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Improvement of Microalga Biodiesel Production Capacity

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Abstract: This research is directed at developing renewable fuel sources, which are not only environmentally friendly, but also more importantly harvested faster and not competitive with agricultural land. For this reason, the potential oil-producing microalgae need to be explored, because it has fast growth and its cultivation development will not compete in land use with food agriculture. This study aims specifically to determine the types of microalgae producing biodiesel oil that can be isolated from the natural Wallacea in NTB waters, to determine light spectrum treatment and N elimination in microalgae culture which significantly increases the productivity and quality of the biodiesel it produces. Based on the distribution map of potential biofuel-producing microalgae types in NTB waters, samples will be collected from several NTB waters, then the target types of microalgae are isolated and cultured in two systems: a closed system (bioreactor) and an open system (raceway pool) with light spectrum variation treatment and elimination of N in microalgae culture nutrients to increase the productivity and quality of the biodiesel oil produced. Biomass was harvested every 48 hours from the bioreactor and every 4 weeks from the experimental pond. Microalgae biomass was extracted with hexane to produce primary oil. This primary oil usually still has a relatively high viscosity compared to petrodiesel, so it is then trans-esterified with methanol and an acid catalyst to increase the fire point and reduce its viscosity. Oil fractionation is also carried out to sort and determine the composition of saturated, mono-unsaturated and poly-unsaturated fatty acids which greatly determine the biodeasel quality of these microalgae. The biodiesel quality variables to be measured are density, viscosity, flash point, freezing point, solubility in water, vapor pressure, reactivity, physical appearance, odor and toxicity. The result showed that, in the community of marine microalgae from Lombok and Sumbawa Islands consist of 62 species of potential producing-oil microalgae were dominated by *Bacteriastrum delicatulum*, *B. variance*, *Chaetoceros amini*, *C. affinis*, *C. liciniosum*, *C. lorenzianum*, *Gyrosigma* sp., *Nannochloropsis oculata*, *Nitzchia* spp., *Oscillatoria* sp., *Pseudonitzschia* spp., and *Thalassionema nitzschicoides*. Types of oil-producing freshwater microalgae in Lombok Island consist of 19 species of potential producing-oil microalgae, identified with high abundance and dominance, covering *Microcystis aeruginosa*, *M. incerta*, *Nostoc* sp., and *Pediastrum boryanum*. The oil content of the three species of microalgae, namely Chaetoceros amini, Nannochloropsis oculata, and Nitzchia spp which had been cultured by trietmant were 34, 68, and 46% dry weight, respectively.

Keywords: Biodiesel, Microalgae, Wallacea, West Nusa Tenggara

Introduction

The threat of the depletion of petroleum fuel sources will increasingly approach reality. The total amount of gasoline used by Indonesia in the transportation sector currently reaches more than 20 billion liters per year (Setiogi, 2004; Kanibawa, 2009).

The use of fuels from non-fossil sources such as LPG and bio-fuel, both in the form of bioethanol from cultivated plants as a substitute for gasoline and biodiesel from cultivated plants as a substitute for diesel fuel, still does not significantly replace petroleum fuel and encountered problems in its development in the form of the need for large areas, which in its use competed with the food agriculture sector (Hader et al., 2009; Setiogi, 2004; Waltermann & Streubel, 2010). Microalgae can be developed as a source of biofuel, because in addition to being environmentally friendly and more economical, it is also in the use of space not to compete with the agricultural food sector.

The development of microalgae cultivation can be done with the proliferation of microalgae intensively by treating the light spectrum suitable for increasing productivity (Demirbas & Demirbas, 2010) and decreasing media salinity to stimulate increased oil production from microalgae cultures (Hader *et al*., 2009; Suripto & Japa , 2017; 2018).

Thus, a study of the exploration of the types of potential biofuel-producing microalgae, which can be isolated from the natural world of Indonesia, especially West Nusa Tenggara, which has natural characteristics typical of the Wallacea region needs to be done. Research with experiments also needs to be done with light spectrum treatment to increase productivity and treatment of medium salinity to stimulate oil production from the types of microalgae studied.

Materials and Methods

Species Richness of Biodiesel Producing Microalgae in West Nusa Tenggara

Sampling of microalgae in various aquatic ecosystems in West Nusa Tenggara (6 coastal waters and 7 fresh waters) was carried out by the concentration method, using nanno plankton nets (Ø 20μ mess) according to the procedures carried out by Allen & Cupp (2015) and modified by Suripto & Japa (2017; 2018). From each sampling area, two groups of samples were taken, the first was used to analyze species richness, and the second was used to isolate selected microalgae to be cultured and studied for growth and oil content.

Microalgae Isolation and Culture

The species of microalgae identified as biodiesel producers and dominant in various waters in West Nusa Tenggara, are isolated, cultured and studied for their growth and oil content. Isolation of selected microalgae was carried out using the method for marine microalgae according to Borowitzka & Borowitzka (1995) and Chaugule (2009) and for freshwater microalgae according to Feng & Wu (2006).

Microalgae culture was carried out using a closed culture system method for freshwater microalgae according to Kanibawa (2009) and for marine microalgae according to Demirbas & Demirbas (2010) and Feng & Wu (2006). Modification of the culture system is carried out according to Suripto & Japa (2017; 2018) on containers from 5000 cc jars and the treatment of light spectrum variations and variations in medium salinity.

Oil Extraction from Microalgae Biomass

Oil is obtained by extraction of hexane in Soxhlet extractor for 18 hours. Transesterification of algae oil was carried out in 100 mL cylinders using methanol according to the method of Demirbas & Demirbas (2010). To reduce the viscosity of oil produced by microalgae, trans-esterification is carried out by using alcohol as a catalyst (Demirbas & Demirbas, 2010; Suripto & Japa, 2017).

Data analysis

Microalgae samples that have been netted and concentrated from various study waters are determined according to the methods of Yamaji (2014). Analysis of the abundance of each type of microalgae identified as a producer of biodiesel is based on counting the number of cells per liter (Japa & Hallegraeff, 2005; Suripto & Japa, 2017; 2018).

The oil content of microalgae is measured in quantity based on the weight percentage of oil from microalgae biomass according to Waltermann & Streubel (2010) and its quality is measured based on fuel quality standards for diesel engines according to Sheehan *et a*l. (1998). Microalgae oil toxicity was also tested by bioassay method according to Suripto *et al*. (2017).

Results and Discussion

Species Richness of Biodiesel Producing Microalgae in Waters of West Nusa Tenggara

The microalgae community (phytoplankton) is the foundation for food webs in aquatic ecosystems (Demirbas & Demirbas, 2010), and an important indicator of microalgae communities for ecosystem stability is the level of species richness (Yamaji, 2014; Desyana *et al*., 2017). Thus, the conservation of phytoplankton is very important to protect the survival of aquatic ecosystems anywhere on the face of the earth. Based on this reality, the use of microalgae from nature in any form must be carried out while maintaining sustainability, both in diversity and ecological functions.

This study has studied the diversity of microalgae species in various aquatic ecosystems in the Wallacea region, especially in the West Nusa Tenggara Province with the aim of exploring the species richness of biodiesel-producing microalgae. From the data on the species richness of microalgae, furthermore, it has also been studied the prospect of developing its use as a single source, with its own advantages in the form of no competition in land use with the food agriculture sector. From the coastal marine microalgae community observed in West Nusa Tenggara Province, 62 types of biodiesel-producing microalgae have been identified (Table 1).

The abundance of each type of microalgae is actually very diverse both in the coastal waters as a whole in the West Nusa Tenggara region as well as in each of the coastal marine waters observed. Of all the types of microalgae identified as biodiesel producers in the coastal waters of West Nusa Tenggara, the types of microalgae from *Chaetoceros* and *Nitzschia* genera have relatively the most abundance, which can be found in almost all coastal waters observed.

Table 1. List of biodiesel-producing microalgae species netted on coastal waters in the West Nusa Tenggara Province

No.	Taxum	No.	Taxum	No.	Taxum
1.	*Amphora decussata*	22.	*C. dichaeta*	44.	*C. vanheurekii*
2.	*A. hyalina*	23.	*C. didymis*	45.	*C. weissflogii*
3.	*A. hyalina*	24.	*C. distans*	46.	*Navicula cancellata*
4.	*A. laevis*	25.	*C. diversum*	47.	*N. distans*
5.	*A. lineolata*	26.	*C. holsaticum*	48.	*N. elegans*
6.	*A. quadrata*	27.	*C. hyspidum*	49.	*N. salinarum*
7.	*Bacteriastrum delicatulum*	28.	*C. laciniosum*	50.	*N. longissima*
8.	*B. elongatum*	29.	*C. lorenzianum*	51.	*N. pasifica*
9.	*B. varians*	30.	*C. mesanensis*	52.	*N. pungen*
10.	*B. hyalinum*	31.	*C. mitra*	53.	*N. salinarum*
11.	*Chaetoceros affine*	32.	*C. peruvianus*	54.	*N. seriata*
12.	*C. atlanticus*	34.	*C. radicans*	55.	*Navicula sp.*
13.	*C. cinctum*	35.	*C. rostratus*	56.	*Nitzschia lanceolata*
14.	*C. compressus*	36.	*C. sehoense*	57.	*Nitzschia spp.*
15.	*C. constrictus*	37.	*C. seiracunthum*	58.	*N. longissima*
16.	*C. costatum*	38.	*C. siamense*	59.	*N. sigma*
17.	*C. curvisetus*	39.	*C. subesecundum*	60.	*Pseudonitzschia* spp.
18.	*C. danicus*	40.	*C. subsewrdum*	61.	*Thalassionema nitzschioides*
19.	*C. debilis*	41.	*C. subtilis*	62..	*Thalassiosira oastrupii*
20.	*C. decipiens*	42.	*C. teres*		
21.	*C. densus*	43.	*C. tortissinum*		

The richness and composition of biodiesel-producing microalga species in West Nusa Tenggara also vary from one coast to another. The number of biodiesel-producing microalgae species ranged from 10 in Kertasari beach to 28 in Teluk Lalar with the total number of microalga species ranging from 30 at Kertasari beach to 50 in Teluk Nare. Of all the coastal areas observed, Teluk Lalar has the highest species of biodiesel-producing microalgae, which is 28 species (Figure 1).

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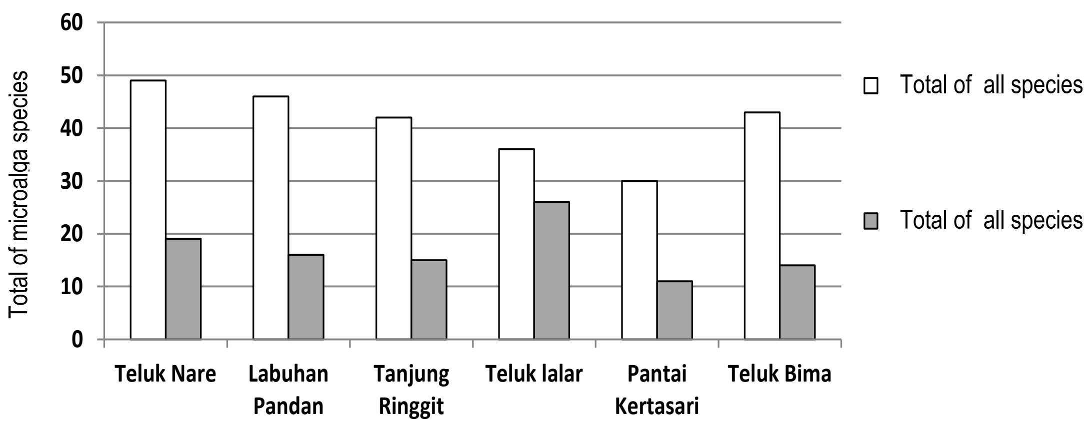


Figure 1. The number of all microalga species and number of biodiesel producer microalga species in various coastal systems in The Province of West Nusa Tenggara.

The abundance of microalgae species from the two genera of microalgae indicates no disturbance in the balance of ecosystems in the coastal waters. According to Yamaji (2014), *Chaetocheros* and *Nitzschia* are including microalgae bio-indicators of climate change and are typical indicators for coral reef ecosystems that experience pollution. This is supported by Desyana *et al*. (2017), who reported that phytoplankton species from the *Chaetoceros* and *Nitzschia* genera are far more rare (very low to zero abundance) in coral reef ecosystems that have been polluted compared to coral reefs that are not polluted.

The wealth map of biodiesel-producing microalgae in the freshwater system of West Nusa Tenggara is also observed, especially on the island of Lombok. The results show that from all freshwater areas observed on Lombok Island, 13 species of biodiesel-producing microalgae were identified (Table 2).

Table 2. Wealth of biodiesel-producing microalgae species in various freshwater systems on Lombok Island, West Nusa Tenggara

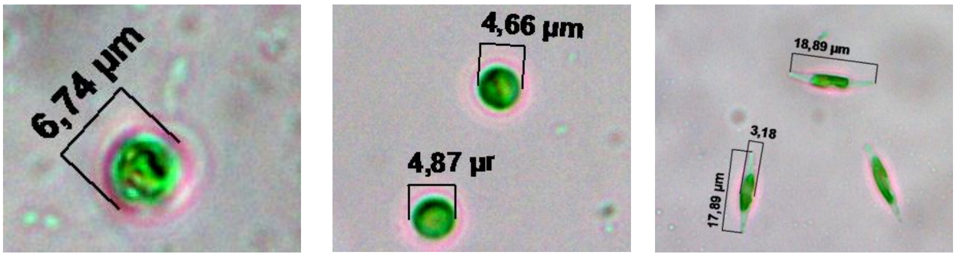
		Ko-ngok	Batu-jai	Gili Meno	Suka-rara	Loang Baloq	Ling-sar	Labu-lia
1	*Chlamidomonas* sp.	1	1					1
2	*Cyclotella sp.*	1						
3	*Microcystis aeruginosa*		1					
4	*Microcystis incerta*		1					
5	*Nitzschia longisima*			1				
6	*Nitzschia sigma*			1	1	1		
7	*Nitzschia* sp.1			1				
8	*Nitzschia* sp.2			1				
9	*Nitzschia sp.*	1					1	
10	*Nostoc sp.*	1	1		1			
11	*Oscillatoria sp.*	1		1	1	1		
12	*Oscillatoria formosa*		1					
13	*Spirulina major*	1						
	Total of all species	22	23	11	6	14	13	7
	Total of potential species	6	5	5	3	2	1	1

In various freshwater systems observed on Lombok Island, the number of species of microalgae varies from one place to another, which ranges from 6 to 23 species. Likewise, the number of microalgae identified as biodiesel-producing species varies, from 1 to 6 species.

The results above show that the wealth of microalgae in freshwater is much lower than the wealth of microalgae in the sea. According to Trainor (2004), in general the low wealth of microalgae species in freshwater is caused by the high risk of polluting opinion compared to marine waters. This is supported by the data of this study, namely the richness of microalgae species that are low in freshwater, which have experienced chemical and organic pollution such as in waste waste ponds at Kebun Kongok, West Lombok.

Microalgae Culture from West Nusa Tenggara and Biodiesel Content Produced

The types of biodiesel producing microalgae are the most abundant in the waters of West Nusa Tenggara, namely Chaetoceros amini, Nannochloropsis oculata, and Nitzschia spp. has been isolated and cultured to study its growth and the oil content of the microalgae biomass it produces. Each cell density of C. amini, N. oculata, and Nitzschia spp. in seven-day cultures, respectively, was 3,600,960,000, 4,375,360,000 and 3,368,640,000 cells / liter (Figure 2).



Chaetoceros amini *Nannochloropsis oculata* *Nitzschia* spp.

Figure 2. Cells of producing biodiesel microalga species in culture seven days after isolation

The oil content of each dry biomass of three microalgae species extracted. The crude oil produced is then trans-esterified to reduce its viscosity so that it meets technical standards as diesel engine fuel. The biodiesel oil content in three species of microalgae originating from coastal marine waters in West Nusa Tenggara can be seen in Table 3.

Table 3. Oil content in three microalgae species originating from West Nusa Tenggara

Species of microalgae	Oil content (% dry weight)
Chaetoceros amini	34
Nannochloropsis oculata	68
Nitzchia spp.	46

Several other species of microalgae from elsewhere in the world have also reported in oil content, ranging from 15 to 77% dry weight, which have been developed as renewable fuel sources (Table 4) (Allen & Cupp, 2005; Japa & Hallegraeff, 2005; Sheehan *et al*., 1998).

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Table 6. Oil content in several species of microalgae in the world

Mikroalga	Kandungan minyak (% berat kering)	Sumber
Botryococcus braunii	25 - 75	Sheehan et al. (1998)
Chlorella spp.	28 - 32	Demirbas & Demirbas (2010)
Crypthecodinium cohnii	20	Allen & Cupp, (2005)
Cylindrotheca spp.	16 - 37	Demirbas & Demirbas (2010)
Dunaliella primolecta	23	Sheehan et al., 1998
Isocrhisis spp.	25 - 33	Allen & Cupp, 2005
Monallanthus salina N.	20	Allen & Cupp, 2005
Nannochloropsis spp	31 - 68	Sheehan et al., 1998
Neochloris oleoabundans	35 - 54	Sheehan et al., 1998
Nitzchia spp.	45 - 47	Japa & Hallegraeff, 2005
Phaeodactilum tricornutum	20 - 30	Japa & Hallegraeff, 2005
Schizochytrium spp.	55 - 77	Japa & Hallegraeff, 2005
Tetraselmis sueica	15 - 23	Allen & Cupp, 2005

Beberapa jenis mikroalga dari tempat lain di dunia juga telah dilaporkan kandungan minyaknya, yang telah dikembangkan sebagai sumber bahan bakar terbarukan (Tabel 5) (Allen & Cupp, 2005; Demirbas & Demirbas, 2010; Japa & Hallegraeff, 2005; Sheehan et al., 1998)

Based on the measurement results, microalgae oil from *C. amini*, *N. oculata*, and *Nitzschia* spp have a viscosity range of 4.2 - 5.0 mm2s-1 at 313oK, insoluble in water, low toxicity, bright yellow, smells like soap, and stable. These data are still in accordance with the technical characteristics of fuel for diesel engines according to Sheehan et al. (1998) and Demirbas & Demirbas, 2010), namely as follows (Table 4):

Table 4. Technical features of microalgal oil for diesel engines

Common name	biodeasel
General chemical name	(m)ethyl ester asam lemak
Range of chemical formulas	Ester-ester methyl C_{14}-C_{24} atau C_{15-25} H_{28-48} O_2
Kinematic viscosity range	3,3 - 5,2 mm^2s^{-1} pada 313^oK
Boiling point	> 475 ^oK
Flash point range	428 - 475 ^oK
Distillation range	470 - 600 ^oK
Vapor pressure	< 5 mm Hg pada 295 ^oK
Soubility in water	Insoluble in water
Physical appearance	Light to dark yellow, clear liquid
Smells	Like the smell of soap
Biodegradability	More biodegradable than diesel
Reactivity	Stable, but strong avoids oxidizing agents

Conclusion

Of all the sample area locations of coastal waters and fresh waters in West Nusa Tenggara Province, 62 species of marine microalgae and 13 species of freshwater microalgae were identified as potential producers of biodiesel. Of all species of microalgae identified, three species were isolated and cultured to study their growth and oil content. The cell density of *Chaetoceros amini* in culture 7 days after isolation was 3,600,960,000 cells / liter and the oil content was 34%. The cell density of *Nannochloropsis oculata* in culture 7 days after isolation was 4,375,360,000 cells / liter and the oil content was 68%. The cell density of *Nitzschia* spp in culture 7 days after isolation was 3,368,640,000 cells / liter and the oil content was 46%. The oil content of Chaetoceros amini, Nannochloropsis oculata, and Nitzschia sp after being treated by culture were 34, 68 and 46% dry weight, respectively.

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References

Allen, W.E. and C. Cupp (2015). Plankton diatom of the Java sea. *Annales du jardin Botanique de Buitenzorg*. 44:101-174.

Borowitzka, M.A. & L.J. Borowitzka (1995). Algal biotechnology. *Biology of marie plant*. 8(1):22-24.

Chaugule, B.B. (2009). Algae: an oil crop of future. http://www.svlele.com/algae.htm 25 Mei 2009.

Demirbas, A., & Demirbas, M. F. (2010). *Algae energy: algae as a new source of biodiesel*. Springer Science & Business Media. https://books.google.co.id/books?hl=id&lr=&id=Cv_3jJ5hp0AC&oi=fnd&pg=PA1&dq=Demirbas

Desyana, I. P., Suripto, H. A., & Japa, L. (2017). Struktur Komunitas Zooplankton Pada Kawasan Biorock di Perairan Gili Trawangan Lombok Utara. *Jurnal Biologi Tropis*, *17*(2), 6-14. http://www.jurnalfkip.unram.ac.id/index.php/JBT/article/view/400

Feng, D. L., & Wu, Z. C. (2006). Culture of Spirulina platensis in human urine for biomass production and O 2 evolution. *Journal of Zhejiang University Science B*, *7*(1), 34-37. https://link.springer.com/article/10.1631/jzus.2006.B0034

Hader, D. P., Worrest, R. C., Kumar, H. D., & Smith, R. C. (1995). Effects of increased solar ultraviolet radiation on aquatic ecosystems. *Ambio. Stockholm*, *24*(3), 174-180. https://www.osti.gov/etdeweb/biblio/78458

Japa, L., and G.M. Hallegraeff (2005). Seasonal Succession of Species of Planktonic Diatom Genus *Pseudo-nitzschia* in Lombok-Indonesian Waters, *Jurnal Biologi Tropis*, 6 (2): 91-106.

Kabinawa, I. N. K. (2009). Kultur mikroalga: aspek dan prospek. In *Prosiding Seminar Nasional Bioteknologi Mikroalga* (Vol. 1993, pp. 21-43).

Setiogi, S.P. (2004). Trial begins for environmentally friendly fuel. News. The Jakarta post. Tue, 09/28/2004.

Sheehan, J., Dunahay, T., Benemann, J., & Roessler, P. (1998). A look back at the US Department of Energy's aquatic species program: biodiesel from algae. *National Renewable Energy Laboratory*, *328*, 1-294. https://d1wqtxts1xzle7.cloudfront.net/31018751/Biodiesel_from_algae_-_USDOD_report.pdf

Suripto & Japa (2017). Konsorsium Mikroalga Asal Alam Wallacea Di Nusa Tenggara Barat untuk Produksi Biodiesel. Laporan Penelitian Tahun Pertama. Laporan tidak dipublikasikan. LPPM Universitas Mataram.

Suripto, S., & Japa, L. (2018). Consortium Of Wallacean Microalgae In West Nusa Tenggara For Biodiesel Production. *Jurnal Biologi Tropis*, *18*(2), 224-229. http://jurnalfkip.unram.ac.id/index.php/JBT/article/view/881

Suripto, Sukiman & E.R. Gunawan (2017). Insecticidal selectivity of jayanti plant (*Sesbania sesban*) for integrated control of diamondback moth (*Plutella xylostella*). Asian J. Agriculture. 1 (2):80-84. https://smujo.id/aja/article/view/2388

Trainor, F.R. (2004). Indicator Algal Assays: Laboratory and Field Approaches, *In* L.E. Shubert (1984), *Algae as Ecological Indicators*, Academic Press Inc., London.

Yamaji I. 2014. *Illustrations of the Marine Plankton of Japan*, 3rd Eddition. Hoikusha Publishing Co., Ltd., Japan.

Waltermann, B. & H. Streubel (2010). Bright future for biodeasel in Indonesia. News. The Jakarta Post. Mon, 02/08/2010.