-1, 5-2, 5-3 shows the comparison of the cost of biofuel production.

The cost of biofuel production is almost same as (220 ¥/L) on each condition.

Table 5-5 shows the cost of biofuel production using *N. oceaninca* IMET1 when BG11, BG11 with used urea instead of nitrate and BG11 with used all carbon sources instead of glucose is used.

Fig. 5-4 shows the comparison of the cost of biofuel production from each condition.

When nitrate is used as medium component, the cost of biofuel production is low value (220 ¥/L). In previous studies, the cost of biofuel production is 2.95 USD/L [1-15]. In present, this cost is equivalent to 368 ¥/L. This result indicated that *N. oceanica* IMET1 is highly possible to produce biofuel at low cost. When glucose is used, the cost is 305 ¥/L and lower than previous study.

5.3 Conclusion

The cost of biofuel production from *N. oceaninca* IMET1 is 220 ¥/L which is lower than the cost reported at present, when BG11 is used as medium. It was found that *N. oceaninca* IMET1 are possible to produce biofuel in low cost.

Table 5-1 Major items for the cost calculation[5-3)]

Microalage		
Cultivation condision	Temperature [℃]	
	Light lintensity [μmol•m^{-2}•s^{-1}]	
	CO_2 concentration [%]	
Medium		
Additive		
Cultivate process(A)	Production scale [×10^8 L]	A1
	Broth [¥/L]	a1
	Required culture vessel [×10^6 reactor]	a2
	Incubator volume [×10^{-4} m^3]	a3
	Algae growth rate [×10^3 g/m^3/day]	a4
	Oil content [%]	a5
	Oils specific gravity [g/m^3]	a6
	Operating days of one year [day/year]	a7
	Broth cost one year [×10^8 ¥/L/year]	A2
	Electricity charges [M¥]	A3
Concentration process(B)	Flocculant unit-price	b1
	Flocculant / algae [g/kg_dry]	b2
	The amount produced by one reactor one year [g]	b3
	Required culture vessel [×10^6 reactor]	b4
	Incubator volume [×10^{-4} m^3]	b5
	Concentrated energy [kW/m^3]	b6
	Flocculant costs [×10 G¥]	B1
	Electricity charges [×10 k¥]	B2
Drying process(C)	Latent heat of vaporization [kJ/mol]	c1
	Incubator volume [×10^{-4} m^3]	c2
	Required culture vessel [×10^6 reactor]	c3
	Electricity unit-price[¥/kwh]	c4
	Electricity charges [k¥]	C
Extraction process(D)	Water unit-price[¥/ton]	d1
	Cooling water [Ton/Ton_ raw materials]	d2
	The amount produced by one reactor one year [g]	d3
	Required culture vessel [×10^6 reactor]	d4
	Electricity unit-price[¥/kwh]	d5
	Power [kWh/Ton_ raw materials]	d6
	Hexane unit-price[¥/L]	d7
	Extraction solvent make-up amount [L/Ton_dry alga]	d8
	Water rates [M¥]	D1
	Electricity charges [k¥]	D2
	Hexane cost [M¥]	D3
Manufacturing cost [¥/L]		Cost

Table5-2 Effect of CO_2 concentration on biofuel production cost

Microalage		*N.oceanica* IMET1				
Cultivation condision	Temperature [℃]	30	30	30	30	
	Light lintensity [μmol•m^{-2}•s^{-1}]	100	100	100	100	
	CO_2 concentration [%]	0.03	2	10	20	
Medium		BG11	BG11	BG11	BG11	
Additive		-	-	-	-	
Cultivate process(A)	Production scale [×10^8 L]	1.73	1.73	1.73	1.73	A1
	Broth [¥/L]	6.12	6.12	6.12	9.98	a1
	Required culture vessel [×10^6 reactor]	2.72	2.59	2.94	3.98	a2
	Incubator volume [×10^{-4} m^3]	5.00	5.00	5.00	5.00	a3
	Algae growth rate [×10^3 g/m^3/day]	3.53	3.70	3.26	2.41	a4
	Oil content [%]	12	12	12	12.00	a5
	Oils specific gravity [g/m^3]	0.8	0.8	0.8	0.8	a6
	Operating days of one year [day/year]	240	240	240	240	a7
	Broth cost one year [×10^8 ¥/L/year]	6.65	6.35	7.20	9.74	A2
	Electricity charges [M¥]	10	10	10	10	A3
Concentration process(B)	Flocculant unit-price	7	7	7	7	b1
	Flocculant / algae [g/kg_dry]	5	5	5	5	b2
	The amount produced by one reactor one year [g/year]	424	444	391	289	b3
	Required culture vessel [×10^6 reactor]	2.72	2.59	2.94	3.98	b4
	Incubator volume [×10^{-4} m^3]	5	5	5	5	b5
	Concentrated energy [kW/m^3]	0.1	0.1	0.1	0.1	b6
	Flocculant costs [×10 G¥]	3.80	3.80	3.80	3.80	B1
	Electricity charges [×10 k¥]	18.47	17.64	20.01	27.05	B2
Drying process(C)	Latent heat of vaporization [kJ/mol]	40.69	40.69	40.69	40.69	c1
	Incubator volume [×10^{-4} m^3]	5	5	5	5	c2
	Required culture vessel [×10^6 reactor]	2.72	2.59	2.94	3.98	c3
	Electricity unit-price[¥/kwh]	17	17	17	17	c4
	Electricity charges [k¥]	1.8	1.8	2.0	2.7	C
Extraction process(D)	Water unit-price[¥/ton]	40	40.0	40.0	40.0	d1
	Cooling water [Ton/Ton_ raw materials]	11	11	11	11	d2
	The amount produced by one reactor one year [g/year]	424	444	391	289	d3
	Required culture vessel [×10^6 reactor]	2.72	2.59	2.94	3.98	d4
	Electricity unit-price[¥/kwh]	17	17	17	17	d5
	Power [kWh/Ton_ raw materials]	25	25	25	25	d6
	Hexane unit-price[¥/L]	1200	1200	1200	1200	d7
	Extraction solvent make-up amount [L/Ton_dry alga]	2	2	2	2	d8
	Water rates [M¥]	5.06	5.06	5.06	5.06	D1
	Electricity charges [k¥]	489	489	489	489	D2
	Hexane cost [M¥]	2.76	2.76	2.76	2.76	D3
Manufacturing cost [¥/L]		220	220	221	221	Cost

Table5-3 Effect of temperature on biofuel production cost

Microalage		*N.oceanica* IMET1			
Cultivation condision	Temperature [℃]	10	23	30	
	Light lintensity [μmol•m^{-2}•s^{-1}]	100	100	100	
	CO_2 concentration [%]	2	2	2	
Medium		BG11	BG11	BG11	
Additive		-	-	-	
Cultivate process(A)	Production scale [×10^8 L]	1.73	1.73	1.73	A1
	Broth [¥/L]	6.12	6.12	6.12	a1
	Required culture vessel [×10^6 reactor]	3.83	2.90	2.59	a2
	Incubator volume [×10^{-4} m^3]	5.00	5.00	5.00	a3
	Algae growth rate [×10^3 g/m^3/day]	2.50	3.31	3.70	a4
	Oil content [%]	12	12	12	a5
	Oils specific gravity [g/m^3]	0.8	0.8	0.8	a6
	Operating days of one year [day/year]	240	240	240	a7
	Broth cost one year [×10^8 ¥/L/year]	9.39	7.09	6.35	A2
	Electricity charges [M¥]	10	10	10	A3
Concentration process(B)	Flocculant unit-price	7	7	7	b1
	Flocculant / algae [g/kg_dry]	5	5	5	b2
	The amount produced by one reactor one year [g/year]	300	397	444	b3
	Required culture vessel [×10^6 reactor]	3.83	2.90	2.59	b4
	Incubator volume [×10^{-4} m^3]	5	5	5	b5
	Concentrated energy [kW/m^3]	0.1	0.1	0.1	b6
	Flocculant costs [×10 G¥]	3.80	3.80	3.80	B1
	Electricity charges [×10 k¥]	26.07	19.69	17.64	B2
Drying process(C)	Latent heat of vaporization [kJ/mol]	40.69	40.69	40.69	c1
	Incubator volume [×10^{-4} m^3]	5	5	5	c2
	Required culture vessel [×10^6 reactor]	3.83	2.90	2.59	c3
	Electricity unit-price[¥/kwh]	17	17	17	c4
	Electricity charges [k¥]	2.6	2.0	1.8	C
Extraction process(D)	Water unit-price[¥/ton]	40	40.0	40.0	d1
	Cooling water [Ton/Ton_ raw materials]	11	11	11	d2
	The amount produced by one reactor one year [g/year]	300	397	444	d3
	Required culture vessel [×10^6 reactor]	2.72	2.59	2.94	d4
	Electricity unit-price[¥/kwh]	17	17	17	d5
	Power [kWh/Ton_ raw materials]	25	25	25	d6
	Hexane unit-price[¥/L]	1200	1200	1200	d7
	Extraction solvent make-up amount [L/Ton_dry alga]	2	2	2	d8
	Water rates [M¥]	5.06	5.06	5.06	D1
	Electricity charges [k¥]	489	489	489	D2
	Hexane cost [M¥]	2.76	2.76	2.76	D3
Manufacturing cost [¥/L]		221	221	220	Cost

Table5-4 Effect of light intensity on biofuel production cost

Microalage		*N.oceanica* IMET		
Cultivation condision	Temperature [℃]	30	30	
	Light lintensity [μmol•m^{-2}•s^{-1}]	53	100	
	CO_2 concentration [%]	2	2	
Medium		BG11	BG11	
Additive		-	-	
Cultivate process(A)	Production scale [×10^8 L]	1.73	1.73	A1
	Broth [¥/L]	6.12	6.12	a1
	Required culture vessel [×10^6 reactor]	2.90	2.59	a2
	Incubator volume [×10^{-4} m^3]	5.00	5.00	a3
	Algae growth rate [×10^3 g/m^3/day]	3.31	3.70	a4
	Oil content [%]	12	12	a5
	Oils specific gravity [g/m^3]	0.8	0.8	a6
	Operating days of one year [day/year]	240	240	a7
	Broth cost one year [×10^8 ¥/L/year]	7.09	6.35	A2
	Electricity charges [M¥]	10	10	A3
Concentration process(B)	Flocculant unit-price	7	7	b1
	Flocculant / algae [g/kg_dry]	5	5	b2
	The amount produced by one reactor one year [g]	397	444	b3
	Required culture vessel [×10^6 reactor]	2.90	2.59	b4
	Incubator volume [×10^{-4} m^3]	5	5	b5
	Concentrated energy [kW/m^3]	0.1	0.1	b6
	Flocculant costs [×10 G¥]	3.80	3.80	B1
	Electricity charges [×10 k¥]	18.47	17.64	B2
Drying process(C)	Latent heat of vaporization [kJ/mol]	40.69	40.69	c1
	Incubator volume [×10^{-4} m^3]	5	5	c2
	Required culture vessel [×10^6 reactor]	2.90	2.59	c3
	Electricity unit-price[¥/kwh]	17	17	c4
	Electricity charges [k¥]	1.8	1.8	C
Extraction process(D)	Water unit-price[¥/ton]	40	40.0	d1
	Cooling water [Ton/Ton_ raw materials]	11	11	d2
	The amount produced by one reactor one year [g]	397	444	d3
	Required culture vessel [×10^6 reactor]	2.72	2.59	d4
	Electricity unit-price[¥/kwh]	17	17	d5
	Power [kWh/Ton_ raw materials]	25	25	d6
	Hexane unit-price[¥/L]	1200	1200	d7
	Extraction solvent make-up amount [L/Ton_dry alga]	2	2	d8
	Water rates [M¥]	5.06	5.06	D1
	Electricity charges [k¥]	489	489	D2
	Hexane cost [M¥]	2.76	2.76	D3
Manufacturing cost [¥/L]		221	220	Cost

Table 5-5 Comparison of the production costs on different nutrition

Microalage		*N.oceanica* IMET1			
Cultivation condision	Temperature [℃]	30	30	30	
	Light lintensity [μmol•m^{-2}•s^{-1}]	100	100	100	
	CO_2 concentration [%]	2	2	2	
Medium		BG11	BG11 without nitrate	BG11	
Additive		-	urea	glucose, streptmysin	
Cultivate process(A)	Production scale [×10^8 L]	1.73	1.73	1.73	A1
	Broth [¥/L]	6.12	6.12	9.98	a1
	Required culture vessel [×10^6 reactor]	2.59	8.35	822.14	a2
	Incubator volume [×10^{-4} m^3]	5.00	5.00	5.00	a3
	Algae growth rate [×10^3 g/m^3/day]	3.70	2.34	10.00	a4
	Oil content [%]	12	5.89	14.00	a5
	Oils specific gravity [g/m^3]	0.8	0.8	0.8	a6
	Operating days of one year [day/year]	240	240	240	a7
	Broth cost one year [×10^8 ¥/L/year]	0.64	0.89	328.33	A2
	Electricity charges [M¥]	10	10	10	A3
Concentration process(B)	Flocculant unit-price	7	7	7	b1
	Flocculant / algae [g/kg_dry]	5	5	5	b2
	The amount produced by one reactor one year [g/reactor]	444	281	1.00	b3
	Required culture vessel [×10^6 reactor]	3	8	822.14	b4
	Incubator volume [×10^{-4} m^3]	5	5	5	b5
	Concentrated energy [kW/m^3]	0.1	0.1	0.1	b6
	Flocculant costs [×10 G¥]	3.80	7.74	3.26	B1
	Electricity charges [×10 k¥]	1.76	5.68	5590.60	B2
Drying process(C)	Latent heat of vaporization [kJ/mol]	40.69	40.69	40.69	c1
	Incubator volume [×10^{-4} m^3]	5	5	5	c2
	Required culture vessel [×10^6 reactor]	2.59	8.35	822.14	c3
	Electricity unit-price[¥/kwh]	17	17	17	c4
	Electricity charges [k¥]	17.6	56.8	559.1	C
Extraction process(D)	Water unit-price[¥/ton]	40.0	40.0	40.0	d1
	Cooling water [Ton/Ton_ raw materials]	11	11	11	d2
	The amount produced by one reactor one year [g/reactor]	444	281	1.00	d3
	Required culture vessel [×10^6 reactor]	2.59	8.32	822.14	d4
	Electricity unit-price[¥/kwh]	17	17	17	d5
	Power [kWh/Ton_ raw materials]	25	25	25	d6
	Hexane unit-price[¥/L]	1200	1200	1200	d7
	Extraction solvent make-up amount [L/Ton_dry alga]	2	2	2	d8
	Water rates [M¥]	5.06	10.32	4.34	D1
	Electricity charges [k¥]	489	997	419	D2
	Hexane cost [M¥]	2.76	5.63	2.37	D3
Manufacturing cost [¥/L]		220	449	379	Cost

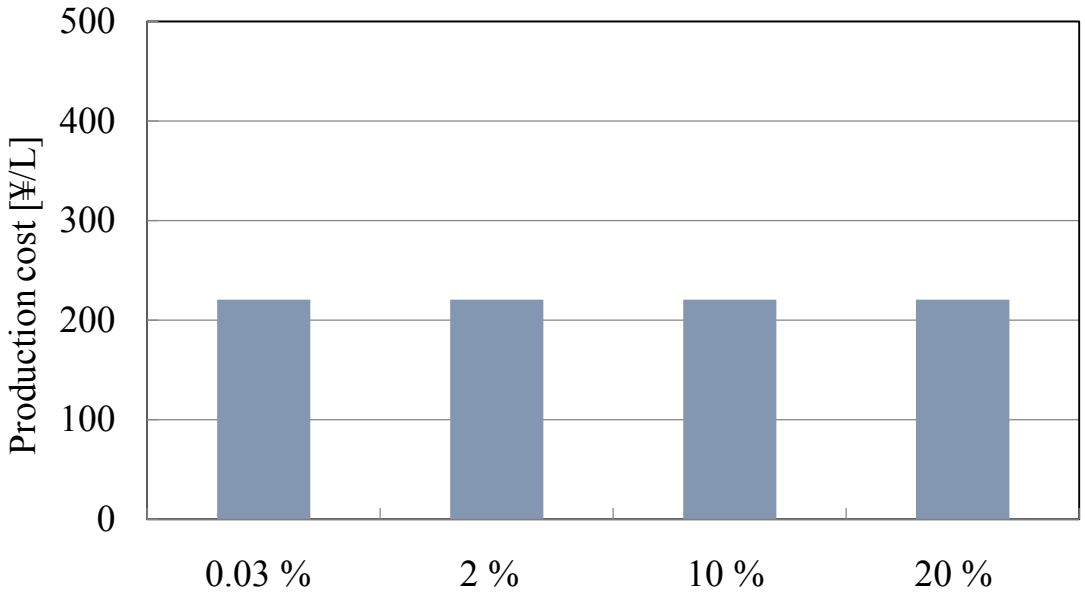

Fig. 5-1 Comparison of biofuel production costs under different CO_2 concentration

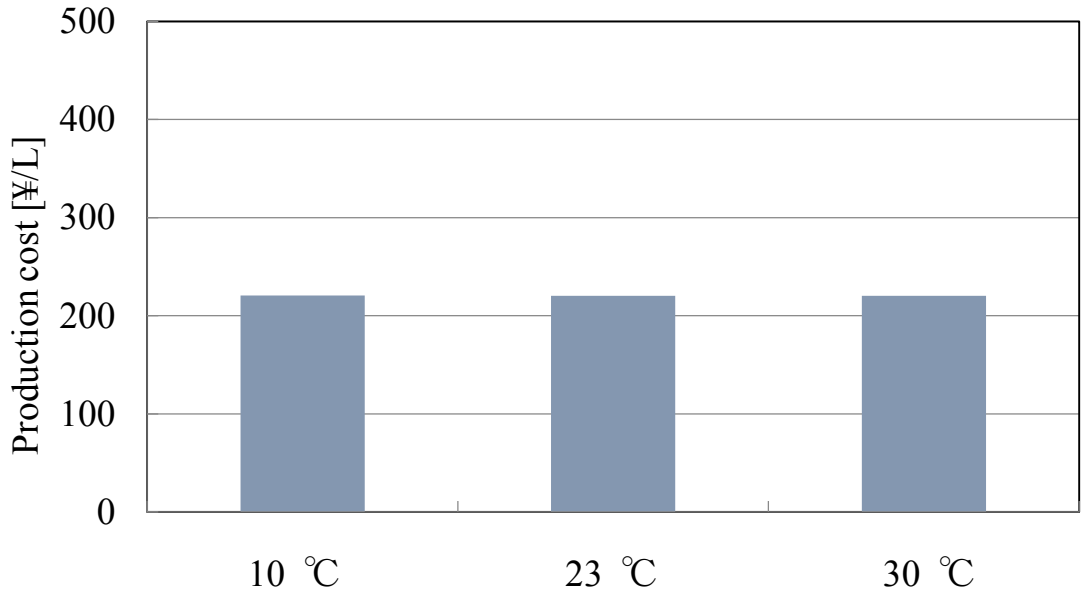

Fig. 5-2 Comparison of biofuel production under different temperature

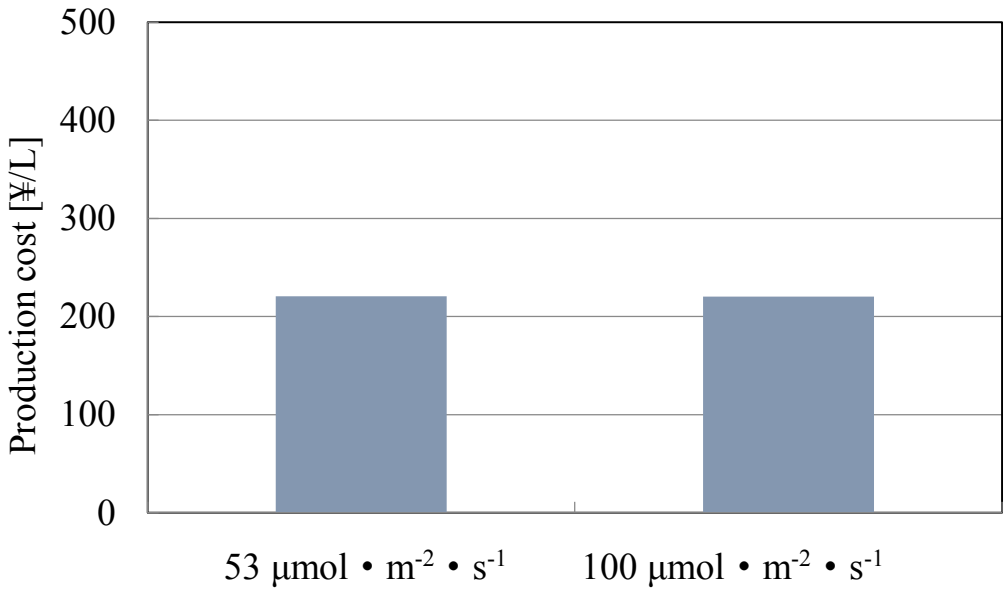

Fig. 5-3 Comparison of biofuel production under different light intensity

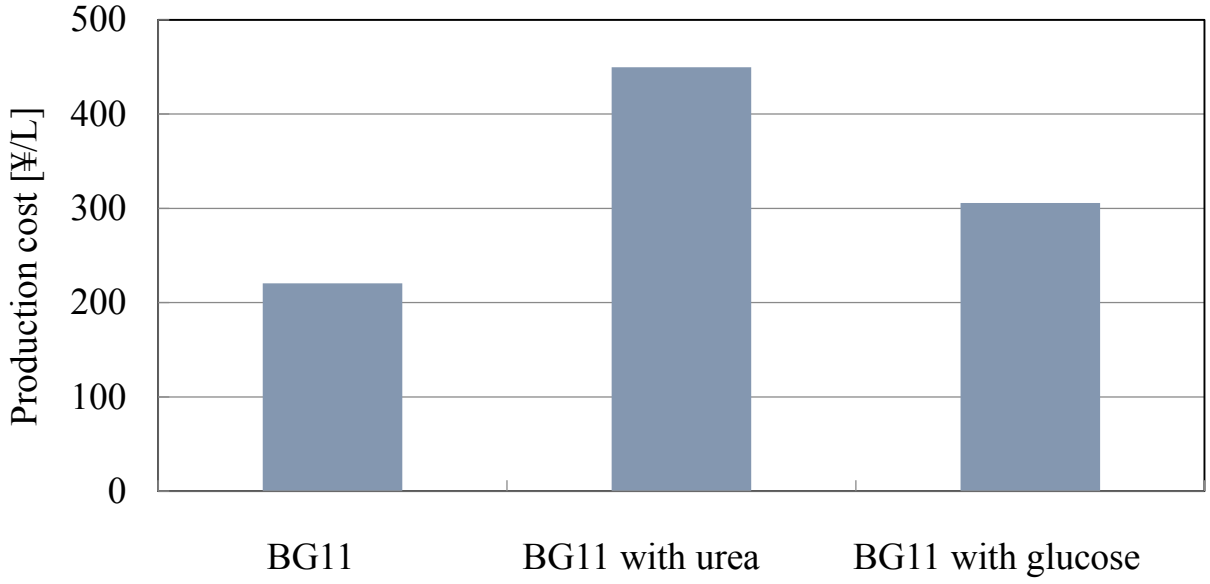

Fig. 5-4 Comparison of production cost on different nutrition

References

5-1) U.S. Energy Information Administration HP
5-2) Agency for Natural Resources and Energy HP
5-3) Council on Competitiveness-Nippon HP, final report of the project of development of fuel using microalgae.

6. Conclusions

This study aims at the production of biofuels at a lower cost and investigation of culture condition for the production of biofuel using *Nannochloropsis oceanica* IMET1.

Chapter 1 explains about *Nannochloropsis oceanica* IMET1 such as characteristics, advance production of biofuel, etc.. In addition, the previous studies of microalgae for producing biofuel and the issue of using microalga are explained.

Chapter 2 explains the growth performance of five microalgae including *Nannochloropsis oceanica* IMET1, *Nannochloropsis gaditana* CCMP526, 527, 1775, and 1894. *N. oceanica* IMET1 shows best growth performance and highest growth rate of the five microalgae. *N. gaditana* CCMP526, 1894 also shows good growth performance and growth rate. As there are previous studies of *N. gaditana* CCMP526, it is selected as a comparison of *N. oceanica* IMET1.

Chapter 3 explains the effect of the photosynthetic impact factor (temperature, CO_2, light) on growth performance of *N. oceanica* IMET1 with a comparison of *N. gaditana* CCMP526. The growth performance of *N. oceanica* IMET1 was better than that of *N. gaditana* CCMP526. In particular, the growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526, regardless of culture medium under CO_2 concentrations > 10 %. The optimum growing conditions for *N. oceanica* IMET1 was a CO_2 concentration of 2%, light intensity of 53 μmol・m^{-2}・s^{-1}, and 30 °C when MBG11 medium was used.

Chapter 4 explains the effect of nutrition in the medium (nitrogen, carbon sources) on biomass and biofuel production. The higher growth rate of *N. oceanica* IMET1 was evident in the flask cultures. The yield of lipid products obtained by *N. oceanica* IMET1 can produce a high yield of lipid products in comparison to *N. gaditana* CCMP526. *N. oceanica* IMET1 seems to contain higher amount of C16:0 fatty acid than *N. gaditana* CCMP526. Nitrates increased dry cell weight, biomass yield, and lipid weight in comparison with urea. Replacing nitrate with urea had a negative effect on dry cell weight and lipid yield. Fatty acid composition was affected by nitrogen source. Nitrate culture produced mainly C16:0 (6.3 %) and C18:2 (14.9 %) and urea produced culture mainly C16:0 (6.8 %), C18:3 (8.6 %), and C22:0 (33.1 %) fatty acids.

Fatty acid composition differed when different nitrogen sources were used. The effect of glucose on growth performance and lipid product. It was decreased by the effect of streptomycin for controlled bacterium. The yield of biofuel, however, was increased. This study found that the growth performance of *N. oceanica* IMET1 is higher than that of other *Nannochloropsis* spp, and the biofuel yield of *N. oceanica* IMET1 is high. Therefore *N. oceanica* IMET1 can produce high efficiently biofuel.

Chapter 5 explain the cost of biofuel production from *N.oceanica* IMET1. As the result of the calculations, the cost of biofuel production from *N. oceaninca* IMET1 is 220 ¥/L. It is lower than the cost reported by other researchers, when BG11 is used as medium. It was found that *N. oceaninca* IMET1 is a possible candidate to produce low cost biofuels.

Future issues are to clarify of produced biofuel mechanism for the improvement of biofuel yield, and to basically study the production technology of biofuel for practical microalgae.

Research records

Paper

1. Title : Effect of nitrogen source on biomass and lipid production of *Nannochloropsis oceanica* IMET1
   Author : Yongxue Chi, Feng Chen, Yasuyuki Takiguchi
   Green and Sustainable Chemistry, Vol.5, (2015.5) pp. 101~106

2. Title : Comparison of the growth performance of *Nannochloropsis oceanica* IMET1 and *Nannochloropsis gaditana* CCMP526 under various culture conditions
   Author : Yongxue Chi, Yasuyuki Takiguchi
   Journal of Plant Sciences, Vol.3, No.1 (2015.1) pp. 9~13

International Conference

1. Title : Effect of nitrogen source on biomass and lipid production of *Nannochloropsis oceanica* IMET1
   Author : Yongxue Chi, Feng Chen, Yasuyuki Takiguchi
   Abstract of 8th international Algae congress (1-3, Dec. 2014)
   in Florence, Italy

2. Title : Growth performance of two oleaginous microalgae *Nannochloropsis oceanica* IMET1 and *N. gaditana* CCMP526 under different culture conditions
   Author : Yongxue Chi, Yasuyuki Takiguchi, Feng Chen
   Abstract of 10th International Phycological Congress (4-10 Aug. 2013)
   in Orlando, Florida, USA

Domestic Conference

1. Title : *Nannochloropsis oceanica* IMET1 の増殖とオイル生産への培地中の窒素化合物有無の影響
   著者：遅永雪，陳峰，野島稜加，滝口泰之
   第 38 回日本藻類学会，東邦大学習志野キャンパス,2014 年 3 月 14〜16 日
   藻類，62 巻，1 号，p. 68（2014 年 3 月）

2. Title : 種々の培養条件における脂質含有微細藻類*Nannochloropsis oceanica* IMET1および *Nannochloropsis gaditana* CCMP526 の増殖

著者：遅永雪，陳峰，劉徴，滝口泰之

第 36 回日本藻類学会，北海道大学学術交流会館, 2013 年 7 月 13～15 日

藻類，60 巻，2 号，p. 68 （2012 年 7 月）

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