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Consortium Of Wallacean Microalgae In West Nusa Tenggara For Biodiesel Production

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Abstract : This study aims to explore the species richness of biodiesel-producing microalgae in the waters of West Nusa Tenggara to develop renewable energy source, environmentally friendly, and not compete in land use with the agricultural sector. Microalga samples were collected from various waters in West Nusa Tenggara Province using the concentration method. Species determination and data analysis of microalgae was carried out at the Biology Laboratory, University of Mataram to calculate the abundance of each species. The dominant species of biodiesel-producing microalgae were isolated and cultured by a closed culture system method. The oil content of the microalgae biomass produced was extracted by a liquid-liquid extraction method using n-hexane as a solvent. The results show that of the 49 species of marine microalgae identified in the waters of West Nusa Tenggara, there are 12 species of microalgae which are biodiesel producers. Of the 30 freshwater microalga species, only 8 species of microalgae are producers of biodiesel. The microalgae community of marine waters of West Nusa Tenggara is dominated by *Bacteriastumdelicatulum*, *B. variance*, *Chaetocerosaffinis*, *C. liciniosum*, *C. lorenzianum*, *Gyrosigma* sp., *Oscillatoria* sp., *Pseudonitzschia* spp., and *Thalassionemanitzschicoides*, while freshwater microalgae communities are dominated by *Microcystis aeruginosa*, *M. incerta*, *Nostoc* sp., *Pediastrumboryanum*, and *Staurastumcristatum*. The dominant types of biodiesel-producing microalgae isolated and cultured are *Chaetocerosamini*, *Nannochloropsisoculata* and *Nitzschia* spp. Each cell density of *C. amini*, *N. oculata*, and *Nitzschiaspp* in culture 7 days after isolation was 3,600,960,000, 4,375,360,000, and 3,368,640,000 cells / liter respectively with oil content 34, 68, and 46%.

Key words: 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 bio-ethanol 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 (\varnothing 20 μ 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 al.* (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. | <i>Amphora decussata</i> | 22. | <i>C. dictyota</i> | 44. | <i>C. vanheurekii</i> |
| 2. | <i>A. hyalina</i> | 23. | <i>C. didymis</i> | 45. | <i>C. weissflogii</i> |
| 3. | <i>A. hyalina</i> | 24. | <i>C. distans</i> | 46. | <i>Navicula cancellata</i> |
| 4. | <i>A. laevis</i> | 25. | <i>C. diversum</i> | 47. | <i>N. distans</i> |
| 5. | <i>A. lineolata</i> | 26. | <i>C. holsaticum</i> | 48. | <i>N. elegans</i> |
| 6. | <i>A. quadrata</i> | 27. | <i>C. hysspidum</i> | 49. | <i>N. salinarum</i> |
| 7. | <i>Bacteriastrium delicatulum</i> | 28. | <i>C. laciniosum</i> | 50. | <i>N. longissima</i> |
| 8. | <i>B. elongatum</i> | 29. | <i>C. lorenzianum</i> | 51. | <i>N. pastifica</i> |
| 9. | <i>B. varians</i> | 30. | <i>C. mesanensis</i> | 52. | <i>N. pungen</i> |
| 10. | <i>B. hyalinum</i> | 31. | <i>C. mitra</i> | 53. | <i>N. salinarum</i> |
| 11. | <i>Chaetoceros affinis</i> | 32. | <i>C. peruvianus</i> | 54. | <i>N. seriata</i> |
| 12. | <i>C. atlanticus</i> | 34. | <i>C. radicans</i> | 55. | <i>Navicula sp.</i> |
| 13. | <i>C. cinctum</i> | 35. | <i>C. rostratus</i> | 56. | <i>Nitzschia lanceolata</i> |
| 14. | <i>C. compressus</i> | 36. | <i>C. sehoense</i> | 57. | <i>Nitzschia spp.</i> |
| 15. | <i>C. constrictus</i> | 37. | <i>C. seiracanthum</i> | 58. | <i>N. longissima</i> |
| 16. | <i>C. costatum</i> | 38. | <i>C. siamense</i> | 59. | <i>N. sigma</i> |
| 17. | <i>C. curvisetus</i> | 39. | <i>C. subsecundum</i> | 60. | <i>Pseudonitzschia spp.</i> |
| 18. | <i>C. danicus</i> | 40. | <i>C. subseawardum</i> | 61. | <i>Thalassionema nitzschioides</i> |
| 19. | <i>C. debilis</i> | 41. | <i>C. subtilis</i> | 62. | <i>Thalassiosira oestrupii</i> |
| 20. | <i>C. decipiens</i> | 42. | <i>C. teres</i> | | |
| 21. | <i>C. densus</i> | 43. | <i>C. tortissimum</i> | | |

The richness and composition of biodiesel-producing microalgae 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 TelukLalar with the total

number of microalga species ranging from 30 at Kertasari beach to 50 in TelukNare. Of all the coastal areas observed, TelukLalar has the highest species of biodiesel-producing microalgae, which is 28 species (Figure 1).

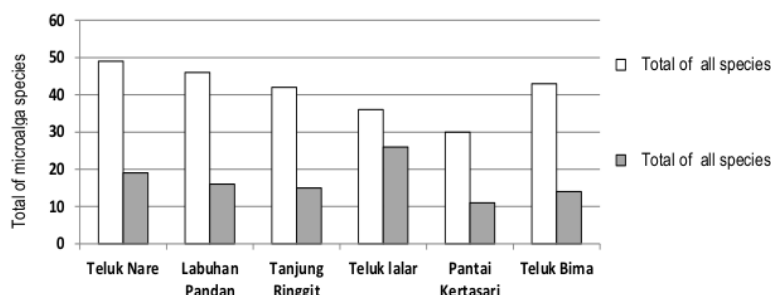


Figure 1. The number of all microalga species and number of biodiesel producer microalga species in various coastal systems in The Province of West Nusa Tenggara.

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 | <i>Chlamidomonas</i> sp. | 1 | 1 | | | | | 1 |
| 2 | <i>Cyclotella</i> sp. | 1 | | | | | | |
| 3 | <i>Microcystis aeruginosa</i> | | 1 | | | | | |
| 4 | <i>Microcystis incerta</i> | | 1 | | | | | |
| 5 | <i>Nitzschia longissima</i> | | | 1 | | | | |
| 6 | <i>Nitzschia sigma</i> | | | 1 | 1 | 1 | | |
| 7 | <i>Nitzschia</i> sp.1 | | | 1 | | | | |
| 8 | <i>Nitzschia</i> sp.2 | | | 1 | | | | |
| 9 | <i>Nitzschia</i> sp. | 1 | | | | | 1 | |
| 10 | <i>Nostoc</i> sp. | 1 | 1 | | 1 | | | |
| 11 | <i>Oscillatoria</i> sp. | 1 | | 1 | 1 | 1 | | |
| 12 | <i>Oscillatoria formosa</i> | | 1 | | | | | |
| 13 | <i>Spirulina major</i> | 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)

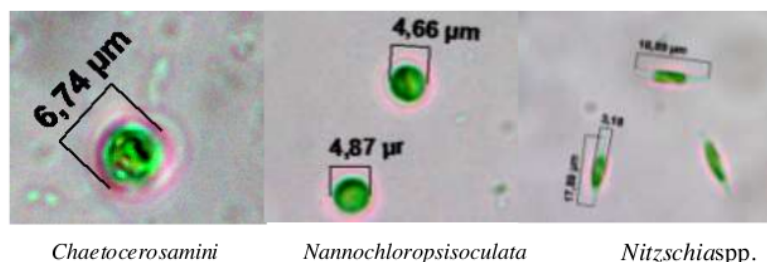


Figure 2.Cells of producing biodiesel microalga species in culture seven days after isolation

The oil content of each dry biomass of three microalgae species extracted. The crude oil produced is then transesterified 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) |
|-------------------------------|----------------------------|
| <i>Chaetocerosamini</i> | 34 |
| <i>Nannochloropsisoculata</i> | 68 |
| <i>Nitzschia spp.</i> | 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 (Allen & Cupp, 2005; Japa & Hallegraeff, 2005; Sheehan *et al.*, 1998).

Based on the measurement results, microalgae oil from *C. amini*, *N. Oculata*, and *Nitzschiaspp* 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 asamlemak |
| Rentang formula kimia | Ester-ester methyl C ₁₄ -C ₂₄ atau C ₁₅₋₂₅ H ₂₈₋₄₈ O ₂ |
| Kinematic viscosity range | 3,3 - 5,2 mm ² s ⁻¹ pada 313°K |
| The density range | 860 - 894 kg m ⁻³ pada 288°K |
| Boiling point | > 475°K |
| Flash point range | 428 - 475°K |
| Distillation range | 470 - 600°K |
| Vapor pressure | < 5 mm Hg pada 295°K |
| Soubility in water | Tidaklarutdalam air |
| Physical appearance | Terangsampaikuningtua, cairbening |
| Smells | Sepertibausabun |
| Biodegradability | Lebih biodegradable daripada solar |
| Reactivity | Stabil, tetapikuatmenghindariagen-agen yang mengoksidasi |

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* sp. 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* sp. in culture 7 days after isolation was 3,368,640,000 cells / liter and the oil content was 46%.

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