Research Journal of Life Science
AUGUST-2018 Volume 5 NO. 2 (128-139)
journal homepage: www.rjls.ub.ac.id

Influence of Light Intensity on Lipid Productivity and Fatty Acids Profile of *Choricystis* sp. LBB13-AL045 for Biodiesel Production

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KEYWORDS

Light intensity
Lipid productivity
Fatty acids profile
Choricystis sp.
Biodiesel properties

Abstract The influence of various light intensity on the lipid productivity, fatty acids profile, and biodiesel properties of *Choricystis* sp. AL045 were examined. *Choricystis* sp. LBB13-AL045 was a high lipid content microalgal strain (up to 42.49 ± 0.41% per dry weight basis) with great performance of its growth (specific growth rate and biomass productivity were up to 0.802 ± 0.013 day^{-1} and 108.57 ± 8.07 mg L^{-1} day^{-1}, respectively). Such results indicated its high lipid productivity and its potency to be used for biodiesel production. The treatment of various light intensities on the microalgal culture resulted obvious differences in lipid productivity and fatty acids composition. Maximum lipid productivity (46.13 ± 3.43 mg L^{-1} day^{-1}) was at 405 µmol m^{-2} s^{-1} of light intensity. The fatty acids profiles of four light intensities treatments were quite similar; the major components of fatty acid obtained from *Choricystis* sp. LBB13-AL045 oil were oleic acid (C18:1) and palmitic acid (C16:0), which provided a strong indication of high-quality biodiesel. Biodiesel properties were determined by empirical equations and found to be within the limits of biodiesel standard SNI 7182:2015, ASTMD6751-08 and EN14214.

Introduction

Microalgae are promising feedstock for biodiesel generation due to escalating in fuel price and emerging concern about global warming associated with burning fossil fuels (Chisti, 2007). Microalgae, unlike conventional oil crops, grow extremely rapid and many are exceedingly rich in oil. Therefore, oil productivity as the product of lipid content and biomass productivity become key characteristic for choosing microalgal species for biodiesel production (Griffiths & Harrison, 2009). Some microalgal species have been widely known for their high lipid productivity, including *Nannochloropsis*, *Tetraselmis* (Rodolfi et al., 2008), *Amphora* (De la Pena, 2007), *Neochloris oleoabundans* (Li, Horsman, Wang, Wu, & Lan, 2008) and *Ankistrodesmus falcatus* (Griffiths & Harrison, 2009). Another promising microalgal species as lipid producer was also known, i.e. *Choricystis minor*, and such species pay less attentions despite the fact that it store great potency as biodiesel feedstock (Mazzuca & Chisti, 2009).

In regards to economic viability of microalgae-based application, strategies are needed to improve its biomass productivity, especially optimization cultivation condition (Adams, Godfrey, Wahlen, Seefeldt, & Bugbee, 2013). Optimization of the culture conditions would be a more effective approach to obtain a high biomass and lipid productivity of microalgae (Seo et al., 2017). Several factors, such as light intensity (Seo et al., 2017; Wahidin et al., 2013), CO_2 (Kassim & Meng, 2017; Thawechai et al., 2016), pH (Qiu et al., 2017), temperature (Darvehei et al., 2018) and nutrient compositions (Lasimone et al., 2018; Zhuang et al., 2018), have already been tested in the cultivation system to achieve high microalgal biomass and lipid productivity. Light intensity is one of the critical factor for microalgal

How to cite this article: Praharyawan, S., Rahman, D. Y., Susilaningsih, D. (2018). *Influence of Light Intensity on Lipid Productivity and Fatty Acids Profile of Choricystis sp. LBB13-AL045 for Biodiesel Production.* Research Journal of Life Science 5 (2), 128-139. https://doi.org/10.21776/ub.rjls.2018.005.02.7

photosynthetic which could be highly species-specific (Ramanna, Rawat, & Bux, 2017; Seo et al., 2017). Therefore, such growth critical factor needs to be optimized for particular microalgal strain (*Choricystis*) under consideration to achieve a higher biomass and lipid productivity.

Accordingly, this study was aiming at evaluating Choricystis sp LIPI-LBB13-AL045 as biodiesel feedstock for biodiesel production, with the focus on the influence of varied light intensity on lipid productivity and on the evaluation of the biodiesel quality.

Materials and methods

Microalgal strain and cultivation condition.

Choricystis sp. LBB13-AL045 was obtained from the culture collection of the Bioenergy and Bioprocess Laboratory, Research Center for Biotechnology, Indonesian Institute of Sciences, Indonesia. The microalgal strain was isolated from freshwater Lake in Bengkulu province, Indonesia. *Choricystis* sp. LBB13-AL045 was unicellular with the small cell size. The strain showed distinguish shape of oval based on the observation under the microscope (Figure 1). The cell walls of microalgal strain was smooth with no mucilage envelope. Further observation of this strain under the microscope found that the microalgal cells proliferate by means of asexual reproduction, autospores.

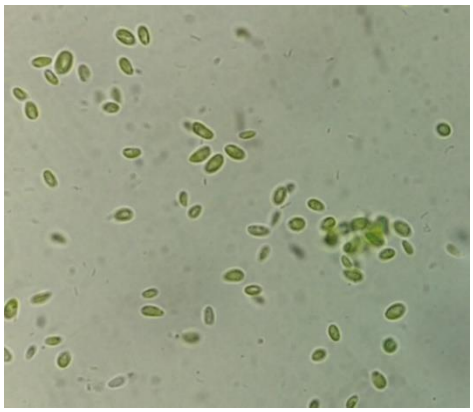

Figure 1. Microscopic photograph of *Choricystis* sp. LBB13-AL045, examined at 1000x magnification

The culture was maintained and grown in a medium containing (mM) 5.95 $NaHCO_3$; 1.65 $NaNO_3$; 0.47 $(NH_4)_2SO_4$; 0.12 $MgSO_4.7H_2O$; 0.074 KH_2PO_4; 0.029 K_2HPO_4; 0.008 $C_6H_5FeO_7$; and 0.01 $C_6H_8O_7$. Cells were grown in a 600-mL bottle containing 500 mL of the microalgae culture with aeration and under continuous illumination with varied light intensity. White LED flood light (50 Watt) was used as a light source and its light intensity was measured at the outer-surface of the bottle (on the middle of the culture's height) by using light meter. The laying distance of the microalgal culture from the light source corresponds to its value of light intensity used, which were 135, 270, 405, and 675 µmol m^{-2} s^{-1}. Every light-intensity treatment of cultivation was conducted in duplicate. The biomass productivity and total lipid content were measured in order to determine lipid productivity.

Microalgal growth property.

The microalgal strains of *Choricystis* sp. LBB13-AL045 were inoculated to 500 mL aerated bottle and cultured at room temperature under continuous illumination at varied light intensity started at 135 to 675 µmol.m^{-2} s^{-1} until it reached late stationary stage in their growth. The growth of microalgae was determined by using cell counting (haemocytometer) under light microscope. The specific growth rate of each strain was calculated based on the equation:

$$\mu = \ln (N_y/N_x)/ (t_y - t_x)$$

N_x : the cell amount at the start (t_x) of the logarithmic growth phase

N_y : the cell amount at the end (t_y) of the logarithmic growth phase

Biomass productivity (P_{dwt}) was determined as the dry biomass produced during the logarithmic growth phase. While lipid productivity (L_p) was calculated according to the equation (Nascimento et al., 2013):

$$L_p = P_{dwt} \times L_c$$

E-ISSN 2355-9926

L_p : Lipid productivity (mg/L/day).
P_{dwt} : Biomass productivity (mg/L/day).
L_c : Lipid content (%).

Determination of lipid content.

Lipid content reported as percentage of the total biomass (in % dry weight). Lipid extraction was conducted by adapting the modified method from Ryckebosch et al. (2012). Chloroform: methanol = 1:1 were used as solvent in the microalgal lipid extraction. 6 mL of solvent was added to 100 mg microalgae biomass and the tube was vortex mixed for 30 s. 2 mL of solvent and water were then added and the tube was vortex mixed again and subsequently centrifuged at 2000 rpm for 10 min. The aqueous layer was removed and the solvent layer was transferred into the clear tube. The remaining solid were re-extracted with 4 mL solvent. The re-extraction of lipid was repeated until the remaining-microalgae biomass turned to be colorless. The solvent was removed by letting it evaporated in the open air and the lipid content was determined gravimetrically. The extraction was performed in quadruplicate. The resulting percentage of extracted lipids is the sum of three extractions performed in series.

Direct transesterification and fatty acids methyl esters (FAMEs) profile analysis.

The determination of fatty acid methyl esters (FAMEs) were conducted by the direct transesterification of microalgae wet biomass (Harwati et al., 2012). Briefly, 200 mg of wet biomass and 3 mL of transesterification reaction mix (methanol/hydrochloric acid/chloroform, 10:1:1 vol/vol) were added. Cells were vortexed for 10 s and were placed at 90°C for 120 min. After the process was completed, the samples were removed and allowed to cool to room temperature. Water (1 mL) was then added and vortexed for 10 s. FAMEs were extracted via the addition of 3 x 2-mL aliquots of hexane, vortexed and separated. The FAMEs were subsequently analysed using gas chromatography equipped with a mass spectrophotometer detector (GC-MS) with a FAMEWAX column and an autosampler. The initial oven temperature was set at 40°C, and held for 2 min, then raised to 320°C at a rate of 6°C/min, and held at 320°C for 15 min, while the injector and detector temperature were set at 310°C and 320°C, respectively. The carrier gas (helium) was controlled at 5 mL/min. The compounds were identified in the Wiley Registry 9th edition + NIST 2011 Mass Spectral Database. The percentage of fatty acids were calculated based on the total known fatty acids by using the area normalization method.

Estimation of biodiesel fuel properties based on FAME profiles.

Biodiesel is a renewable transportation fuel consisting of fatty acid methyl esters (FAMEs). FAMEs profiles, especially size distribution and the degree of unsaturation, could significantly influence the physical and chemical properties of biodiesel (Hoekman et al., 2012). At present, many equations based on FAME profiles have been built to predict biodiesel properties (Francisco et al., 2010; Hoekman et al., 2012; Nascimento et al., 2013). Especially, the equations of Hoekman et al. (2012) was widely accepted due to the calculated values by the equation were more close to the measured values (Ma et al., 2014). In this study, the equations of (Hoekman et al., 2012) were selected to predict the biodiesel properties and detailed calculations are as follows:

Average degree of unsaturation (ADU) computed from compositional profiles in Table 2 (Eq. 3):

$$ADU = \sum M \times Y_i$$

ADU : the average degree of unsaturation of microalgal oil.
M : the number of carbon-carbon double bonds in each fatty acid constituent.
Y_i : the mass fraction of each fatty acid constituent.

For example, the mass fraction of each fatty acid constituent of potential microalgae

E-ISSN 2355-9926

cultivated under 135 µmol.m^{-2} s^{-1} in Table 2 was 29.98% (C16:0); 13.74% (C16:1); 1.75% (C16:2); 5.27% (C16:4); 0.99% (C18:0); 36.95% (C18:1); 8.36% (C18:2); 2.96% (C18:4), respectively. According to the Eq 3, calculations are as follows:

$$\begin{aligned} ADU &= \sum M \times Y_i \\ &= 0 \times 29.96\% + 1 \times 13.74\% + 2 \times 1.75\% \\ &\quad + 4 \times 5.27\% + 0 \times 0.99\% + 1 \times \\ &\quad 36.95\% + 2 \times 8.36\% + 4 \times 2.96\% \\ &= 1.04 \end{aligned}$$

The relationships between average degree of unsaturation (x) and biodiesel properties (y) including kinematic viscosity, specific gravity, cloud point, cetane number, iodine value, and higher heating value (HHV) are as shown in Eqs. (4–8), respectively (Hoekman et al., 2012).

$$y = -0.6316x + 5.2065 \quad (4)$$
$$y = 0.0055x + 0.8726 \quad (5)$$
$$y = -13.356x + 19.994 \quad (6)$$
$$y = -6.6684x + 62.876 \quad (7)$$
$$y = 74.373x + 12.71 \quad (8)$$
$$y = 1.7601x + 38.534 \quad (9)$$

Results and discussion

Growth and lipid productivity.

Choricystis sp. LBB13-AL045 was cultured in photobioreactor until it reached 7 days after initial day of stationary growth phase. The results of microalgal specific growth rate, biomass productivity, lipid content, and lipid productivity at different light intensities are shown in Table 1. The best specific growth rate of *Choricystis* sp. LBB13-AL045 was shown at the light intensity of 270 µmol m^{-2} s^{-1}. At the light intensity above 270 µmol m^{-2} s^{-1}, the specific growth rate was gradually decreased. This result showed that the growth of *Choricystis* sp. LBB13-AL045 start to inhibit at the light intensity of 405 µmol m^{-2} s^{-1}. The phenomenon of photoinhibition under high light intensity could be recognized as a reasonable explanation. At such light intensity, *Choricystis* sp. LBB13-AL045 accumulated lipid in high percentage per dry weight, indicated lipid accumulation occurred when microalgae at light-stress condition. Different characteristics was shown by *Chlorella vulgaris* which accumulated lipid at optimum light condition (150 µmol m^{-2} s^{-1}), while its growth started to inhibit at 200 µmol m^{-2} s^{-1} (Jiang et al., 2016).

Before reaching the stage of photo inhibition, the biomass productivity and lipid content of microalgal strain increased with rising light intensity. When the light intensity supplied too much (up to 675 µmol m^{-2} s^{-1}), photoinhibition appeared, thereby caused the decline of biomass and lipid content as well (He et al., 2015). With the increase of light intensity, the biomass productivity, lipid content, and lipid productivity gradually increased (Table 1), but above a certain threshold (405 µmol m^{-2} s^{-1}) it did not increase anymore. *Choricystis* sp. LBB13-AL045 has higher sensitivity to photoinhibition compared to *Scenedesmus* (Masojidek et al., 1999; Sanchez et al., 2008) and *Chlamydomonas* (Davis et al., 2013). The present work showed similar results with the work of Solovchenko et al. 2008. Solovchenko et al. (2008) analysed the effect of different light intensities on the growth and lipid content of *Parietochloris incisa*, and found that the optimal growth rate and lipid content were all under the light intensity of 400 µmol m^{-2} s^{-1}. However, in this present work, the optimal biomass and lipid productivity of *Choricystis* sp. LBB13-AL045 were under the light intensity of 405 µmol m^{-2} s^{-1}. At such light intensity, win-win in biomass and lipid content could be achieved as the desired purpose. Further increase in light intensity to 675 µmol m^{-2} s^{-1}, a decrease in biomass and lipid productivity was observed. This result demonstrated that the light regime is an important factor controlling biomass and lipid production in *Choricystis* sp. LBB13-AL045.

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Table 1. Growth kinetics, lipid content, and lipid productivity of oleaginous microalgal strain of *Choricystis* sp. LBB13-AL045 cultivated under different light intensities

Light intensity ($\mu mol.m^{-2}\ s^{-1}$)	Specific growth rate (day^{-1})	Biomass productivity ($mg\ L^{-1}\ day^{-1}$)	Lipid content (%)	Lipid productivity ($mg\ L^{-1}\ day^{-1}$)
135	0.743 ± 0.016	79.05 ± 4.87	36.38 ± 0.90	28.75 ± 1.,77
270	0.802 ± 0.013	77.69 ± 2.94	38.95 ± 0.60	30.26 ± 1.14
405	0.489 ± 0.005	108.57 ± 8.07	42.49 ± 0.41	46.13 ± 3.43
675	0.362 ± 0.006	82.54 ± 3.60	38.27 ± 0.81	31.58 ± 1.38

Fatty acid profiles properties

Through analysis of the fatty acids (FAs) composition data in Table 2, a useful comparison of various conditions of light intensity with respect to the saturated, monounsaturated, and polyunsaturated compounds is provided in Figure 2. Fatty acid profiles of *Choricystis* sp. LBB13-AL045 under different light regimes were quite similar, except for the condition of light intensity at 675 µmol $m^{-2}\ s^{-1}$. At the light intensity up to 405 µmol $m^{-2}\ s^{-1}$, monounsaturated fatty acid (MUFA) content was at dominant percentage over saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA), but at the light intensity of 675 µmol $m^{-2}\ s^{-1}$ the percentage of MUFA was relatively at the same level to SFA and PUFA percentage. At 675 µmol $m^{-2}\ s^{-1}$, compared to the other conditions of light intensity, the percentage of MUFA was relatively decreased and of PUFA was increased, while of SFA was at constant percentage. According to Hoekman et al. (2012) and Knothe (2009) the most favorable biodiesel would have rather low levels of polyunsaturated fatty acids (PUFAs) and low levels of saturated fatty acids (SFAs) to decrease oxidative stability and cold flow problems. Therefore, monounsaturated fatty acids (MUFAs) were capable of giving the finest compromise between oxidative stability and cold flow (Hoekman et al., 2012; Knothe, 2009). In this study, the results indicated that the microalgae which were cultivated at the light intensity of 675 µmol $m^{-2}\ s^{-1}$ would produce oil that was less suitable to be used as biodiesel feedstock compared to another oil produced from different light intensities due to its lowest MUFA content compared to others.

Table 2. Fatty acid profiles of *Choricystis* sp. LBB13-AL045 at different light intensities (% of total FAME)

Fatty Acids	Light Intensity ($\mu mol.m^{-2}\ s^{-1}$)			
	135	270	405	675
C14:0	-	0.6	0.63	0.68
C16:0	29.98	31.97	31.02	32.21
C16:1	13.74	9.42	10.05	-
C16:2	1.75	1.97	2.13	1.68
C16:3	-	-	-	7
C16:4	5.27	5.16	5.32	5.08
C18:0	0.99	0.9	0.61	0.94
C18:1	36.95	37.42	46.22	37.7
C18:2	8.36	8.78	-	9.84
C18:4	2.96	3.19	3.33	4.03
C22:0	-	0.59	0.69	0.84
∑ SFA	**30.97**	**34.06**	**32.95**	**34.67**
∑ MUFA	**50.69**	**46.84**	**56.27**	**37.70**
∑ PUFA	**18.34**	**19.10**	**10.78**	**27.63**

E-ISSN 2355-9926

Investigations showed that the most common feedstocks suitable for biodiesel production were enriched in the six most common C16-C18 fatty acids, namely, palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids (Knothe, 2009; Schenk et al., 2008). The data in Table 2 showed that the microalgae strain treated with 4 variations of light intensity possessed significant amounts of C16 and C18 species, around 98% to 100%. Overall, in the microalgal oil of *Choricystis* sp. LBB13-AL045, oleic acid (C18:1) was the most common of the FAs, ranging from 36.95% to 46.22%. High amounts of oleic acid (C18:1) contained in *Choricystis* sp. LBB13-AL045 indicated its potency to be used as biodiesel feedstock (Nascimento et al., 2013). Moreover, the algal oil contained PUFAs at low percentage, less than 20% except for the microalgal oil from the treatment with highest light intensity (675 µmol m^{-2} s^{-1}). Palmitic acid (C16:0) appeared to be the second most common FA in the microalgal oil treated with varied light intensity, followed by palmitoleic acid (C16:1) in the third place. Palmitoleic acid content in the microalgal oil would be benefited due to the biodiesel produced would possess properties of low oxidative potential, high cetane number (CN) and good cold flow characteristics (Nascimento et al., 2013). At highest light intensity treatment (675 µmol m^{-2} s^{-1}) used in this study, palmitoleic acid content was absent and linoleic acid (C18:2) was increased concomitantly. It would contribute to the escalation of total PUFAs percentage and reduction of total MUFAs percentage (Figure 2), which eventually would negatively influence the properties of the biodiesel. Fatty acid composition could significantly influence biodiesel fuel properties. It is difficult to describe clearly the suitability of fatty acid profiles due to the diversity and conflicting impacts of fatty acid profiles on biodiesel properties. Hence, an analysis of fatty acid profiles is needed to evaluate biodiesel fuel properties.

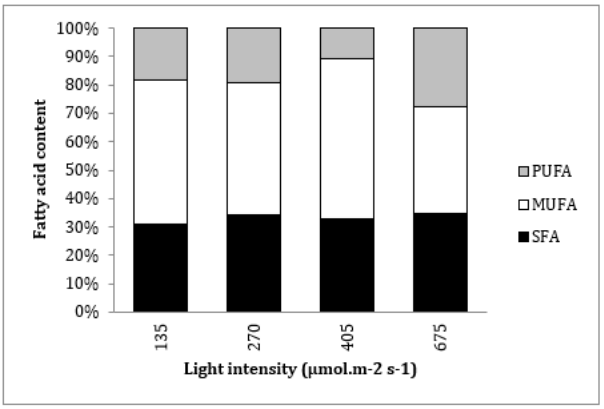

Figure 2. The percentage of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in fatty acid compositions of *Choricystis* sp. LBB13-AL045 microalgal oil cultivated under different conditions of light intensiity. SFA = saturated fatty acids (14:0, 16:0, 18:0, 22:0); MUFA = monounsaturated fatty acids (16:1, 18:1); PUFA = polyunsaturated fatty acids (16:2, 16:3, 16:4, 18:2, 18:4).

Biodiesel's properties from microalgal oil

Table 3 gives comparison of seven properties of biodiesel from *Choricystis* sp. LBB13-AL045 microalgal oil cultivated under different conditions of light intensity, SNI biodiesel (Indonesia), ASTM biodiesel (America), and EN biodiesel (Europe) standard. Six important biodiesel properties besides average degree of unsaturation (ADU) of the algal oil produced under different light regimes were analyzed. The biodiesel property of average degree of unsaturation was proved to have high correlation with several other properties, such as higher ADU leads to lower cetane number and poorer oxidation stability, but improved low temperature performance. Six important biodiesel properties were computed based on the relationships built across a range of realistic biodiesel types (Hoekman et al., 2012). With respect to degree of unsaturation of fatty acids, the only estriction

E-ISSN 2355-9926

Table 3. Comparison of seven properties of biodiesel from *Choricystis* sp. LBB13-AL045 microalgal oil, SNI biodiesel, ASTM biodiesel and EN biodiesel standard.

Property	Light Intensity (µmol.m^{-2} s^{-1})				Biodiesel[a]	SNI 7182:2015	ASTM D6751-08	EN14214
	135	270	405	675				
Kinematic viscosity 40°C (mm^2.s^{-1})	4.55	4.56	4.61	4.46	4-5	2.3 – 6.0	1.9 – 6.0	3.5 – 5.0
Specific gravity (kg L^{-1})	0.878	0.878	0.878	0.879	0.87-0.89	0.85–0.89	0.85-0.90	-
Cloud point (°C)	6.13	6.41	7.32	4.21	-	max 18.00	-	-
Cetane number	56.0	56.1	56.6	55.0	45-55	min 51	min 47	min 51
Iodine number (gI_2/100 g)	89.9	88.4	83.3	100.6	-	max 115	-	max 120
HHV (MJ/kg)	40.4	40.3	40.2	40.6	38-41	-	-	-
Avg. unsaturation	1.04	1.02	0.95	1.18	0.6-1.6	-	-	-

[a] The data about biodiesel were taken from published literature as indicated in the text. Average degree of unsaturation (ADU) computed from compositional profiles in Table 2. Kinematic viscosity, specific gravity, cloud point, cetane number, iodine value, and higher heating value (HHV) computed from relationships between biodiesel ADU and the properties in Section Materials and Methods (Estimation of biodiesel fuel properties based on FAME profiles).

is the European biodiesel standard EN 14214, which stipulates a maximum acceptable limit of 12% for linolenic acid (C18:3) and 1% for fatty acids with more than three double bonds (Francisco et al., 2010). In this study, it is noteworthy that the algal oil obtained from four treatments of light intensity did not contain linolenic acid and had slightly higher levels of PUFAs (more than three double bonds) compared to the European biodiesel standard.

According to three quality standards for biodiesel, SNI 7182:2015 in Indonesia, ASTM D6751-08 in the US and EN 14214 in Europe, and the range of qualities occurring in common biodiesel feedstocks (Hoekman et al., 2012), the values of kinematic viscosity, specific gravity (fuel density), cloud point (definite only in SNI biodiesel standard), cetane number and iodine number of the algal oil obtained from four treatments of light intensity almost satisfied the specifications. Average degree of unsaturation of all algal oil produced in this study were in range of common biodiesel feedstock (Table 3). Therefore, all estimated biodiesel properties were satisfied the three biodiesel standards.

Among three biodiesel standards, only SNI definite the specification of cloud point (CP) which was stipulated at the maximum acceptable limit of 18°C. Cloud point is the temperature at which the least soluble biodiesel component crystallizes from solution. Low temperature performance is one of the most important properties for biodiesel. Poor low temperature performance may result in filter plugging due to wax formation, and engine starving due to reduced fuel flow. In general, poor cold flow properties result from the presence of long chain, saturated FAME present in biodiesel (Hoekman et al., 2012). In this study, the results showed that the estimated cloud point for microalgal biodiesels varied from 4.21°C to 7.32°C. The microalgal oil obtained from the treatment of light intensity at 405 µmol.m^{-2} s^{-1} showed the highest cloud point, but still in range of SNI specification, may due to its high SFA and low PUFA content simultaneously.

E-ISSN 2355-9926

The iodine number is an indicating parameter of the degree of unsaturation involving the weighted sum of the masses of MUFA and PUFA, important for the biodiesel oxidative stability (Nascimento et al., 2013). According to Wang et al. (2012), iodine number greatly influences the deposit formation in diesel engine injectors. Islam et al. (2013) reported that the lower the iodine number of biodiesels leads to the higher cetane number, which means better quality of the biodiesel, and vice versa. The lower iodine number is required to reduce the emission of nitrogen oxides from the engine (Altun, 2014)**.** The results of this study indicated that microalgal oil derived from the light intensity of 675 µmol m^{-2} s^{-1} showed the highest iodine number 100.6 gI_2/100 g, while the microalgal oil obtained from the light intensity of 405 µmol.m^{-2} s^{-1} showed the lowest iodine number at 83.3 gI_2/100 g. The biodiesel standard, SNI and EN, stipulated a maximum acceptable limit of iodine number at 115 and 120 gI_2/100 g, respectively.

The cetane number (CN) is the ability of the fuel to ignite quickly after being injected and a higher value indicates a better ignition quality of the fuel (Abbasi & Diwekar, 2014). CN is one of the important critical parameters in the selection of biodiesel feedstock (Chuah et al., 2016). CN decreased with an increase of ADU and it indicated that the more unsaturation present in the oil, the less CN (Demirbas, 2005). In this study, the CN varied within a narrow range of 55.0-56.6 for microalgal biodiesels from *Choricystis* sp. LBB13-AL045 cultivated under different light intensity. Microalgal culture cultivated under 405 µmol m^{-2} s^{-1} of light intensity showed the highest CN may due to its higher MUFA and SFA content, while microalgal culture with 675 µmol m^{-2} s^{-1} of light intensity had the lowest CN due to its highest content of PUFA among other treatments.

Higher heating value (HHV) is a measure of the energy produced when the fuel is burned completely, which also determines the suitability of biodiesel as an alternative diesel fuel. Neither the Indonesian, US nor European biodiesel standards include a specification for heating value, but in EN 14213 (biodiesel for heating purpose) a minimum of 35 MJ/kg is required (Arias-peñaranda et al., 2013). The HHV value of biodiesel produced from the oil of *Choricystis* sp. LBB13-AL045 was within narrow range of 40.2 to 40.6 MJ/kg. Biodiesel produced from the microalgae cultivated under 675 µmol m^{-2} s^{-1} possessed highest value of HHV due to its highest PUFA content.

Taken together, according to three quality standards for biodiesel, SNI 7182:2015 in Indonesia, ASTM D6751 in the US and EN 14214 in Europe, and the range of qualities occurring in common biodiesel feedstocks (Hoekman et al., 2012), the values of kinematic viscosity, specific gravity, cloud point, iodine number, cetane number and HHV of the *Choricystis* sp. LBB13-AL045's biodiesel derived from varied conditions of light intensity completely satisfied the specifications. The microalgae strain of *Choricystis* sp. LBB13-AL045 cultivated under 405 µmol m^{-2} s^{-1} of light intensity produced the best oil in terms of fatty acids profile to be utilized as biodiesel feedstock. While microalgal biodiesel of *Choricystis* sp. LBB13-AL045 derived from cultivation under 675 µmol m^{-2} s^{-1} of light intensity showed the least quality of biodiesel properties among others, however, all of its values still within the limits established by the standards related to biodiesel quality.

Conclusion

Choricystis sp. LBB13-AL045 isolated from Bengkulu province, Indonesia, was potential to be used as biodiesel feedstock due to its high lipid productivity and favourable biodiesel properties when cultivating under high light intensity, regarding Indonesia as tropical

E-ISSN 2355-9926

country with abundant sunlight throughout the year. Among the four treatments of light intensity, the cultivation of *Choricystis* sp. LBB13-AL045 under 405 µmol.m^{-2} s^{-1} of light intensity showed the best lipid productivity and also the highest quality of biodiesel. At such condition, lipid productivity was 46.13 ± 3.43 mg L^{-1} day^{-1} and biodiesel properties of higher cetane number (56.6) and lower iodine number (833.3) could be achieved.

Acknowledgements

The authors gratefully acknowledged to technician in Bioenergy and Bioprocess Laboratory, Research Centre for Biotechnology, LIPI (Ms. Fitriyani) for her good assistance and also for all laboratory members for their great support.

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