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Morphological Observation, Identification and Isolation of Tropical Marine Microalgae from Ambon Bay, Maluku

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Abstract

Microalgae are photosynthetic microorganisms that are widely distributed in waters. They have the potential as an alternative source of pigments, lipids, proteins, and carbohydrates. In this study, identification and isolation of single-cell microalgae from Ambon Bay were carried out by serial dilution method and inoculation of the culture by appearance/spread on the surface of the solidified agar medium. The results showed that the waters of Ambon Bay have physical parameters such as temperature, pH, salinity, and TDS, which are still within the threshold specified in the seawater quality standard for biota. The water conditions at the 10 sampling locations had different salinity, pH, temperature, and TDS. This condition affects the distribution of microalgae in the waters in Ambon Bay. The image from light microscopy shows that there are 27 species of microalgae scattered in the waters of Ambon Bay, which belong to the classes Chlorophyceae, Flagilariophyceae, Bacillariophyceae (diatoms), Coscinodiscophyceae, and Dinophyceae. Microalgae isolation obtained five species (single-cell) including four species from the class Bacillariophyceae (*Navicula* sp, *Nitzschia* sp, *Cyclotella* sp) and one species from the class Chlorophyceae (*Chlorella* sp).

Keywords: Identification, isolation, morphology, tropical marine microalgae,

INTRODUCTION

The ocean has forest areas that are high in biodiversity. Organisms that are widely distributed in this area are mostly able to absorb carbon dioxide (CO_2) so that they can overcome the problem of global warming. This ability is owned by photosynthetic organisms such as seaweed and microalgae. Microalgae are microscopic algae that do not have roots, stems, and leaves. Microalgae convert CO_2 into biomass through the process of photosynthesis. The ability of microalgae to absorb CO_2 is 233 times higher than the ability of land plants (Singh & Ahluwalia, 2013). Because microalgae are very small in size and distributed in water, the ability of microalgae to capture sunlight per unit cell surface area is large and efficient so that photosynthesis is easy to take place. Microalgae habitats are scattered in various waters such as fresh, brackish, and marine waters. Indonesian ocean with an area of 62.9% of the total area of the country is a very diverse tropical marine microalgae habitat.

Marine microalgae are known to have potential as alternative sources of natural photosynthetic pigments and have a wide variety of pigments and other secondary metabolites as cosmetic and medicinal ingredients (Telussa, Rusnadi, & Nurachman, 2019; Yusuf & Nafie, 2016; Nurachman, et al., 2015), lipids as biodiesel material (Nurachman, Brataningtyas, Hartati, & Panggabean, 2012; Kusumaningtyas, Nurbaiti, Suantika, & Amran, 2017), carbohydrates as bioethanol material (Putnarubun, Suratno, & Adyaningsih, 2012; Negara, et al., 2019; (Agustini & Febrian, 2018), biosilica as bionanomaterial (Vona, et al., 2016; Abo- Shady, Zalat, Al-Ashkar, & Ghobara, 2019), and cells as bioreduction and bioremediation (Nurillah, Raya, & Maming, 2016; Teheni, Nafie, & Dali, 2016).

In addition, microalgae have advantages over other plants, including having a short life span and does not require a large area for cultivation ((Barsanti & Gualtieri, 2006)). The ocean where microalgae live is very dynamic, therefore the quantity of microalgae in water is largely determined by oceanographic and meteorological conditions. The microalgae communities that are commonly found in the Maluku oceans consist of the classes Bacillariophyceae, Dinophyceae, Chrystophyceae, and Cyanophyceae.

Microalgae from Ambon Bay waters are endemic to the Ambon Sea, so they are easier to obtain and develop in Indonesia. Therefore, in this research, morphological observations, identification, and isolation of endemic marine microalgae in Ambon will be carried out which can be used as a source of biofuels, bionanomaterials, and cosmetic ingredients.

METHODOLOGY

Materials and Instrumentals

All the chemicals used in this research are pro-analysis grade (Merck, Germany): $FeCl_3$, $NaH_2PO_4.2H_2O$, Na_2EDTA, $Na_2SiO_3.5H_2O$, KNO_3, and Bakto Agar. The equipment used includes glassware, analytical balance, refractometer, falcon tube, Eppendorf micropipette size 10-100 and 100-1000 µL, Nikon YS-100 light microscope, hemocytometer, Tomy ES-315 autoclave, Thermo Scientific S16 centrifuge, pH meter, lux meter, and plankton net.

Methods

Place and time of research

The study was conducted in the waters of Ambon Bay in December 2020. The waters of Ambon Bay include the inner Ambon Bay and the outer Ambon Bay (Figure 1).

Figure 1. Research Location Map

Sample Collection

Ambon Bay waters is a place for sampling microalgae. Sampling was carried out at 10 different points, using a plankton net. The depth of sampling is 1.5 meters from the water surface.

Morphological Observations and Identification of Microalgae

Observation of the morphology of microalgae samples using a light microscope (Nikon YS-100), with 400 times magnification. To determine the microalgae species, the observed morphology was identified using the Algae Resource Database.

Microalgae Isolation

Isolation of microalgae using serial dilution method aims to obtain a single microalgae colony. Samples containing microalgae were put into the first dilution tube (1/10 or 10^{-1}) aseptically after the sample was entered then dissolved by shaking it until homogeneous. Take 1 ml from tube 1 with a measuring pipette then transferred to tube 2 aseptically and then homogenized using a vortex mixer. The transfer is continued until the last dilution tube in the same way. Furthermore, planting is carried out by taking the suspension from the last dilution tube. Then spread on the surface of the agar media. then incubate for 4 days at room temperature with a light intensity of 5000 lux.

RESULTS AND DISCUSSION

Water Parameter Condition in Ambon Bay

Microalgae sampling was carried out at 10 locations along Ambon Bay (Figure 2), where stations 1-3 were the outer Ambon Bay and stations 4-10 were the inner Ambon Bays. At each sampling location, the water conditions showed differences in pH, salinity, temperature, and Total Dissolved Solids (TDS) (Table 1). Based on the results of pH measurements in Ambon Bay waters, it is classified as high with a value of 7.1-10. In the waters of the outer Ambon Bay (station 1-3) the pH value (8.6-9.3) is higher than the pH (7.1-7.8) in the waters of the inner Ambon Bay (stations 4-10), but generally the ideal pH of marine waters at a pH of 8.1-8.4.

Figure 2. Microalgae Sampling Location

Based on the Decree of the Minister of Environment No. 51 of 2004 concerning seawater quality standards for biota, the pH in the waters of the inner Ambon Bay (station 4-10) is still at the specified threshold, which is between 7-8.5. This indicates that the difference between industrial and household waste is a factor that can cause the pH of the water to fluctuate. Meanwhile, the salinity in Ambon Bay waters ranges from 29-32 ppt which indicates the degree of salt concentration dissolved in the water is generally distributed with very small variations (Figure 3(a)), this means that the salinity is relatively homogeneous. The same thing with salinity, the temperature values in the waters ranged from 28-32°C (Figure 3(b)). In general, based on the Decree of the Minister of Environment No. 51 of 2004 concerning seawater quality standards for biota, the temperature and salinity in the Ambon Bay waters are still at tl specified threshold.

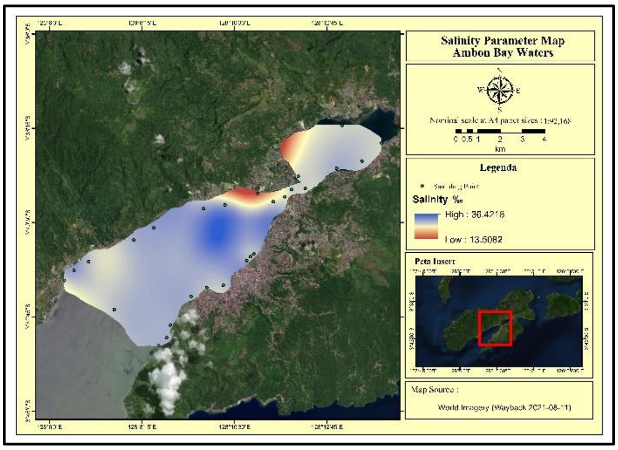
TDS measurement shows the solubility of soli in water which is measured in units of Parts per Millic (ppm) or the ratio of the weight of ions to water. Base on the measurement results, the TDS value in tl waters of Ambon Bay ranges from 696-938 mg L The results of this TDS measurement have n exceeded the class II water quality standard, which 1000 mg/l (PP 82 of 2001 concerning Water Quali Management and Water Pollution Control). Tl conditions of these physical and chemical paramete affect the growth of microalgae species that grow at each station in the waters of Ambon Bay.

Table 1. Results of Analysis of Chemical and Physical Parameters in Ambon Bay Waters

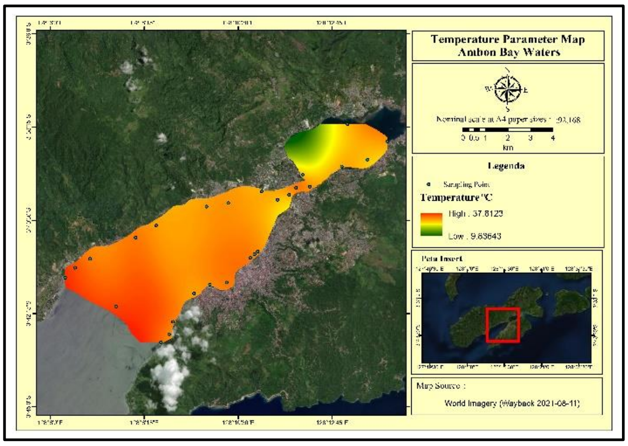
Sta	Coordinate point			Physical Parameters		
	Latitude (South)	longitude (East)	pH	Salinity (ppt)	Temperature (°C)	(n
1	03°42.266'	128°09.445'	9.3	31	31	
2	03°40.629'	128°08.536'	8.7	32	31	
3	03°40.315'	128°09.468'	8.6	31	31	
4	03°39.715'	128°11.886'	7.8	29	33	
5	03°39.689'	128°12.217'	7.8	29	231.5	[illegible]
6	03°39.203'	128°12.978'	7.5	31	30	938
7	03°39.036'	128°13.597'	7.7	30	28.5	938
8	03°38.598'	128°14.071'	7.1	29	31	938
9	03°38.180'	128°13.118'	7.2	29	29	807
10	03°39.400'	128°12.046'	8.5	29	29	696

Isolation and Identification of Microalgae

Microalgae samples from 10 locations along the waters of Ambon Bay, were identified microalgae morphology using a light microscope with 400 times magnification. Microscopic observations aim to identify the types of living microalgae and to determine the purity of microalgae samples (Atıcı & Tokatli, 2014).



(a)



(b)

Figure 3. Map of the chemical and physical parameters of the waters at the sampling location; (a) salinity, (b) temperature

The light microscopy image shows that there are several types of microalgae scattered in the waters. Furthermore, the results of observations of microalgae morphology were identified based on data in the Algae Resource Database. Based on the identification, 27 species are belonging to the classes Chlorophyceae, Flagilariophyceae, Bacillariophyceae (diatoms), Coscinodiscophyceae, and Dinophyceae (Figure 2, Table 1).

Figure 4. Microalgae light micrograph from Ambon Bay waters

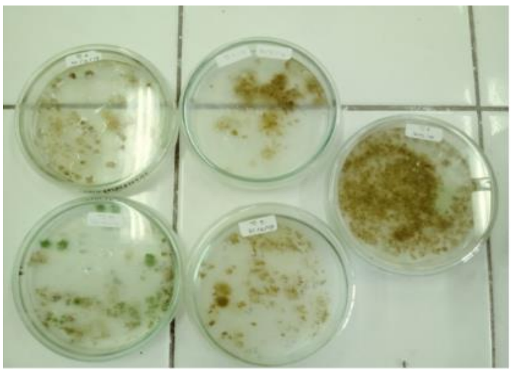
Wherein, classes Chlorophyceae and Bacillariophyceae are this group of microalgae that are high in abundance in seawater. The isolation of microalgae from the waters of Ambon Bay was carried out using the serial dilution method. The purpose of serial dilution is to minimize or reduce the number of microbes suspended in the liquid. Determination of the amount or number of dilution levels depends on the estimated number of microbes in the sample. A ratio of 1: 9 was used for the sample and the first and subsequent dilutions so that the next dilution contained 1/10 of the microorganism cells from the previous dilution (Figure 5a).

Table 2. Types of microalgae from Ambon Bay waters identified

Symbol	Class	Species
a	Bacilariophyceae	*Nitzschia* sp.
b	Bacilariophyceae	*Pseudo-Nitzschia* sp.
c	Bacilariophyceae	*Nitzschia bizertensis*
d	Bacilariophyceae	*Nitzschia navis-varingica*
e	Bacilariophyceae	*Chaetoceros diversum*
f	Bacilariophyceae	*Chaetoceros affinis*
g	Bacilariophyceae	*Chaetoceros laciniosum*
h	Fragilariophyceae	*Thalassionema nitzschioides*
i	Bacilariophyceae	*Navicula* sp.
j	Bacilariophyceae	*Navicula transitans*
k	Bacilariophyceae	*Navicula* sp.
l	Bacilariophyceae	*Pinnularia* sp.
M	Bacilariophyceae	*Amphora bigibba*
n	Bacilariophyceae	*Navicula* sp.
o	Coscinodiscophyceae	*Coscinodiscus* sp.
p	Bacilariophyceae	*Skeletonema* sp.
q	Bacilariophyceae	*Cymbella lanceolata*
r	Bacilariophyceae	*Bacteriastrum* sp.
s	Bacilariophyceae	*Nitzschia panduriformis*
t	Chlorophyceae	*Chlorella* sp.
u	Bacilariophyceae	*Navicula forcipata*
v	Bacilariophyceae	*Skeletonema* sp.
w	Bacilariophyceae	*Cyclotella* sp.
x	Dinophyceae	*Prorocentrum lima*
y	Bacilariophyceae	*Bacillaria* sp.
z	Chlorophyceae	*Haematococcus pluvialis*

The results of serial dilutions were continued with cultivation aimed at obtaining single colonies in suspension from several dilution tubes. Then, the microalgae culture was inoculated on the solidified agar medium by spreading it on the surface of the agar medium (Figure 5b). Microalgae isolation results obtained five species (single-cell) including four species from the class Bacillariophyceae (2 species *Navicula* sp., *Nitzschia* sp., *Cyclotella* sp.) and one species from the class Chlorophyceae (*Chlorella* sp.) (Figure 6). In general, two types of diatoms are differentiated based on their morphological characteristics, namely centric and pennate diatoms.

(a)



(b)

Figure 5. Microalgae isolation process (a) Serial dilution, (b) Planting on the solidified agar medium

Centric diatoms are characterized by the shape of cells that have radial or concentric symmetry with one central point and usually live planktonic like *Cyclotella* sp. In contrast, pennate diatoms have bilateral symmetry, which is generally elongated or sigmoid in shape and usually live benthic, such as *Navicula* sp., *Nitzschia* sp. Observation of *Navicula* sp. and *Chlorella* sp. which will be developed as a source of biofuel, cosmetic, and biosilica materials were also observed with an electron microscope (SEM), which showed the characteristic feature of *Navicula* sp. with oval-shaped frustules and nanopores that lay on the surface for the exchange of nutrients or other materials (Fig. 7(a)). The abundance of diatoms in the world contributes greatly to the diversity of frustules with nanopores scattered on the surface of diatom frustules that show the characteristics of each diatom (Strelnikova, Fourtanier, & Kociolek, 2004).

(a)

(b)

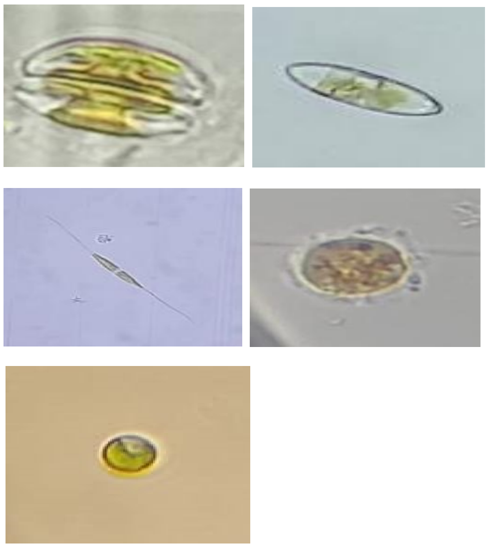
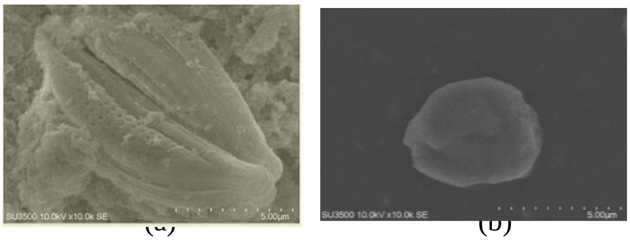


Figure 6. Microscopic image of a single colony that was successfully isolated. (a) class Bacillariophyceae (*Navicula* sp., *Nitzschia* sp., *Cyclotella* sp.), (b) class Chlorophyceae (*Chlorella* sp.)

Meanwhile, *Chlorella* sp. showed round cells with a smooth surface without pores (Figure 7(b)). *Chlorella* has a size of about 5-10 µ and when dried, they aggregate to form larger particles. Cell shells containing about 30% cellulose are present on the surface of Chlorella particles (Indhumathi, Soundararajan, Shabudeen, Shoba, & Suresh, 2013; Azaman, Nagao, Yusoff, & Tanand, S.W, 2017).



(a) (b)

Figure 7. SEM image of isolated microalgae (a) *Navicula* sp. (b) *Chlorella* sp.

CONCLUSION

The waters of Ambon Bay have physical parameters such as temperature, pH, salinity, and TDS which are still at the threshold set in the seawater quality standard for biota. There are 27 species of microalgae scattered in Ambon Bay waters belonging to the classes Chlorophyceae, Flagilariophyceae, Bacillariophyceae (diatoms), Coscinodiscophyceae, and Dinophyceae. Microalgae isolation obtained five species (single-cell) including four species from the class Bacillariophyceae (2 species *Navicula* sp, *Nitzschia* sp., *Cyclotella* sp.) and one species from the class Chlorophyceae (*Chlorella* sp.).

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