Appl Biochem Biotechnol (2017) 182:1605–1618
DOI 10.1007/s12010-017-2421-8

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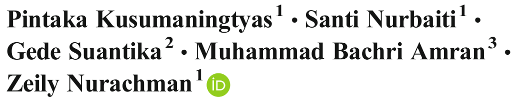
Enhanced Oil Production by the Tropical Marine Diatom *Thalassiosira* Sp. Cultivated in Outdoor Photobioreactors



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Received: 7 December 2016 / Accepted: 18 January 2017 /
Published online: 23 January 2017
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Abstract Microalgae-derived oils have potential as a biofuel feedstock. To produce microalgal oils at a large scale, large amounts of nutrients and energy are needed to grow the algae. In this study, we evaluated three types of agricultural fertilizer (AF)-based culture media (AF1, AF2, and AF3) based on a previously published enriched seawater (ES) medium to produce biomass and oils from *Thalassiosira* sp. Under laboratory conditions, the highest cell productivity of *Thalassiosira* sp. was obtained with the AF3 medium. *Thalassiosira* sp. cultured in the AF3 medium produced 10.4 ± 0.9 mg L^{-1} day^{-1}oils, which is significantly higher than the 5.8 ± 0.7 mg L^{-1} day^{-1}produced in the ES medium. The higher production was due to the presence of nitrate and trace elements, both of which played roles in enhancing biomass and oil content, respectively. During cell growth, resting spores appeared inside the cells and were a marker to harvest the cells. Because of the abundant availability of sunlight in the tropics during the year, the oil production of *Thalassiosira* sp. in the AF3 medium was scaled up using outdoor photobioreactors under different weather conditions (rainy and dry seasons). *Thalassiosira* sp. produced more unsaturated fatty acids during the rainy season and produced more saturated fatty acids during the dry season. This study also demonstrated that it was possible to culture *Thalassiosira* sp. under outdoor conditions using a low-cost

Dedicated to the memory of Dr. Santi Nurbaiti (†29 May 2016)

Electronic supplementary material The online version of this article (doi:10.1007/s12010-017-2421-8) contains supplementary material, which is available to authorized users.

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agricultural fertilizer-based culture medium (AF3 medium) to produce biodiesel feedstock with an annual production of 8.1 ± 0.4 t ha^{-1} during the dry season and of 23.9 ± 6.8 t ha^{-1} during the rainy season.

Keywords Agricultural fertilizer-based medium · Oil productivity · Outdoor cultivation · *Thalassiosira* sp. · Tropical marine diatom

Introduction

The conversion of solar energy to biofuel through photosynthesis by microalgae has received tremendous attention for decades due to the higher growth rates and richer in oil content of microalgae (30–60% of dry weight) compared to terrestrial crops [1–3]. Biodiesel from microalgae is known as one of the most environmentally friendly renewable energy resources because of the capability to reduce greenhouse gas emissions [4, 5]. Currently, one of the main obstacles to the commercial production of microalgae oils is the cost of biomass production, including additional nutrients, electricity, and water, which is higher than the cost to produce common crop oils [1, 3]. Hence, intensive studies to reduce the costs of microalgae biomass production should be conducted.

Agricultural fertilizer-based medium used for culturing microalgae is critical to producing biofuel owing to its high availability and low cost [6, 7]. Agricultural fertilizers contain phosphorus and nitrogen, which are essential nutrients for marine diatoms [8, 9] and green algae [10]. Previously, we found that the tropical marine *Thalassiosira* sp. grew well in an enriched seawater (ES) medium [11]. However, the availability of additional nutrients may hinder the use of ES medium for large-scale algal biomass production. Thus, enhancing biomass production using an agricultural fertilizer-based medium is interesting.

During the production of algal biomass at a large scale, microalgae cultured under direct sunlight exposure may reduce energy costs. Some outdoor models have been developed to culture microalgae from small to economic scales [12–15], especially in areas of the subtropics with mild climates, such as Spain [16], Japan [17], China [14, 18], Australia [19, 20], and Israel [21]. The microalgal productivity ranged from 10 to 30 g m^{-2} day^{-1} depending on geographic area and the prevailing weather conditions [15, 16, 18, 22]. As a marine country in the tropics, Indonesia has the potential for microalgal biofuel production because the basic requirements for growing microalgae, such as sunlight, seawater, and favourable ambient air temperature, are abundant and available all year long. However, reports on outdoor algal oil productivity in the tropics have been very limited thus far. Outdoor microalgal cultivation in the tropics faces many challenges due to environmental stress including sunlight intensity, evaporation, temperature, and contamination risks. Thus, to produce microalgal biomass outdoors, closed photobioreactors are more effective than open-culture systems [17, 23–25].

The tropical marine diatom *Thalassiosira* sp. is a good candidate for producing oils [11]. The natural oils of *Thalassiosira* sp. contain a mixture of free fatty acids (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)), neutral lipids, glycolipids, phospholipids, and other components. In this study, we evaluated the development of *Thalassiosira* sp. in agricultural fertilizer-based media to grow biomass followed by observation of the resting spores of *Thalassiosira* sp. to identify oil accumulation in the cells. The best medium to produce *Thalassiosira* sp. biomass in the outdoor photobioreactors was chosen, and the oil content, including total fatty acids produced by the

cells in both rainy and dry seasons, was measured. This paper reports the growth profile of *Thalassiosira* sp. in different agricultural fertilizer-based media, the characteristics of intracellular oil accumulation, and the oil productivity of *Thalassiosira* sp. cultured outdoors.

Materials and Methods

Chemicals and Reagents

All chemicals used for media preparation (both Walne medium and ES medium), lipid extraction, esterification, and fatty acids profile analysis were pro-analysis grade (Merck, Germany). Agricultural fertilizers of urea, TSP-36, NPK™, and Si-P-(PG)™ were purchased from the local agriculture market, and industrial grades of $FeCl_3$, anhydrous Na_2SiO_3, and anhydrous Na_2EDTA were purchased from a local supplier. Nile red, capric acid (C10:0) as an internal standard, and fatty acid methyl ester (FAME) mix C4–C24 as an external standard were purchased from Sigma-Aldrich, USA.

Microalgae Strain and Culture Maintenance

The source and maintenance of the tropical marine diatom *Thalassiosira* sp. have been described previously [11]. The cultures were periodically regenerated to remain in the growth phase.

Evaluation of Agricultural Fertilizer-Based Media

To obtain a $10^{\times}$ cell/mL cell density of *Thalassiosira* sp., three types of agricultural fertilizer-based media, namely AF1 medium, AF2 medium, and AF3 medium, were first evaluated in indoor conditions (Table 1). *Thalassiosira* sp. cells that were cultured in the medium containing a similar N/P/Si ratio of 11.7:1:1.34 (*w*/*w*/*w*) and the enriched seawater (ES) medium were used as the control [11]. *Thalassiosira* sp. cells were cultured in simple air-lift photobioreactors made of transparent glass bottles with a working volume of 800 mL. Cultures with an initial density of 2×10^5 cell mL^{-1} were illuminated under a light intensity of 95 μmol m^{-2} s^{-1} at room temperature with a photoperiod of light/dark = 12:12 h, salinity of 28 ppt, pH 8.0–8.7, and free air bubbling. The cells were counted daily under a light microscope using an improved Neubauer-haemocytometer (Germany) to determine cell density, specific growth rate, and doubling time. The experiments were performed in triplicate, and the results were given as the average values and standard deviation. Statistical analysis of the data was carried out using the Microsoft Excel 2013 software. A one-way analysis of variance was used to compare each data set with a confidence level of 95% and P values <0.05. Significant differences among the data were further analysed using a least significant difference test. Finally, the best medium was chosen to produce biomass in outdoor cultivation.

Determination of Biomass and Oil Productivity under Laboratory Conditions

The growth of *Thalassiosira* sp. in urea-ES medium (ES medium with urea as the N source), nitrate-ES medium (ES medium with $NaNO_3$ as the N source), and TM-ES medium (ES medium containing additional trace minerals) was evaluated separately. All of the media contained a final nitrogen concentration of 11.7 mg L^{-1}. The amount of trace minerals in 1 L medium was 0.02 mg $CuSO_4.5H_2O$, 0.021 mg $ZnCl_2$, 0.36 mg $MnCl_2.4H_2O$, and 0.02 mg

Table 1 Nutrient composition and concentration of media

Media	Concentration (mg L^{-1})
ES medium	
Urea (p.a.)	25
$NaH_2PO_4.2H_2O$ (p.a.)	5
$Na_2SiO_3.5H_2O$ (p.a.)	10
$FeCl_3.6H_2O$ (p.a.)	6
$Na_2EDTA.2H_2O$ (p.a.)	70
AF1 medium	
Urea fertilizer (containing 46% N)	25
TSP-36™ fertilizer (containing 36% P_2O_5)	5.4
Si-P-(PG)™ fertilizer (containing 6–8% P_2O_5, and 36–42% SiO_2)	8
$FeCl_3$ (industrial grade)	3.3
Na_2EDTA (industrial grade)	70
AF2 medium	
NPK fertilizer ™ (containing 4.5% $NaNO_3$, 20.5% urea, 5% Na_2HPO_4, and 20% K_2O)	115
Si-P-(PG)™ fertilizer	8
$FeCl_3$ (industrial grade)	3.3
Na_2EDTA (industrial grade)	70
AF3 medium	
NPK fertilizer ™	115
Na_2SiO_3 (industrial grade)	6
$FeCl_3$ (industrial grade)	3.3
Na_2EDTA (industrial grade)	70

$CoCl_2.6H_2O$. *Thalassiosira* sp. cells cultured in the AF3 medium were used as a control. The cells were cultured under the same conditions as mentioned above. The change in cell number and cell morphology (in particular, resting spore formation) was monitored daily under a light microscope. Intracellular neutral lipid accumulation was measured using the Nile red dye fluorescence spectrophotometer method [26]. *Thalassiosira* sp. cells harvested on the 13th day were centrifuged at 15,000×*g* at 4 °C for 30 min, and algal oil was extracted from the cells using the method of Bligh and Dryer [27]. The productivity of biomass (P_B) and oil (P_O) of *Thalassiosira* sp. was determined according to Eqs. 1 and 2, respectively. The experiments were performed in triplicate, and all data were analysed using a two-tailed Student's *t* test. A significant difference among treatments was determined by a degree of error of $P < 0.05$.

$$P_B\left(\text{mg L}^{-1}\ \text{day}^{-1}\right) = \frac{\text{Dried weight biomass (mg)}}{\text{Volume of culture (L)} \times \text{Time (day)}} \tag{1}$$

$$P_O\left(\text{mg L}^{-1}\ \text{day}^{-1}\right) = \frac{\text{Dried biomass yield}\left(\frac{\text{mg}}{\text{L}}\right) \times \%\text{Oil content}\left(\frac{\text{mg oil}}{\text{mg biomass}} \times 100\right)}{\text{Time (day)}} \tag{2}$$

Determination of Biomass and Oil Productivity in Outdoor Conditions

To scale up the outdoor algal biomass production, *Thalassiosira* sp. cells were cultivated in AF3 medium using air-lift column photobioreactors based on Indonesian patent no. ID P0030250 with

a diameter of 15 in. and a height of 1 m. Ambient air was bubbled through the bottom of each photobioreactor at a flow rate of 25 L min^{-1} using a compressor pump. Before use, all medium and photobioreactors were sterilized by soaking them in 63 mg L^{-1} NaClO for 24 h, followed by $Na_2S_2O_3$ neutralization with a final concentration of 95 mg L^{-1} for 16 h and free air bubbling. Activated cells of *Thalassiosira* sp. obtained from indoor cultures were cultivated in outdoor photobioreactors with an initial density of 5×10^5 cell mL^{-1} and the cells grew under direct natural sunlight. The incident light intensity and temperature of the medium were recorded at 5-min intervals using data logger equipment. The cells were counted daily at 09.00 a.m. The changes in cell number and cell morphology (in particular, resting spore formation) were monitored daily under a light microscope. The presence of resting spores was indicated by cells with dark pigmented small circles and fragmented chloroplasts. The outdoor experiments were conducted on a campus (6° 51′ 21.3″ S, 107° 36′ 42.5″ E) over two different seasons (between July and October 2014). Cultures at the stationary phase were harvested by a filtration technique using Masini™ cotton cloth and then weighed. The biomass was freeze-dried, and lipids were extracted [27]. The biomass and oil productivity were determined as described above. Areal oil productivity (P_{OA}) of *Thalassiosira* sp. was calculated according to Eq. 3. The conversion factor from P_O to P_{OA} was 3.65, derived from 365 (converting day to year) multiplied by 10^{-9} (converting biomass yield from mg to ton) and 10^7 (converting culture volume from L to ha).

$$P_{OA}\left(\text{ton ha}^{-1}\ \text{year}^{-1}\right) = P_O \times \text{Conversion factor} \tag{3}$$

Fatty Acid Analysis

FAME was prepared by esterification of *Thalassiosira* sp. oil. A mixture of 40 mg *Thalassiosira* sp. oil and 2 mmol NaOH in methanol solution was incubated for 12 h at 55–60 °C with continuous stirring or shaking, followed by addition of 4 mL of 2.5% H_2SO_4 and incubation for 1 h at 55–60 °C with continuous stirring. Prior to FAME extraction, 10 mg of capric acid methyl ester, 3 mL n-hexane, and 6 mL 0.5% NaCl (*w*/*v*) were added to the mixture, and the FAME was centrifuged at 15,000×*g* at 4 °C for 10 min. The supernatant was analysed using gas chromatography (GC) equipped with a flame ionization detector (FID) on an HP5 column, 30 m × 0.25 mm i.d × 0.25 μm. The injector and detector temperature were 260 °C. The oven temperature was set at 140 °C and increased from 180 to 270 °C at a rate of 4 °C min^{-1} and remained at 270 °C for 7.5 min. A set FAME standard (containing 37 different components of FAME) was also injected into the GC-FID under the same conditions. To confirm the FAME components, GC-MS of the FAME standard was also carried out.

Results and Discussion

Growth of *Thalassiosira* sp. in Agricultural Fertilizer Media

To obtain a low-cost culture medium for large-scale cultivation of *Thalassiosira* sp., three types of agricultural fertilizer-based medium (AF1, AF2, and AF3 medium) were evaluated, and an ES medium was used as a control (Table 1). Starting with an initial cell density of 2×10^5 cell mL^{-1}, the *Thalassiosira* sp. cells cultured in the ES medium required 6 days to reach the end of the exponential phase, with a doubling time of 1.5 days and then a stationary

phase of 2 days (Fig. 1). Maximum cell density determined on the 6th day was 5.60×10^6 cell mL^{-1} (Table 2). Compared with cells grown in the ES medium, the maximum cell density in the AF2 medium (5.49×10^6 cell mL^{-1}) was not significantly different, but it was significantly different from the cell density in the AF1 medium (4.32×10^6 cell mL^{-1}) ($P < 0.05$). In terms of cell density, *Thalassiosira* sp. cells showed a better development in the AF2 medium than in the AF1 medium, as the phosphorus source in the AF2 medium was of a more soluble form. Conversely, the source of phosphorus in the AF1 medium, TSP™ fertilizer, was obtained from phosphate rock and had low solubility. This result indicated that the availability of dissolved phosphorus in the medium was essential for cell development.

Surprisingly, *Thalassiosira* sp. cells cultured in the AF3 medium remained in the exponential phase on the 5th day, whereas the cells cultured in the AF2 medium had reached the stationary phase by the 5th day. The cells grew well in the AF3 medium with soluble silicate and phosphate but did not grow well in the AF2 medium, as the silicate was supplied from Si-P-PG™ fertilizer, which is available in the form of hard granulated solid with low solubility. This result indicated that soluble silicate played an essential role in cell development, particularly during the formation of the cell wall. The maximum cell density of *Thalassiosira* sp. cells cultured in the AF3 medium on the 7th day was 1.4 times higher than that in the AF2 medium. To scale up the cultivation of *Thalassiosira* sp. cells outdoors, the AF3 medium was chosen for subsequent experiments. In terms of additional nutrient cost per litre medium, AF3 medium costs IDR 117, and the ES medium costs IDR 547. (Note: 1 IDR is roughly equal to US$0.000074). Beal et al. [15] reported that the cost to produce 1 L of algae oils was US$2. Thus, the AF3 medium was found to be cost-effective to produce a large amount of *Thalassiosira* sp. biomass.

Characteristics of Intracellular Oil Accumulation

To understand which minerals in the AF3 medium enhance biomass productivity, the growth of *Thalassiosira* sp. cells was further evaluated. Commercial NPK™ fertilizer used in the AF3

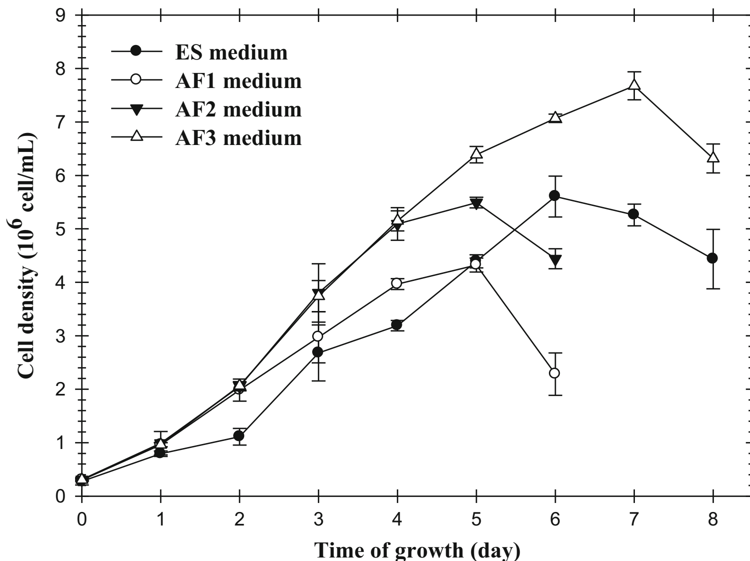

Fig. 1 Growth profiles of *Thalassiosira* sp. in four different agricultural fertilizer-based media

Table 2 Characteristics of *Thalassiosira* sp. growth

	Medium			
	ES	AF1	AF2	AF3
Specific growth rate (day^{-1})	0.46 ± 0.03	0.51 ± 0.01	0.57 ± 0.04	0.43 ± 0.01
Max. cell density (×10^{6} cell mL^{-1})	5.60 ± 0.38	4.32 ± 0.13	5.49 ± 0.10	7.68 ± 0.26
Doubling time (day)	1.50 ± 0.10	1.35 ± 0.02	1.22 ± 0.09	1.60 ± 0.02
Cost per L (IDR)	547	10	117	117

Data are mean ± standard deviation of triplicates

medium contained two types of N in the forms of nitrate and urea, as well as additional trace minerals (B, Ca, Co, Cu, Fe, Mg, Mn, Mo, S, and Zn) in unknown amounts. Here, we compared the growth of *Thalassiosira* sp. cells cultured in the urea-ES medium, nitrate-ES medium, and urea-TM-ES medium. Then, the biomass productivity, the oil content, and the fatty acid composition were determined. Figure 2 shows *Thalassiosira* sp. cells growing in all media reached the end of the exponential phase by the 10th day. The *Thalassiosira* sp. cells cultured in the medium containing nitrate (nitrate-ES or AF3 medium) were more concentrated than those cultured in the medium containing urea (urea-ES or urea-TM-ES medium). These findings indicated that nitrate was easily absorbed by *Thalassiosira* sp. cells and resulted in good cell development. This result is consistent with the cell development of other diatoms [28] and green microalgae [29–33], both of which require nitrate as the N source, as previously reported. Diatoms have traditionally been thought to be the primary consumers of nitrate in the environment, so they can grow very rapidly under high nitrate concentration conditions and cause massive blooms [34].

To understand whether cell development is related to oil accumulation, we monitored oil accumulation daily during the development of *Thalassiosira* sp. cells using Nile red fluorescence (Fig. 3). During *Thalassiosira* sp. growth, cells photosynthesized to produce energy for

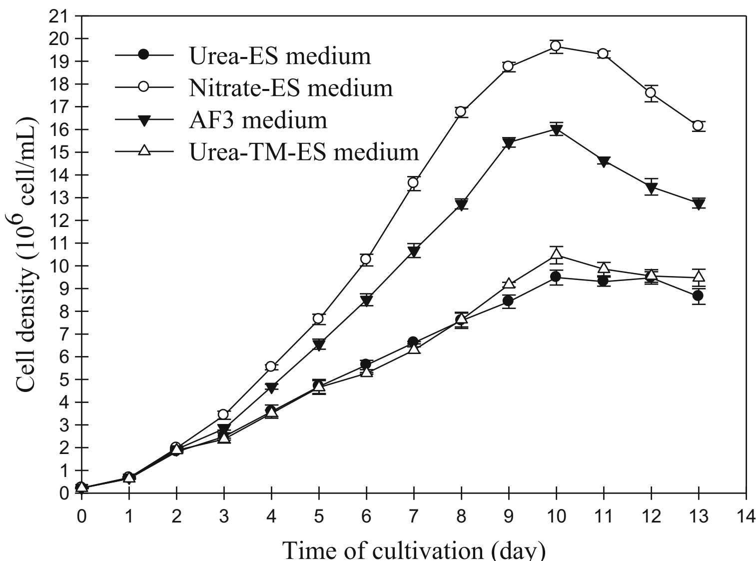

Fig. 2 Growth of *Thalassiosira* sp. in AF3, urea-ES, nitrate-ES, and urea-TM-ES media

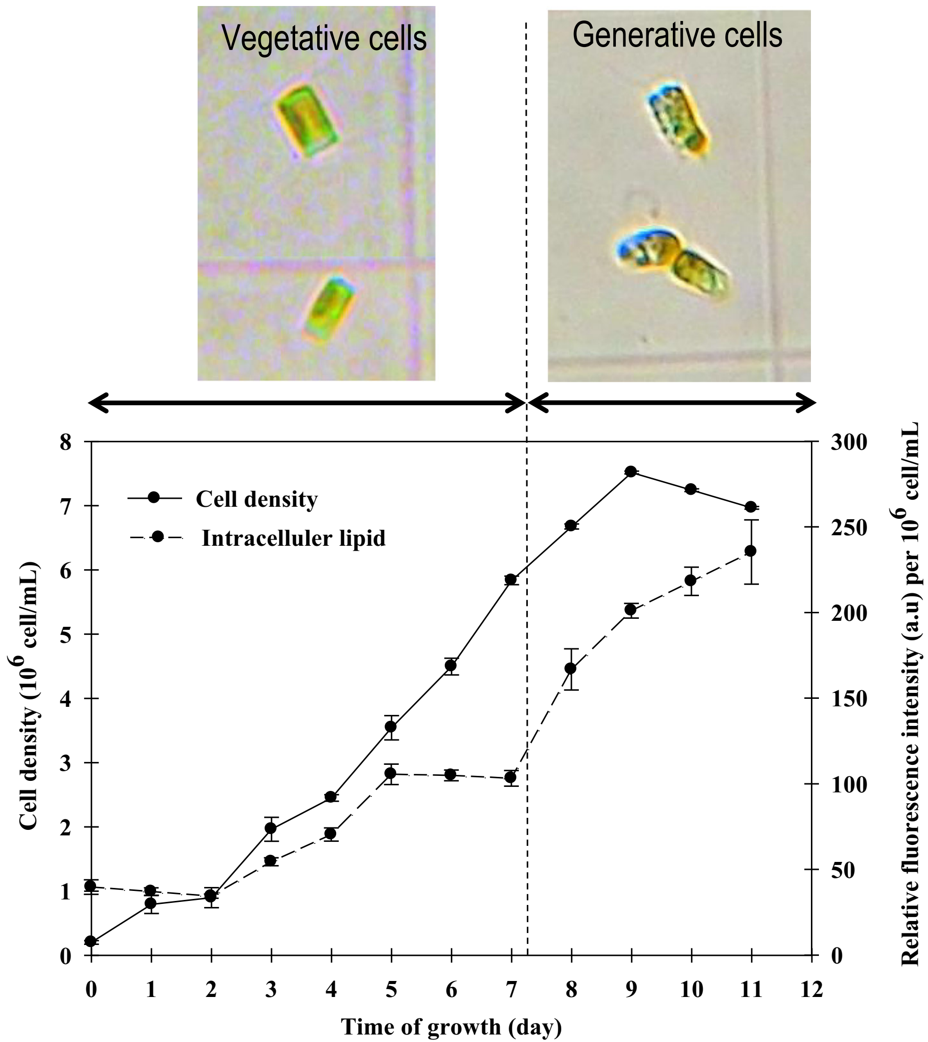

Fig. 3 Accumulation of intracellular neutral lipids in *Thalassiosira* sp. cells. *Thalassiosira* sp. cells without resting spores were observed during 7 days of growth, while cells with resting spores were observed after 8 days of growth

cell division, and the amount of biomass increased. During the exponential phase until the 7th day, *Thalassiosira* sp. cells also produced oils containing phospholipids, glycolipids, and free fatty acids used for the formation of the cell membrane during cell division. Figure 3 shows the morphology of the *Thalassiosira* sp. cells observed during the growth phase until the 7th day, in which the spores are not yet observed. Resting spores (small spherical bodies lying inside diatom cells) of the *Thalassiosira* sp. cells appeared on the 8th day, when the cell division rate declined. Resting spore formation has been commonly observed in centric diatoms including *Thalassiosira* sp. but rarely in pennate marine planktonic diatoms [35]. Resting spores have also been found to occur when diatoms were grown in stressful environments such as areas with depleted nutrients, high light intensity, and temperature changes [36, 37]. The formation of resting spores indicated that the metabolism of the cells had changed from membrane lipid

synthesis to the lipogenic phase to accumulate neutral lipids (oils) as stored energy [38, 39] and as a food reserve [40, 41]. Hence, the resting spores that appeared in the *Thalassiosira* sp. cells were an indicator of the best time to harvest the algal biomass for oil.

Diatoms accumulate lipids when cell division ceases. To enhance oil accumulation in the cells, we evaluated the growth of *Thalassiosira* sp. cells cultured in urea-ES medium with and without additional trace minerals (Fig. 2). *Thalassiosira* sp. cells grown in urea-ES medium showed no significant difference in biomass productivity compared with those in urea-TM-ES medium ($P < 0.05$). The biomass productivity of *Thalassiosira* sp. harvested on the 13th day was 20 mg L^{-1} day^{-1}. However, oil content extracted from the dried-weight *Thalassiosira* sp. cells cultured in both media was significantly different. The yield of oil obtained from *Thalassiosira* sp. cells culture in the urea-ES medium was 28.25% (*w/w*), while the yield from the cells cultured in the urea-TM-ES medium was 37.98% (*w/w*). These results indicated that additional trace minerals within the algal development medium enhanced oil content. Oil accumulation is controlled by the availability of ATP and NADPH in the cell during fatty acid (FA) synthesis, especially during the elongation and desaturation processes [42]. Chemical energy (ATP and NADPH) is generated from light-driven photosynthesis involving the light harvesting complex, the light sensitive complex (photosystem), electron transport proteins, and ferredoxin, all of which require cofactors such as Fe, Mn, Co, Zn, and Cu [37]. The composition of total FA produced by the cultured *Thalassiosira* sp. cells is shown in Table 3. *Thalassiosira* sp. produced SFA dominated by myristic acid and palmitic acid, MUFA dominated by palmitoleic acid, and PUFA dominated by eicosapentaenoic acids (EPA). Interestingly, *Thalassiosira* sp. cells generated more MUFA and PUFA than SFA when grown in the medium containing additional trace minerals (urea-TM-ES and AF3 medium). Hence, trace minerals play critical roles in generating cellular metabolic energy for lipid biosynthesis.

Lipid Production in Outdoor Conditions

To produce lipids of *Thalassiosira* sp. on a large scale, the algal cells were cultivated in outdoor photobioreactors under direct sunlight. The cultures of *Thalassiosira* sp. were scaled up in outdoor photobioreactors in two different seasons, rainy and dry (Fig. 4). Daily sunlight intensity during the rainy season fluctuates greatly depending on the weather conditions, with maximum daily sunlight intensity observed from 766 to 1570 μm photon m^{-2} s^{-1} (Fig. 4a). However, daily sunlight intensity during the dry season was almost two times higher than that during the rainy season, with the maximum daily sunlight intensity fluctuating from 1363 to 1817 μm photon m^{-2} s^{-1} (Fig. 4b). In outdoor culture, the temperature of the medium relied on the intensity of the incident sunlight. During the rainy season, the average daylight temperature of the culture medium ranged from 21.1 to 31.1 °C, with maximum daily temperatures in the range of 26.7 to 34.3 °C (Fig. 4a). The maximum temperature was reached for 10 h (approximately from 6.00 a.m. to 4.00 p.m.) with the temperature increasing at an average rate of 1.1 °C h^{-1}. In contrast, the daily temperature during the dry season was 4 °C higher than that for the rainy season, and the length of daylight was 30 min longer. The average daylight temperature ranged from 21.6 to 33.5 °C, with maximum daily temperatures ranging from 30.1 to 39.1 °C (Fig. 4b). The maximum temperature was reached for 9 h (from approximately 5.00 a.m. to 2.00 p.m.) with the temperature increasing at an average rate of 1.4 °C h^{-1}. Thus, the weather conditions in the tropics were crucial for the acclimatization and adaptation of the *Thalassiosira* sp. cells outdoors.

Thalassiosira sp. cells cultured in both seasonal experiments showed similar growth profile features: they needed to adapt to new environments when transferred from indoor to outdoor

Table 3 Fatty acid composition of *Thalassiosira* sp. cells cultured in different types of media

Types of fatty acids	Types of medium			
	Urea-ES	Nitrate-ES	Urea-TM-ES	AF3
Saturated (SFA)				
C8:0 (caprylic acid)	0.26 ± 0.19	–	–	–
C12:0 (lauric acid)	–	–	0.13 ± 0.05	1.43 ± 0.21
C13:0 (tridecanoic acid)	–	–	–	0.99 ± 0.42
C14:0 (myristic acid)	19.26 ± 0.63	13.10 ± 0.20	12.72 ± 0.85	13.95 ± 0.61
C15:0 (pentadecanoic acid)	0.71 ± 0.25	0.46 ± 0.04	0.64 ± 0.31	0.87 ± 0.56
C16:0 (palmitic acid)	34.76 ± 1.27	35.28 ± 0.23	28.98 ± 0.91	19.37 ± 0.02
C17:0 (heptadecanoic acid)	0.51 ± 0.15	0.29 ± 0.01	1.77 ± 0.08	0.72 ± 0.74
C18:0 (stearic acid)	1.68 ± 0.51	0.76 ± 0.01	1.30 ± 0.18	3.94 ± 0.14
C20:0 (arachidic acid)	–	–	–	0.66 ± 0.62
C22:0 (behenic acid)	0.66 ± 0.06	0.25 ± 0.01	–	1.78 ± 1.43
C23:0 (tricosanoic acid)	–	–	–	0.12 ± 0.07
C24:0 (lignoseric acid)	1.32 ± 0.94	0.45 ± 0.06	0.61 ± 0.02	0.74 ± 0.20
Total SFA	59.2	50.6	46.2	43.9
Monounsaturated (MUFA)				
C14:1 (myristoleic acid)	–	–	–	2.25 ± 0.18
C15:1 (pentadecenoic acid)	–	–	0.22 ± 0.03	0.51 ± 0.06
C16:1 (palmitoleic acid)	32.86 ± 2.07	39.62 ± 1.25	39.3 ± 1.76	33.46 ± 1.71
C17:1 (heptadecenoic acid)	–	–	–	0.54 ± 0.30
C18:1n9c (oleic acid)	1.72 ± 0.29	0.87 ± 0.04	1.69 ± 0.04	1.47 ± 0.13
C18:1n9t (elaidic acid)	1.38 ± 0.56	0.60 ± 0.05	1.46 ± 0.59	2.50 ± 0.03
C20:1 (*cis*-11-eicosenoic acid)	–	–	–	0.80 ± 0.97
Total MUFA	36.0	41.1	42.8	41.5
Polyunsaturated (PUFA)				
C16:3n3 (hexadecatrienoic acid)	1.26 ± 0.16	1.17 ± 0.04	1.84 ± 0.40	2.95 ± 1.42
C18:2n6c (Linoleic acid)	0.28 ± 0.03	0.48 ± 0.01	0.33 ± 0.13	1.27 ± 0.52
C18:2n6t (Linolelaidic acid)	–	0.20 ± 0.00	0.41 ± 0.02	0.44 ± 0.14
C18:3n3 (α-linolenic acid)	0.63 ± 0.45	0.13 ± 0.00	0.15 ± 0.01	0.50 ± 0.12
C18:3n6 (γ-linolenic acid)	–	0.51 ± 0.05	0.59 ± 0.05	0.78 ± 0.27
C20:4n6 (arachidonic acid, AA)	0.36 ± 0.18	0.57 ± 0.09	0.33 ± 0.19	0.71 ± 0.08
C20:5n3 (eicosapentaenoic acid, EPA)	2.31 ± 1.31	4.73 ± 0.75	3.71 ± 0.10	6.32 ± 0.32
C22:2 cis (docosadienoic acid)	–	–	3.41 ± 1.97	0.27 ± 0.32
C22:6n3 (docosahexaenoic acid, DHA)	0.30 ± 0.20	0.53 ± 0.01	0.34 ± 0.05	0.65 ± 0.26
Total PUFA	5.1	8.3	11.1	13.9

conditions. The microalgae cultured outdoors were often exposed to a variety of changes in environmental conditions, particularly sunlight intensity and temperature occurring in circadian and seasonal cycles [43]. In the rainy season, *Thalassiosira* sp. cells needed 4 days to adapt to the outdoor conditions and then grew rapidly, with a specific growth rate of 0.44 day^{-1}(Fig.4a). Meanwhile, in the dry season, the adaptation time of *Thalassiosira* sp. cells was 2 days longer, and the cells grew slowly at a specific growth rate of 0.15 day^{-1}(Fig. 4b). In the dry season, the growth of algal cells was strongly inhibited by high sunlight irradiation and temperature. The excessive light intensity caused photodamage to the chlorophyll of the algal cells but not the carotenoids [44], resulting in decreased oil productivity. Most mesophilic microalgae have been found to grow at optimal temperatures ranging from 18 to 30 °C [45], but temperatures above 35 °C were found to be lethal [46, 47]. The tropical marine diatom *Thalassiosira* sp. cultured in outdoor photobioreactors was able to survive at 35–39 °C for 5 h daily, thus, indicating the thermotolerance of the strain.

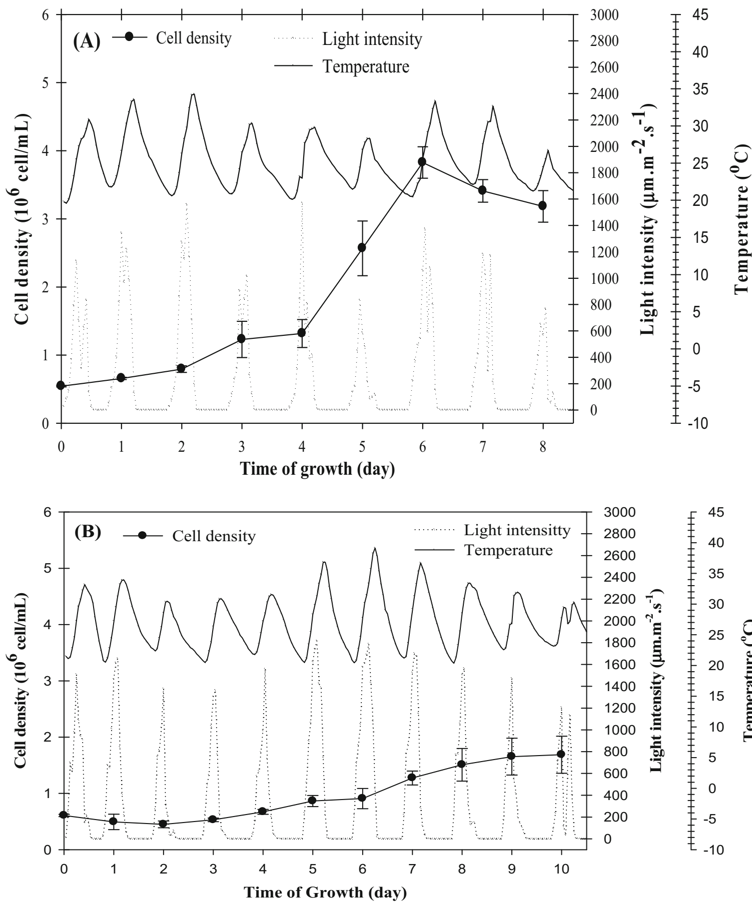

Fig. 4 Growth profile of *Thalassiosira* sp. in outdoor conditions during the rainy season (**a**) and the dry season (**b**)

Thermotolerant microalgae represent a candidate strain to be cultured outdoors for oil production in the tropics [33]. Table 4 shows the biomass and oil productivity of *Thalassiosira* sp. cultivated outdoors. The average biomass productivity of *Thalassiosira* sp. during the dry season was 19.8 and 27.8 mg L^{-1} day^{-1} during the rainy season. The average percent yield of oil produced by *Thalassiosira* sp. during the dry and rainy seasons was 12.0 and 23.3%, respectively. For comparison, the percent oil yield produced by *Fistulifera* sp. in outdoor photobioreactors was 13.0–37.7% [17], *Chlorella zofingiensis* 26.2–58.5% [48], and *Chlorella* sp. 22.8–34.5% [49]. Similar to indoor cultures, *Thalassiosira* sp. cells cultivated in outdoor photobioreactors during the rainy season produced a similar total FA composition dominated by myristic acid, palmitic acid, palmitoleic acid, and EPA (Supplementary Table S2). During the dry season, the total FA composition of *Thalassiosira* sp. was also dominated by myristic acid, palmitic acid, and palmitoleic acid, but the PUFA was dominated by eicosatrienoic acid. In general, *Thalassiosira* sp. produced more unsaturated fatty acids during the rainy season, while they produced more saturated fatty acids during the dry season. The average areal oil productivity of *Thalassiosira* sp. produced during the dry and rainy seasons was 8.1 and 23.9 t ha^{-1} $year^{-1}$, respectively. The oil productivity of *Thalassiosira* sp. was comparable to a palm oil productivity of 5.4 t ha^{-1} $year^{-1}$ [2]. This result implies that *Thalassiosira* sp. is a

Table 4 Biomass and lipid productivity of *Thalassiosira* sp. in outdoor conditions

Cultivation period	Dry weight biomass (mg L^{-1})	Biomass productivity (mg L^{-1} day^{-1})	Total oil content (% dry weight)	Oil productivity (mg L^{-1} day^{-1})	Areal oil productivity (t ha^{-1} $year^{-1}$)
Rainy season	222 ± 30	27.8 ± 3.4	23.3 ± 3.8	6.6 ± 1.9	23.9 ± 6.8
Dry season	198 ± 12	19.8 ± 1.2	12.0 ± 0.3	2.2 ± 0.1	8.1 ± 0.4

Data are mean ± standard deviation of triplicates

promising tropical microalgal oil feedstock. Currently, Indonesia is the highest palm oil-producing country in the world. As a maritime country, Indonesia may also mass produce algal oil above the surface of the sea. In the future, algal oil together with palm oil will hopefully become renewable energy sources to replace fossil fuel, at least to fulfil our domestic demand.

Conclusion

The present study demonstrated that *Thalassiosira* sp. cells grew well in AF3 medium containing soluble nitrate, phosphate, silicate, and trace minerals. They produced high oil productivity. The AF3 medium, which was easily obtainable and inexpensive, was the best choice medium for generating large-scale *Thalassiosira* sp. biomass. The *Thalassiosira* sp.-produced SFA were dominated by myristic acid and palmitic acid, the MUFA were dominated by palmitoleic acid, and the PUFA were dominated by EPA. The areal oil productivity of *Thalassiosira* sp. outdoor in the tropics ranged from 8.1 to 23.9 t ha^{-1} $year^{-1}$. Further efforts on developing a pilot model to produce algal oil above the surface of the sea are worthwhile.

Acknowledgements We gratefully acknowledge the financial support provided by the Ministry of Research, Technology and Higher Education, the Republic of Indonesia through the Program of Masterplan Percepatan dan Perluasan Pembangunan Indonesia (MP3EI) to ZN, and the Program of Insentif Riset Sistem Inovasi Nasional (INSINAS) to ZN, as well as Beasiswa Pendidikan Pascasarjana Dalam Negeri (BPP-DN) to PK.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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