Screening of high-lipid content microalgae for biodiesel production

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Abstract

Currently, the study of biodiesel production from microalgae is extensively studied due to its potential benefits such as higher lipid productivity, rapid growing time and less cultivated area than the oil producing crops. The aim of this study was to screen microalgae strains to produce lipids. In this research thirteen green microalgae were primarily cultivated at 30 °C, light: dark ratio of 16:8 hours (10-12 klux of light intensity) and aeration supplemented with 2% CO_2 for 7 days. The selected five strains due to high lipid contents and lipid productivities were *Ankistrodesmus* sp. IFRPD No: 1061, 1068, *Monoraphidium* sp. IFRPD No: 1067, 1003 and *Chlamydomonas* sp. IFRPD No 1016 and their lipid contents and lipid productivities were 21.95, 28.45, 19.52, 15.19, 26.40% and 2.56, 1.95, 4.14, 3.61, 2.74 mg/L/d, respectively. Then the microalgae were cultured at the same condition for longer time to study the kinetic of biomass and lipid production. *Ankistrodesmus* sp. IFRPD No 1061 gave the highest lipid content at 29.86 % (w/w) and its lipid productivity was 14.49 mg/L/d. *Ankistrodesmus* sp. IFRPD No. 1068, *Monoraphidium* sp. IFRPD No. 1003, 1067, and *Chlamydomonas* sp. IFRPD No.1016 gave 28.68, 25.34, 29.55 and 17.74 % lipid content, and their lipid productivities were 11.37, 9.73, 7.69 and 2.82 mg/L/d, respectively. Therefore, *Ankistrodesmus* sp. IFRPD No. 1061 was chosen to study further in enhancing lipid productivity for biodiesel production.

Keywords: *Ankistrodesmus* sp. IFRPD No: 1061, biodiesel, lipid productivity, microalgae

Introduction

The current research on algae biofuel is mainly focused on species selection for improving oil production capabilities and the development of more efficient methods for microalgae cultivation. Microalgae have the ability to grow rapidly, synthesize and accumulate high lipids which can be modulated by several biotic and abiotic factors; hence, attracted a lot of attention for biodiesel production and as a potential renewable resource for essential fatty acids in the last decade (Sachitra et al. 2013). Biodiesel, one of the most commonly used biofuels, is recognized as an ideal recyclable energy carrier, and a possible primary energy source (Chisti, 2007). Commercial biodiesel is currently produced from animal fat, waste frying oil and vegetable oil (Barnwal and Sharma, 2005), and its competition with edible vegetable oil for agricultural land is still a controversial issue (Mata et al. 2010). Subsequently, microalgae that can grow rapidly and convert solar energy to chemical energy via CO_2 fixation are now being considered a promising oil source for making biodiesel (Mata et al. 2010). Under suitable culture conditions, some microalgal species are able to accumulate up to 50–70% of oil/lipid per dry weight (Chisti, 2007). The fatty acid profile of microalgal oil is suitable for the synthesis of biodiesel (Gouveia and Oliveira, 2009). The most important reason of using microalgal oil for biodiesel is its tremendous oil production capacity i.e. 58,700 oil liters per hectare, which is one or two magnitudes higher than that of

any other energy crop (Chisti, 2007). However the production of biodiesel from microalgae is still too expensive to meet the market requirements. In addition, it is also necessary, but very difficult, to develop cost-effective technologies that would permit efficient biomass harvesting and oil extraction. Nevertheless, since microalgae production is regarded a feasible approach to mitigate global warming, it is clear that producing oil from microalgal biomass would provide significant benefits, in addition to the fuel. Microalgae have thus been widely recognized as the feedstock for third-generation of biofuels (Chisti, 2007).The aim of this research is to screen suitable strains of microalgae for mass cultivation and enhancing lipid productivity for biodiesel production.

Methodology

Algae and cultivation

Green microalgae strains were obtained from the Institute of Food Research and Product Development (IFRPD), Kasetsart University. Thirteen green microalgae strains used in this study were *Ankistrodesmus* spp.1031, 1101, 1172, 1061, 1068, *Monoraphidium* spp.1067, 1003 and *Chlorococcum* spp.1041, 1137, *Chlamydomonas* sp. 1016, *Dictyosphaerium* sp. 1005, *Ulothrix* sp. 1057 and *Oscillatoria* sp. 1175 and were grown on NS III medium (NS III) which consist of (per liter): 1.01 g KNO_3; 0.28 g $K_2HPO_4•3H_2O$; 0.12g $MgSO_4•7H_2O$; 0.015 g $CaCl_2•2H_2O$; 0.11 g NaCl; Solution A (0.592 g KBr; 0.415 g KI; 0.021 g LiCl; 0.077 g H_3BO_3; 3.0 g HCl, 2 mL); Solution B (0.05 g $MnCl_2•4H_2O$; 3.0 g HCl, 2 mL)Solution C (0.81 g Fe $(NO_3)•9$ H_2O; 0.75 g EDTA, 2 mL). These microalgae were primarily cultivated and maintained at 30 °C, the light: dark ratio at 16:8 hours (10-12 klux of light intensity) and aeration supplemented with 2% CO_2 for 7 days (primary screening). Then these microalgae were cultured at the same condition for longer time to study the kinetics of biomass and lipid production (secondary screening).

Determination of biomass concentration and productivity

Dry cell weight was determined by passing 10 mL of algal culture on diameter 4.7 millimeter (mm) Whatman GF/C microfiber filter, rinsed twice with distilled water and dried at 105°C for 24 h to give the dry cell weight (g).The biomass concentration (g/L) was calculate and the productivity ($P_{Biomass}$, g/L/day) calculated from the equation $P_{Biomass} = (X_t - X_0) / (t_t - t_0)$, where X_t and X_o are the dry biomass concentration (g/L) at time t_t and t_0, respectively (Niels et al. 2012).

Measurement of lipid production

Total lipids were extracted by the method of Bingh and Dyer (1959) with some modifications. The suspended sample (50 mL) was centrifuged at 6000 rpm for 15 min to harvest microalgae cells and then the algae cell was re-suspended in 1 mL of distilled water. The chloroform/methanol/water in ratio of 1/2/1, v/v/v (4 mL) was added. The mixture was sonicated for 15 min at 100W and 20 kHz (VCX 130, Sonics & Materials Inc., CT, USA), and vortexed for 30 s. Additional chloroform (1 mL) and water (1 mL) and the solution was again vortexed for 30 s. The solution was centrifuged at 6000 rpm for 15 min and the bottom phase (chloroform/water) was transferred into a new tube. The upper layer (methanol/cell) was extracted again using the same procedure but with a half amount of solvent. The bottom phase were combined and evaporated for 24 h in a drying oven at 80 °C. The lipid contents (C_{Lipids}) were expressed as the percentage of dry cell weight and the lipid productivity was calculated.

$$C_{\mathrm{Lipid}} = W_{\mathrm{L}}/W_{\mathrm{A}} \times 100\%$$

where W_L (g) is the weight of the extracted lipids and W_A (g) is the dry cell microalgae. The lipid productivity was calculated by

$$P_{\mathrm{Lipid}} = \frac{P_{\mathrm{Biomass}} \times C_{\mathrm{Lipid}}}{100}$$

where P_{lipids} is productivity of lipids, P_{Biomass} is productivity of biomass; and C_{Lipids} is the content of lipids and were given as percent dry weight (Nielset al. 2012).

Nitrate determination

Nitrate concentrations were determined according to standard methods (APHA, 1995) using a spectrophotometer. Dry potassium nitrate (KNO_3) standard was incubated in oven at 105 ^{o}C for 24 h, 0.7218 g of potassium nitrate was dissolved in water and diluted to 1000 mL; 1 mL (100 µg NO^{-}_{3}–N), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL were taken from stock solution and added with 100 µL hydrochloric acid solution (HCl, 1 N) for standard preparation. The solutions were mixed thoroughly and measured the absorbance at wavelength of 220 nm to obtained NO^{-}_{3} - N reading and wavelength of 275 nm to determine interference due to dissolved organic matter.

Calculation:

$$NO_3^-(g/L) = \frac{\Delta(O.D_{220} - O.D_{275})}{0.051} \times \mathrm{Dilution}$$

Statistical analysis

The experiments for the determination of dry weight, growth rates, as well as the biochemical assays were all repeated three times to ensure the authenticity of the data. The statistical analysis was performed by using the software SPSS 11.5. One-way analysis of variance (ANOVA) was used to evaluate the differences among the treatments. If ANOVA effects were significant, comparisons between the different means were made using post hoc least significant differences (LSD). Standard deviation of values and error bar shown in the figures were determined by number of replicates (3 replicates) and the probability values were less than 0.05.

Results

Primary screening of microalgae on the basis of growth attributes and lipid accumulation

A set of thirteen green microalgae isolates from eight genera (five species of each of *Ankistrodesmus* two each of *Monoraphidium, and* one each of *Chlamydomonas, Chlorococcum*sp, *Oscillatoria*, *Dictyosphaerium, Chlorococcum* and *Ulothrix*) were screened under standard laboratory conditions for their biomass and lipid production. The summary of biomass concentration, lipid concentration, lipid content % (w/w) and lipid productivity (on dry weight basis) is given in Table 1. All the species were grown under in NS III medium for the identification of promising strains in relation to biomass and lipid production. These results depicted that *Ankistrodesmus* sp. 1068; *Ankistrodesmus* sp. 1061 and *Chlamydomonas* sp. 1016 had lipid content more than 20%. In addition, *Monoraphidium* sp.1067 and 1003 gave maximum lipid concentration at 0.696 and 0.608 g/L and the lipid productivity i.e. 4.140 and3.618 g/L/d, respectively in 7 days of culture. Five microalgae strains were chosen to study further for their biomass and lipid production.

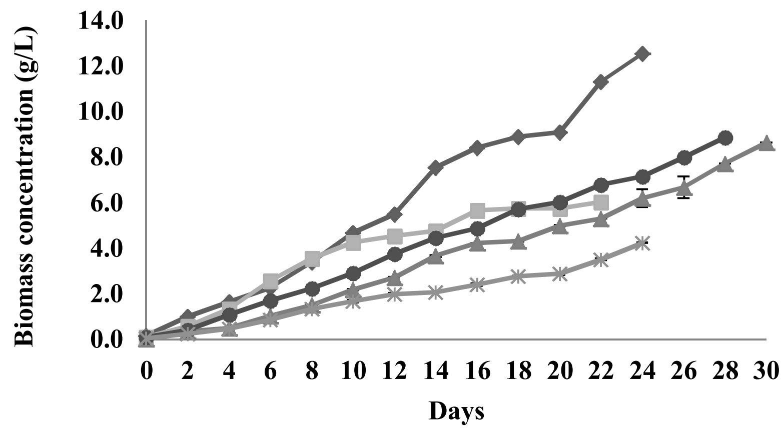
Table1 Kinetic parameters of biomass and lipid production of microalgae.

Strains microalgae IFRPD NO.	Biomass concentration g/L	Lipid concentration g/L	Lipid content %(w/w)	Lipid Productivity mg/L/d
***Ankistrodesmus* sp.1068**	1.157	0.329	28.45	1.958
***Chlamydomonas* sp. 1016**	1.745	0.461	26.42	2.744
***Ankistrodesmus* sp. 1061**	1.965	0.431	21.95	2.567
***Monoraphidium* sp.1067**	3.563	0.696	19.52	4.140
***Monoraphidium* sp.1003**	4.001	0.608	15.19	3.618
***Ankistrodesmus* sp. 1031**	2.075	0.376	18.12	2.238
***Ankistrodesmus* sp. 1101**	1.419	0.255	17.97	1.518
***Ankistrodesmus* sp. 1172**	2.829	0.390	13.79	2.321
***Chlorococcum*sp. 1137**	3.599	0.466	12.95	2.744
***Oscillatoria*sp. 1175**	2.529	0.304	12.02	1.810
***Dictyosphaerium*sp. 1005**	2.589	0.308	11.89	1.832
***Chlorococcum*sp. 1041**	1.643	0.179	10.92	1.068
***Ulothrix*sp. 1057**	1.972	0.154	7.82	0.918

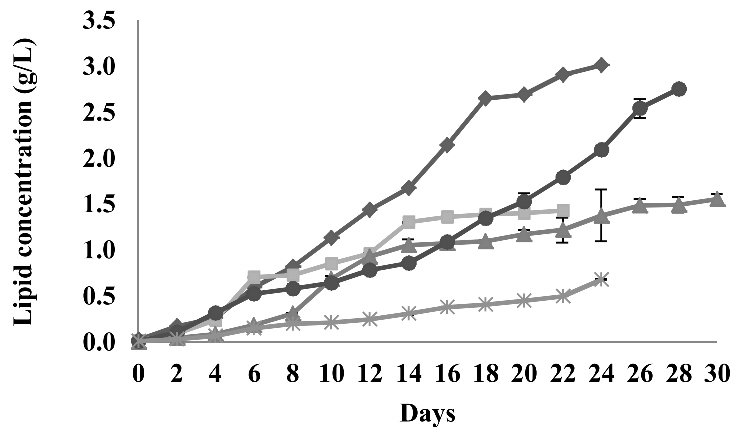
Secondary screening of microalgae on the basis of growth attributes and lipid accumulation

The second part was to study the kinetics of biomass and lipid production of microalgae for secondary screening. Fig. 1 shows the biomass, lipid and nitrate concentration of all the five strain isolates i.e. *Ankistrodesmus* sp. 1068, *Ankistrodesmus* sp. 1061, *Chlamydomonas* sp. 1016 *Monoraphidium* sp.1067 and *Monoraphidium* sp. 1003grown in NS III medium. The cell growth of *Ankistrodesmus* sp. IFRPD No. 1061 significantly enhanced up to 24th day, giving the highest biomass and lipid concentration at 12.523and 3.017g/L at the same time *Ankistrodesmus* sp. IFRPD No. 1068 gave maximum biomass and lipid concentration at 6.017 and 1.435 g/L on 22nd day. *Monoraphidium* sp. IFRPD No. 1067 gave maximum biomass and lipid concentration at8.633 and 1.563 g/L on 30th day. *Monoraphidium* sp. IFRPD No. 1003 provided maximum biomass and lipid concentration at 8.857 and 2.760 g/L on 28nd day. *Chlamydomonas* sp. IFRPD No. 1016 produced maximum biomass and lipid concentration at 4.230 and 0.685 g/L on 24nd day, respectively (Fig.1 (A, B)). In phototrophic culture nitrate concentration drastically reduced to a minimum level within 8 days of growth (Fig. 1 C).*Ankistrodesmus* IFRPD No. 1061 produced maximum biomass concentration, lipid concentration biomass productivity and lipid productivity (12.523, 3.017 g/L and 0.527, 0.145 g/L/d, respectively), (p<0.05) (Table 2).

A



B



C

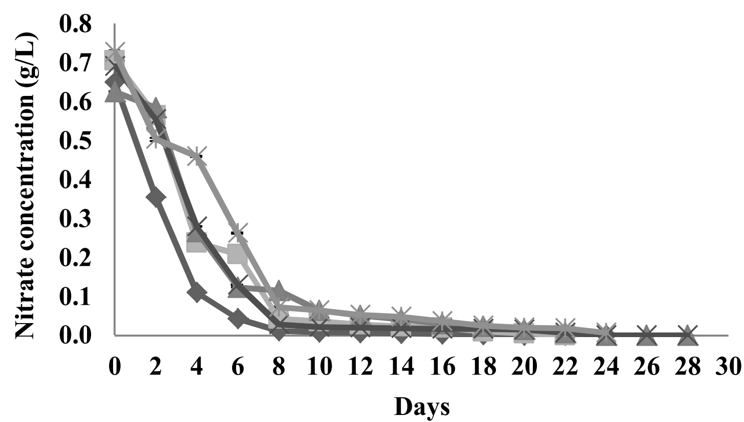


Fig 1: The biomass, lipid and nitrate profiles of five microalgae cultivation. *Ankistrodesmus* sp. IFRPD No. 1061 (◆), *Ankistrodesmus* sp. IFRPD No. 1068 (■), *Monoraphidium* sp. IFRPD No. 1067 (▲), *Monoraphidium* sp. IFRPD No. 1003 (●), *Chlamydomonas* sp. IFRPD No. 1016(*).

Table 2: Kinetic parameters of five microalgae strains cultivation (n=3) [a].

Strains IFRPD No.	Biomass concentration (g/L)	Biomass Productivity (g/L/d)	Lipid concentration (g/L)	Lipid Productivity (g/L/d)	Lipid content % (w/w)
***Ankistrodesmus* sp.1061**	12.523[a]	0.527[a]	3.017[a]	0.145[a]	29.869[c]
***Ankistrodesmus* sp.1068**	6.017[c]	0.433[b]	1.435[c]	0.114[b]	27.477[d]
***Monoraphidium* sp.1003**	8.857[b]	0.312[c]	2.760[b]	0.097[c]	31.163[b]
***Monoraphidium* sp.1067**	8.633[b]	0.287[d]	1.563[c]	0.077[d]	34.240[a]
***Chlamydomonas* sp. 1016**	4.230[d]	0.176[e]	0.685[d]	0.028[e]	17.67[e]

[a] Mean n=3 replicates of microalga cultures; letters in each column indicate significant differences at 5% probability level.

Discussion

Microalgae, a group of fast-growing unicellular or simple multicellular microorganisms, are found in very wide range of environments and offer several advantages, including higher photosynthetic efficiency, enhanced growth rates and higher biomass production compared to other energy crops (Rodolfi et al., 2009). Screening for oil producing microalgae among the isolates is a vital part in the optimisation of biodiesel production. Certain strains of microalgae such as *Botryococcus braunii* have high lipid storage potential (up to 75% lipid/g dry cell weight) but this is accompanied by low productivity. Cultures with moderate lipid accumulation levels (20–50%) but higher lipid productivities are preferred for mass cultivation (Mata et al., 2010; Amaro et al., 2011). In this study, lipid productivity, which combines the dual effects of lipid content and biomass productivity, was used as performance index to examine lipid production efficiency from microalgae (Yeh and Chang, 2012).The lipid productivity for *Ankistrodesmus* IFRPD No. 1061 under phototrophic cultivation was 145 mg/L/d, which was much higher than the average value (50.0 mg/L/d) for oleaginous green algae (Griffiths and Harrison, 2009)or the maximum value (54 mg/L/d) for several *Chlorella* species under different phototrophic, heterotrophic, and mixotrophic conditions (Herrera- Valencia et al., 2011).Therefore, *Ankistrodesmus* IFRPD No. 1061 is an ideal algal strain to produce biodiesel because of its lipid accumulation abilities under phototrophic cultivation. Under stress conditions (chemical stimuli, physical stimuli), microalgae alter their lipid production. The major chemical stimuli are nutrient starvation, salinity and growth-medium pH, while the major physical stimuli are temperature and light intensity (Breur et al., 2012). The lipid content increased from 18% to 40% (in terms of dry cell weight) in *Chlorella vulgaris*, when grown in nitrogen deprivation medium (Illman et al., 2000). The possible reason for this enhancement is the diversion of nutrients for cell growth in the first stage followed by biomass enrichment with lipids, in the absence of nutrient(s) in the second stage (Lin and Lin, 2011). Microalgal oil content can seldom exceed 80% by weight of dry biomass (Chisti, 2007). Therefore, strain selection is critical for biodiesel production. Neutral lipids can be readily converted to biodiesel or other types of fuels through the existing and emerging oil refining processes (Hu et al., 2008).Lourenco et al. (2002) observed that the role of nitrogen starvation in enhancing lipid accumulation needs to be based on selection of

suitable nitrogen source. Navarro-Pérez et al. (2001) observed that lipid content tends to increase when cultures reach stationary phase, especially when silicate and nitrogen become limiting, mainly in diatoms. In the current study, microalgae proved to be in nitrogen deprivation condition which can enhance the total lipids content. In order to fulfil the economical viability of biodiesel projections, there is a need to build up biomass which can provide high rates of lipid productivity. It was first reported by Rodolfi et al. (2009) that in order to increase lipid content and lipid productivity, a two phase cultivation process (a nutrient sufficient phase to produce high algae biomass followed by a nitrogen deprived phase to boost lipid synthesis) was a suitable strategy. It has been postulated that the deficiency of nutrients leads to cessation of growth and the organism channels the metabolic flux generated in photosynthesis to lipid / fatty acid biosynthesis (Ratha et al., 2013). The limited success of algal lipid production stems primarily from the lack of understanding of metabolic pathways and the regulation of algal lipid metabolism in general and neutral lipid synthesis and accumulation in particular, and failure to develop cost-affordable and energy-efficient algal feedstock production systems and processes (Xu et al., 2001). The major obstacle for microalgal oil production is their relatively high costs which may be overcome by the technology developments. Optimization of process is a very important aspect for such technology development (Schenk et al., 2009). This study clearly highlights the different response of the algal species which is variable in both terms of growth and lipid accumulation.

Conclusions

This study provides an effective approach of screening of high-lipid content microalgae for biodiesel production by considering biomass and lipid productivity. *Ankistrodesmus* sp. IFRPD No. 1061 gave maximum biomass and lipid prod maximum biomass concentration lipid concentration, biomass productivity and lipid productivity (12.523, 3.017 g/L and 0.527, 0.145 g/L/d, respectively). The lipid content of *Ankistrodesmus* IFRPD No. 1061 was significantly enhanced to 29% over their controls by this strategy; involving N-limitation. Therefore, selection of this strain for optimization of lipid production through mixotrophic culture to enhance the biomass and lipid yield makes *Ankistrodesmus* IFRPD No. 1061 as a potential source for biodiesel production.

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