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A Preliminary Survey on the Indigenous Microalgae in a Brackish Water Kakaban Lake, East Kalimantan, Indonesia for Potential Biomass Production

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ABSTRACT

Brackish water microalgae exploration has been conducted in Lake Kakaban, Indonesia. This study is a preliminary study of a series of microalgae exploration studies that will utilize sustainable microalgae resources, including isolation and collection. The study was conducted in the waters of Lake Kakaban in July 2024 with sampling points in the North (Area 1) and South (Area 2) areas, each with eight stations. Sample collection was carried out using two approaches: fresh samples and samples preserved with 4% formalin. Fresh samples were cultivated in the laboratory with liquid media enriched with KW21 and F2/Guillard (enriched with silica) and cultured under LED lighting in dark: light conditions (12:12). The isolation of microalgae was conducted using an agar plating and a multilevel cell culture. The identification method was done by observing cells under a microscope, which were identified based on the Algae Resource Database and World Register of Marine Species. There were 46 species of microalgae from 10 classes that were successfully identified and dominated by the diatom group from the *Bacillarophyceae* class (63,04%), green algae (15,2%), Dinoflagellata (13,04%), Golden algae (6,52%) and Euglenophyta (2,17%). Three microalgae species have been successfully isolated and can grow in the laboratory. Of the three isolates, the highest specific growth rate value was obtained in isolate KK1, which was 0.0214 day-1, while in KK3, the SGR value was 0.0206 day-1, and isolate KK9 with an SGR value of 0.0146 day-1 and has the potential for further research development.

INTRODUCTION

Indonesia is known as an archipelagic country that has abundant marine resource potential. One of the potential marine resources that has yet to be widely explored is marine microalgae. Microalgae are prokaryotic and eukaryotic organisms that can photosynthesize and are found in various ecosystems on land and at sea **(Richmond 2004; Mata *et al.*, 2010)**. Microalgae are very diverse microorganisms and can potentially be a source of essential chemicals to be used as feed, food supplements,

cosmetics, medicines, and in the fuel industry **(Olaizola, 2003; Telussa *et al.*, 2019)**. There are thousands or even millions of types of microalgae available in nature, but only a few have been successfully isolated and produced commercially to produce valuable products **(Hannon *et al.*, 2010)**. Hence, bioprospecting microalgae species with commercial potential is an essential and challenging task. Indonesia Ocean, with an area of 62.9% of the country's total area, is a very diverse tropical marine microalga. One unique area that has not been widely explored is Kakaban Lake.

Kakaban Lake, East Kalimantan, is the largest brackish water lake in the world, with an area of about 4km^2, it is formed in the middle of the ocean and is bordered by mangrove forests of the *Bruguiera gymnorrhiza* species **(Becking *et al.*, 2013)**. This lake is an ancient lake formed from seawater trapped by coral reefs due to tectonic activity. This uniqueness is what caused Kakaban Lake to be nominated as a UNESCO world heritage area **(Nurasmi *et al.*, 2021)**. It is suspected that the uniqueness of the habitat will affect the types of microalgae that live there.

The isolation and selection of local microalgae species have advantages, especially in the development of outdoor mass production, because local species have adapted to the local climatic environment **(Larkum *et al.*, 2012)**. Brackish water microalgae can grow in saline or hypersaline conditions, so they have the potential to be developed continuously and more economically compared to freshwater microalgae.

Initial studies related to the exploration of brackish water microalgae species found in Lake Kakaban are essential to be carried out as a source of initial information, so that through isolation and identification of potential microalgae species, data can become the latest source of information so that in the future it can be used as initial data for further development

Microalgae exploration has been carried out in several waters in Indonesia, including microalgae exploration from coastal, ocean, and mangrove areas in Indonesia **(Suyono *et al.*, 2016; Ermavitalini *et al.*, 2021; Hutari *et al.*, 2022; Suhendra *et al.*, 2023)**. However, information about exploration activities for potential microalgae species from Kakaban Island Brackish Waters has yet to be found. This study is preliminary in a series of microalgae exploration studies, including isolation and collection to utilize sustainable microalgae resources.

MATERIALS AND METHODS

Area of study

Microalgae sampling was conducted in 2 locations, namely Location 1 in the North and Location 2 in the South with eight sampling points each using plankton net with a mesh size of 20 microns. Location selection was determined based on the accessibility of the research location (Fig. 1).

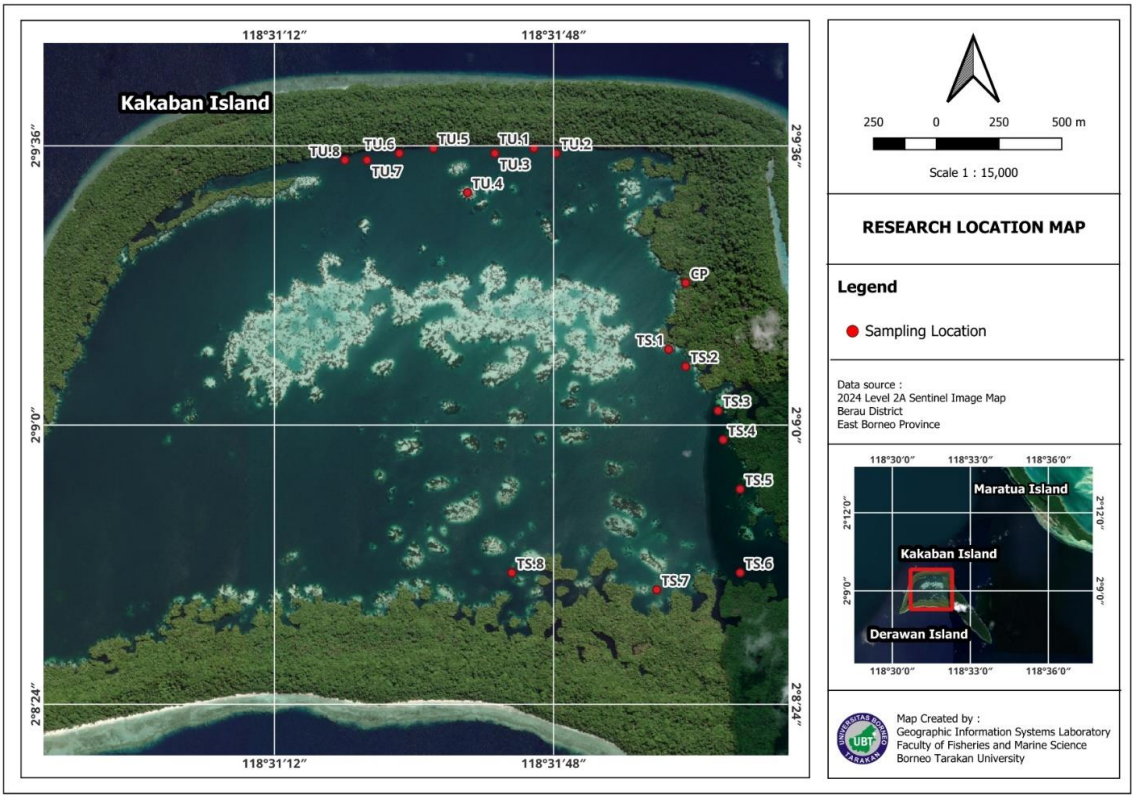

Fig. 1. Sampling location. CP (central point), TU (Area 1) dan TS (Area 2)

Sample collection

Microalgae were isolated at accessible locations by filtering 50 liters of water using a plankton net (20µm) at a depth of 0.5-1m (Fig. 1). The samples were then preserved with 4% formalin **(Arutselvan, 2021)**, while fresh samples were stored in sterile bottles and taken to the Laboratory with a coolbox **(Chaidir *et al.*, 2016; Padmaperuma *et al.*, 2017; Ungwiwatkul & Chantarasiri, 2022; Santos *et al.*, 2023)**.

Water quality measurement

Water quality measurements were carried out *in situ*, including pH (pH meter), salinity (refractometer), brightness (secchi dish), temperature (thermometer), and DO with a DO meter, while BOD and nitrate phosphate content of the waters were *ex-situ* using a spectrophotometer.

Isolation, identification and cultivation

Two methods of samples were collected, namely preserved samples (4% formalin) and fresh samples. Fresh samples were cultured in nutrient-enriched liquid media KW21 and F/2 Guillard (enriched with silica), with a salinity of 24-25ppt, pH 7-8,

and incubated under LED lighting with light-dark conditions (12:12) at a temperature of 24-28°C **(Indrayani *et al.*, 2018)**, with modification. This culture aims to keep the microalgae alive during identification. Preserved samples are prepared to ensure that some microalgae species cannot grow in the laboratory but can still be identified from preserved samples.

Observation of morphology and identification of microalgae samples using a light microscope (Olympus CX21FS1) with a camera (Optilab) with 400 and 1000 time magnification. To determine the microalgae species, the observed morphology was identified using the Algae Resources Database and World Register of Marine Species (WORMS) **(Telussa *et al.*, 2021)**.

Multilevel microalgae cell culture

Live samples from the field were isolated using several methods, namely multilevel dilution and agar plating techniques **(Andersen & Kawachi, 2005)**. The agar medium was prepared by adding 1% agar into the liquid medium before autoclaving. 0,1mL sample was spotted on the middle of the agar plates and spread evenly on the surface using an ose and then sealed with parafilm and incubated in the culture shelve under LED lights with 12h light and 12h dark cycle and at room temperature of 24-28°C. Colonies that emerged on the agar plates were picked up and re-streaked onto fresh agar medium. The pure colonies were then transferred into a medium (10ml liquid enriched with 10µL F/2 medium+silica 10ppm) and incubated for 2 weeks. After 2 weeks of incubation, screening of potential microalgae that could grow well in the laboratory was carried out. Selected isolates were cultured in stages on 200ml media enriched with KW21 and incubated under LED lighting with light-dark conditions (12:12h) at a temperature of 24-28°C for 28 days **(Indrayani *et al.*, 2018)** with modification.

Monitoring of cell growth was conducted by measuring cell density every 2 days using a GENESYS 20 spectrophotometer (OD 680 nm) **(Ungwiwatkul & Chantarasiri, 2022)** with modifications.

Specific growth rate (SGR)

After being cultivated, the SGR (day^{-1}) was calculated using the formula referring to **Indrayani *et al.* (2018)**:

$$\mathbf{SGR} = \frac{ln\left(\frac{N2}{N1}\right)}{t1-t2}$$

Where, N1: OD reading at time 1(t1) , N2: OD reading at time 2 (t2) within the exponensial phase.

RESULTS AND DISCUSSION

1. Water quality in Kakaban Lake

Water quality is one factor affecting the distribution and abundance of microalgae, which can be measured through physical and chemical parameters. The results of water quality in Kakaban (Table 1) show that the highest water temperature in two locations reached 30.4°C, while the lowest temperature was 28.01°C. The high temperature is thought to be influenced by the time the sample was taken at 10.00-12.00 (WITA) Central Indonesian Time.

Water temperature significantly affects the metabolic process and growth of microalgae. The optimal growth temperature for most industrial microalgal strains is commonly between 20 and 30°C **(Ras *et al*., 2013)**. Other studies report that good temperatures for microalgae growth are within the 27-30°C **(Kadim & Arsad, 2016)**. Meanwhile, water temperatures over 35°C have the potential to cause death in microalgae **(Arsad *et al*., 2022)**. The highest water clarity value reached 6.70m, and the lowest was 4.25m. Water clarity is related to light intensity, which plays a direct role in the growth and development of microalgae. Too low clarity results in a small number of microalgae being found. The optimum depth for phytoplankton growth ranges from 5-20m **(Mahmudi *et al*., 2023)**.

Table 1. Water quality in Kakaban Lake

Parameters	Location 1	Location 2
Temperature	28.01-30.4^{0}C	27-30.4^{0}C
Brightness	4.25-6.7 m	4.25-6.7 m
pH	7.75-8.04	7.75-8.16
Salinity	24-25 ppt	24-25 ppt
DO	4.70 mg L^{-1}	4.74 mg L^{-1}
Nitrate	0.31 mg L^{-1}	0.36 mg L^{-1}
Phosphate	0.06 mg L^{-1}	0.08 mg L^{-1}

This study's highest water pH value reached 8.16, while the lowest water pH of 7.75 showed compliance with the quality standard, which is 7-8,5. Alkaline pH in waters can support water productivity and the mesotrophic status of the lake **(Mali & Sharma, 2023)**. This pH value is also still suitable for supporting the life of microalgae, which generally grow at pH 6-8 **(Hadiyanto & Azim, 2012)**. The highest water salinity value was 25ppt, while the lowest salinity was 24ppt. This salinity value is higher than the report from **Nurasmi *et al*. (2021)**, which stated that the salinity of Lake Kakaban was 21ppt. This is thought to be due to changes in the average annual rainfall, which is lower than the evaporation capacity, which triggers the salinity of the lake water to increase to a certain extent. Changes in salinity can cause the extinction of microalgae and affect the abundance of plankton **(Yang *et al*., 2024)**. The water's DO (Dissolved Oxygen) value was obtained at 3.74-4.16mg L-1, indicating that the study area was less fertile because

the DO content was below 5mg L-1. Low DO levels indicate low microalgae density **(Kadim & Arsad, 2016)**.

Nitrate and phosphate are the primary nutrients that are essential for the growth of microalgae. Excessive amounts of nitrate and phosphate can cause a decrease in water quality **(Akinnawo, 2023)** This study's nitrate value in water ranged from 0.31-0.36mg L-1. Meanwhile, the orthophosphate value of water ranged from 0.06-0.08mg L-1. This value also exceeds the orthophosphate standard in seawater up to 0.01mg L-1. However, orthophosphate is generally used for the formation of microalgae cells and is optimal at a level of 0.09-1.08mg L-1 **(Mahmudi *et al.*, 2023)**. An increase in nitrate and phosphate levels will be directly proportional to the rise in microalgae abundance. Too much nutrition can cause an imbalance in the composition of water microalgae **(Zakiyah *et al.*, 2020)**.

2. Checklist of indigenous microalgae Brackhis Water in Kakaban Lake

Forty-six microalgae species of 10 classes were identified and grouped into dinoflagellates, euglenophyta, golden algae, green algae, and diatoms (Table 2). *Bacillariophyceae* class from the diatom group dominates the species of algae identified. Diatoms are divided into two groups, namely centric diatoms and pennate diatoms. Centric diatoms are divided by round or concentric cell shapes with one central point and usually live like planktonic, like in the type *Coscinodiscus* sp. In the pennate type, diatoms are characterized by bilateral symmetry, generally elongated or sigmoid, and typically live as benthic, such as *Navicula* sp. and *Nitzschia* sp. **(Telussa *et al.*, 2021)**.

In this study, using formalin on preserved samples could not protect the specimens well. There are 15 microalgae species identified in preserved samples, while in fresh samples, there are 36 species, and five species of them are identified in both. All types of dinoflagellates identified were obtained from preserved samples. While in fresh samples, no species from the dinoflagellates group was found.

Formalin is widely used in microalgae abundance studies. However, improper use can also cause damage to the microalgae cell walls or cell membranes. This effect can change the cell structure, disrupt its integrity, and cause cell death. Previous research reported the use of 4% formalin plus 5 drops of glycerin in sample preservation obtained 77 species consisting of 51 phytoplankton species and 26 zooplankton species **(Arumwardana, 2014)**. The results of other studies reported that the number of plankton present with formalin preservatives alone was lower than the use of formalin together with $CuSO_4$, Glycerin, and $CaCO_3$ **(Winarsih *et al.*, 2024)**. According to **APHA (American Public Health Association) (1989)**, the most suitable preservative for phytoplankton is Lugol's solution, which dissolves potassium iodide and iodine by adding sodium acetate. Formalin is acidic, so if 4% formalin is used, calcium carbonate or sodium carbonate needs to be added.

Some fragile plankton, especially naked dinoflagellates, are difficult to preserve in good condition **(Tan *et al.*, 2016)** but in this research, dinoflagellates can preserve

good in formalin 4%. *Dinoflagellates* are a group consisting of more than 2000 species of eukaryotic algae which, together with diatoms, play an important ecological role as primary producers at the bottom of aquatic ecosystems **(Taylor & Pollingher, 1987)**. Likewise, in the Chaetoceros class, only 5 species were found in preserved samples. It is suspected that these species cannot survive and grow well in the laboratory. Although dinoflagellates are widely distributed in waters and have high productivity in nature, it isn't easy to cultivate this group of organisms either in fermentors or in synthetic culture media **(Cohen & Ratledge, 2010)**. Both euglenophyta and golden algae were identified as a class. Euglenophyta found the *Trachelomonas* sp. while from Class *Chrysophyceae*, it is suspected from the genus *Ochromonas,* with each having a different number of flagella (Fig. 3).

The green algae group in this study successfully identified 7 species from 5 classes, and several species of green algae can grow well in the laboratory by providing nutrients in the form of KW21, namely *Chlorella* sp. and *Dunalillea* sp. Chlorophyceae, or green algae, are the most diverse algae because some are single-celled and colonial, and some are multicellular. The pigments it possesses are chlorophyll a and b. Most of these algae live in lakes and sometimes in seawater **(Kawaroe *et al.*, 2010)**. There are also those that form colonies that resemble the cormus of higher plants **(Tjitrosoepomo, 1989)** (Table 2).

In fresh samples, it is also highly possible that microalgae do not survive during the transportation process from the field to the laboratory, which stands behind the differences in the microalgae species identified between preserved samples and living samples. Collecting samples from the field and transporting them to the laboratory took more than 4 hours.

Microalgae taken from their natural habitat often experience stress during laboratory collection, transportation, and acclimatization. Temperature, light, or water composition changes can disrupt their physiological balance, affecting their viability and ability to grow in culture.

Table 2. Checklist of microalgae identified in Kakaban Lake

Group	Class	Order	Family	Genus	Spesies	Collection Method	
						Formalin Sample	Fresh Sample
Dinoflagellata	*Dinophyceae*	*Gonyaulacales*	*Pyrocystaceae*	*Alexandrium*	*Gonyaulax* sp./Alexandrium	+	
			Ceratiaceae	*Ceratium*	*Ceratium fusus*	+	
					Ceratium furca	+	
				Alexandrium	*Gonyaulax* sp./*Alexandrium*	+	
		Prorocentrales	*Prorocentraceae*	*Prorocentrum*	*Prorocentrum micans*	+	
		Gymnodiniales	*Gyrodiniaceae*	*Gyrodinium*	*Gyrodinium* sp.		+
Euglenophyta	*Euglenophyceae*	*Euglenales*	*Euglenaceae*	*Trachelomonas*	*Trachelomonas* sp.	+	+
Golden Algae	*Chrysophyceae*	*Ochromondales*	*Ochromonadaceae*	*Ochromonas*	*Ochromonas?*		+
					Ochromonas?		+
					Ochromonas?		+
Green algae	*Chlorophyceae*	*Chlamydomodales*	*Tetrasporaceae*	*Tettraspora*	*Tetraspora* sp.?		+
			Chlamydomondaceae	*Chlamydomonas*	*Chlamydomonas* sp.		+
			Chlamydomondaceae	*Dunaliella*	*Dunaliella* sp.?		+
	Trebouxiophyceae	*Chlorellales*	*Chlorellaceae*	*Chlorella*	*Chlorella* sp.		+
	Ulvophyceae	*Ulotrichales*	*Ulotriceae*	*Ulothrix*	*Ulothrix* sp.	+	+
	Chlorarachniophyceae	*Chlorarachniales*	*Chlorarachniaceae*	*Lotharella*	*Lotharella* sp.		+
	Cyanophyceae	*Chroococcalles*	*Pleurocapsaceae*	*Pleurocapsa*	*Pleurocapsa* sp.		+
Diatom	*Coscinodiscophyceae*	-	-	*Coscnodiscus*	*Coscnodiscus* sp.		+
	Bacillariophyceae	*Achnanthales*	*Cocconeidaceae*	*Cocconeis*	*Cocconeis* sp.		+
		Bacillariales	*Bacillariaceae*	*Cylindrotheca*	*Cylindrotheca* sp.		+
					Cylindrotheca sp.		+
					Cylindrotheca sp.		+
					Cylindrotheca Closterium	+	+
				Nitzschia	*Nitzschia* sp.	+	+
				Psammodictyon	*Nitzchia panduriformis/ Psammodictyon panduriformis*		+
		Cymbellales	*Cymbellaceae*	*Cymbella*	*Cymbella* sp.		+
					Cymbella sp.		+
			Gomphonemataceae	*Gomphonema*	*Gomphonema* sp.		+
		Melosirales	*Melosiraceae*	*Melosira*	*Melosira* sp.		+
		Naviculales	*Naviculaceae*	*Navicula*	*Navicula* sp.	+	+
					Navicula sp.		+
					Navicula sp.		+
			Naviculaceae Pinnulariaceae	*Navicula Pinnularia*	*Navicula cincta*		+
			Diploneidaceae	*Diploneis*	*Pinnularia* sp.		+
					Diploneis didymus/Pinullaria didyma		+
			Stephanodiscaceae	*Cyclotella*	*Cyclotella* sp.		+
		Thalassiosirales	*Thalassiosiraceae*	*Thalassiosira*	*Thalassiosira* sp		+
			Diploneidaceae	*Diploneis*	*Thalassiosira* sp		+
			Stephanodiscaceae	*Cyclotella*	*Thalassiosira* sp		+
			Thalassiosiraceae	*Skeletonema*	*Skeletonema* sp.		+
				Stepanodiscaceae	*Cyclotella* sp.		+
		Chaetocerotanae incertae sedis	*Chaetocerotaceae Chaetocerotaceae*	*Chaetoceros*	*Chaetoceros* sp.	+	
					Chaetoceros sp.	+	
					Chaetoceros sp.	+	
					Bacteriastrum sp.	+	
					Bacteriastrum sp.	+	

Note: Identification and classification reference: Algabase Resouces; World Registres of Marine Species.

The following is a microscopic observation of microalgae from fresh samples that was successfully documented (Figs. 2-3).

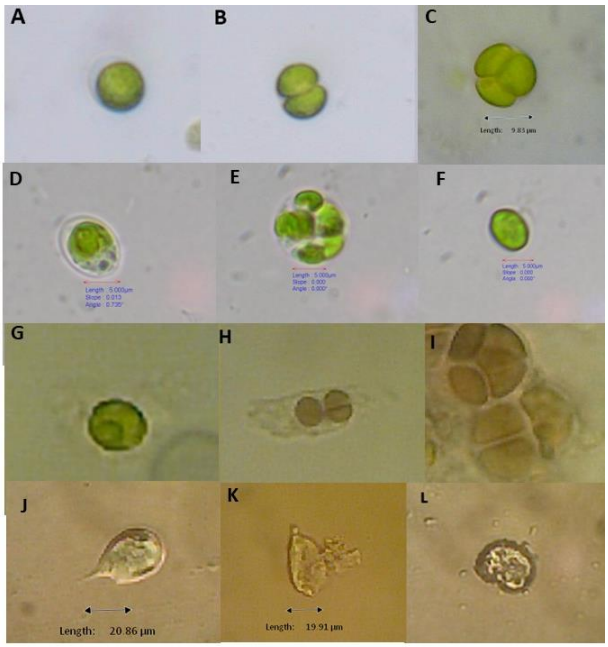

Fig. 2. Green algae group. A-C suspected *Tetraspora* sp.; D-F *suspected Chlamydomonas* sp. G. *Chlorella* sp.; H-I *Pleurocapsa* sp. **Dinoflagellata**. J *Gyrodinium* fusus; K *Prorocentrum* sp. **Euglenophyta.** L *Trachelomonas*

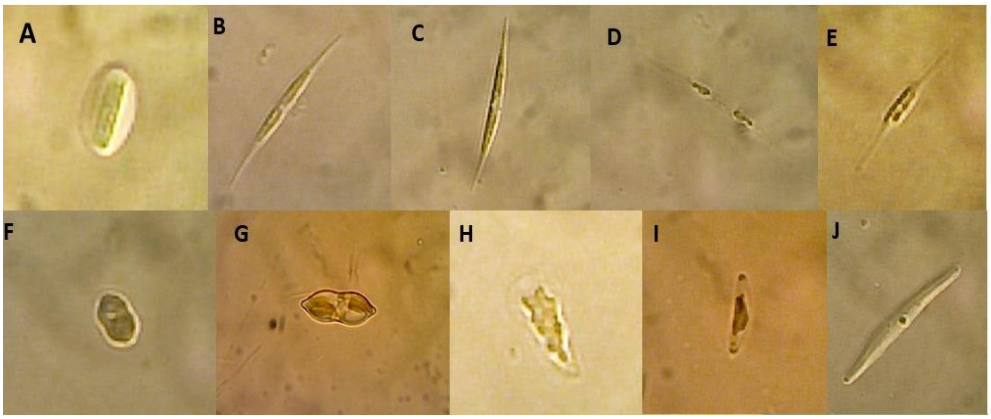

Fig. 3. Diatoms. A *Coconeis* sp. B-D *Cylindrotheca* sp. E *Cylindrotheca Closterium.* F *Nitzschia* sp. G *Nitzchia panduriformis.* H *Gomphonema* sp.I-J *Cymbella* sp.

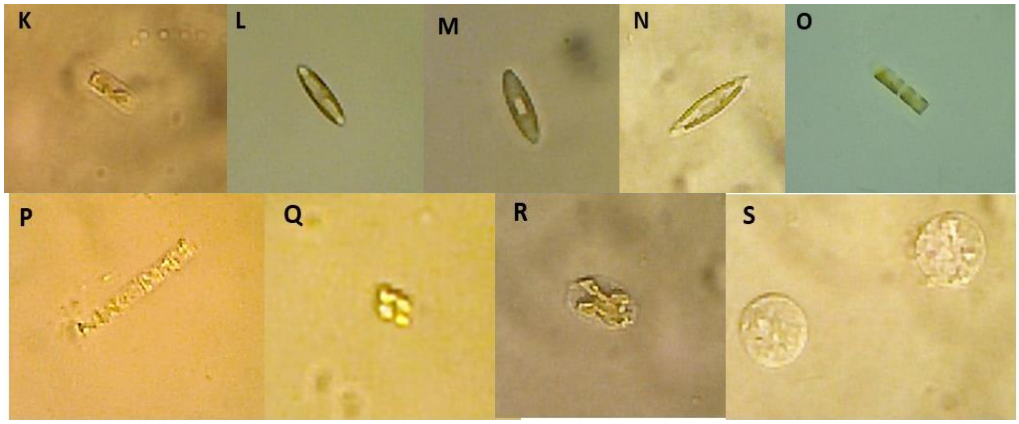

Fig. 3. Diatom. K-M) *Navicula* sp. N *Navicula cincta*. O *Skeletonema* sp. P *Melosira* sp. Q-R *Thalassiosira* sp. S *Coscnodiscus* sp.

3. Percentage of identified microalgae groups

Microalgae from the diatom group were found the most, with a percentage of 63.04%, consisting of *Coscinodiscophyceae* (2.17%) and the *Bacillarophyceae* class (60.87%) (Fig. 4).

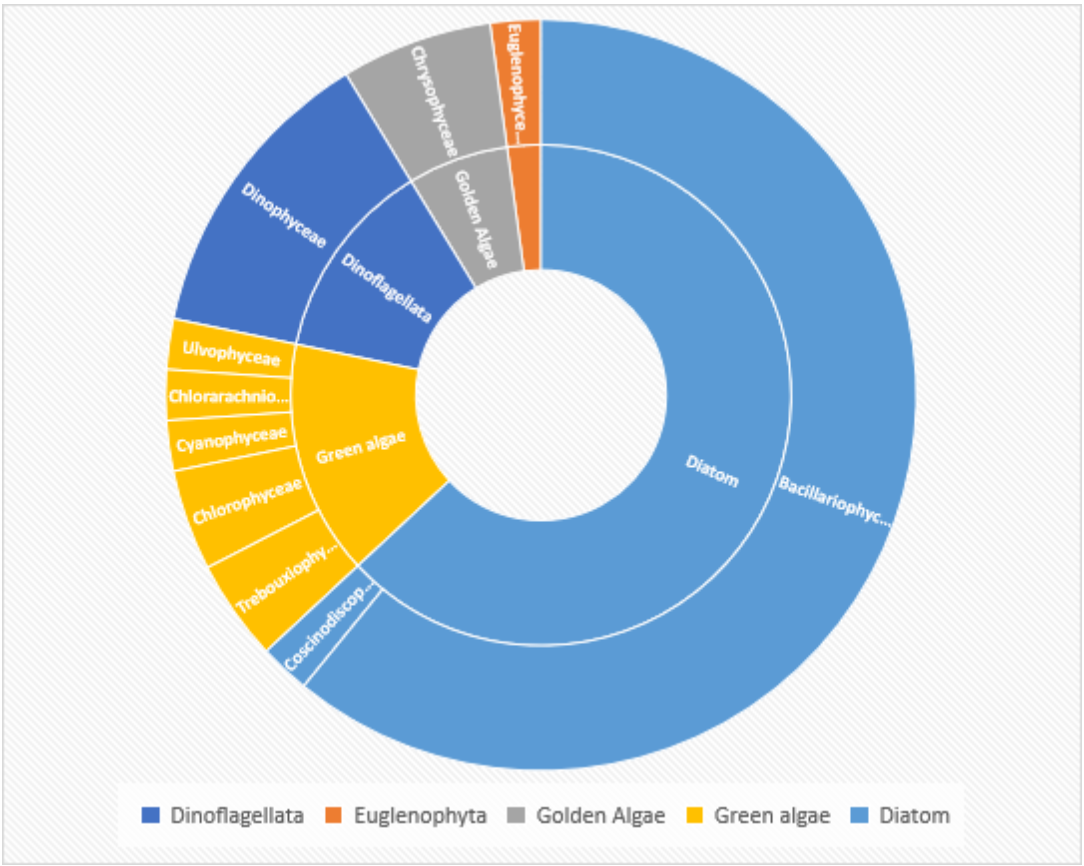

Fig. 4. Percentage of identified microalgae groups

The green algae group ranked 2nd with a percentage of 15.2% divided into five classes *Chlorophyceae* (4,34%) and *Trebouxiophyceae* (4,34%), followed by *Ulvophyceae*, *Chlorarachniophyceae* and *Cyanophyceae* each 2,17%. The 3rd order is at the *Dinoflagellata* group from the *Dinophyceae* class of 13,04%. In the golden algae group, the *Chrysophyceaeclass* was 6,52%, and the lowest identified Euglenophyta consisted of the *Euglenophyceae* class of 2.17% (Fig, 4).

The diatom group of the *Bacillarophyceae* class was found to have the highest percentage of species in this research. Diatoms, also known as golden brown algae, contain more yellow pigments than green pigments. Diatoms are a major, ubiquitous photosynthetic group representing most aquatic ecosystem's microalgal communities **(Rath & Mitbavkar, 2023)**. *Bacillariophyceae* is a class of diatom that dominates the number of phytoplankton in the sea. It is often found in fresh, brackish water, colonies, or unicellular. The Bacillariophyceaediatom class is spread throughout the world and generally dominates the phytoplankton community **(Nasution *et al.*, 2019; Faturohman *et al.*, 2020; Audah *et al*., 2021)**. *Bacillariophyceae* has cosmopolitan nature, is resistant to extreme conditions, is easily adaptable, and has a very high reproductive capacity **(Sachlan, 1982)**. This is also because *Bacillariophyceae* is adaptable so that it can regenerate and reproduce in larger quantities than other species of phytoplankton. This type of plankton is also the most resistant to environmental changes due to tidal influences **(Khaqiqoh *et al*., 2014)**.

Brackish waters usually have quite variable salinity levels. Diatoms can thrive in waters with varying salinities because they are well-adapted to these conditions. They are more tolerant of salinity fluctuations than many other organisms and are more sensitive to environmental changes. The combination of salinity diversity, nutrient availability, and adaptability makes diatoms abundant in brackish waters, making them one of the dominant algal groups in these habitats **(Aseem & Mitbavkar, 2023)**.

Diatom and green algae groups were found with higher percentages compared to other algae groups, which were greatly influenced by environmental conditions. One of the factors is the availability of high nitrate based on water quality data, which is 0.31-0.36mg L^{-1}, while the phosphate content tends to be lower, which is 0.06-0.08mg L^{-1}. Previous studies report that the abundance of phytoplankton in waters is influenced by nutrient nitrate compared to phosphate. Nitrate is the determining factor of phytoplankton abundance, especially green algae **(Darmawan *et al*., 2018; Wijaya & Elfiansyah, 2022)**. Chlorophyceae includes green algae with diverse vegetative and can be found in various types of waters. Microalgae of the genus *Chlorella* are found in almost all geographic areas, including species in freshwater lakes, soil, sea, brackish water, and terrestrial habitats, and some species are also symbionts of lichens, protozoa, and invertebrates **(Luo *et al*., 2010; Bock *et al*., 2011; Darienko *et al.*, 2015)**. The molecular mechanisms underlying survival in these two contrasting environments are still difficult to understand **(Aigner *et al*., 2020)**.

4. Growth of three species of microalgae isolates that can adapt in the laboratory

As many as 47 species of microalgae were successfully identified, and three microalgae species were successfully isolated and could grow in the laboratory. One of them suspected is isolated KK1 (*Pleurocarpsa* sp.), isolate KK3 (*Chlamydomonas* sp.), and isolate KK9 (*Chlamydomonas* sp.). These microalgae isolates were isolated on solid media and adapted to thrive on liquid media for 1 month. The following is the initial growth data on a 200ml scale culture (Fig. 5). It takes a long time to be able to grow local microalgae isolated from nature.

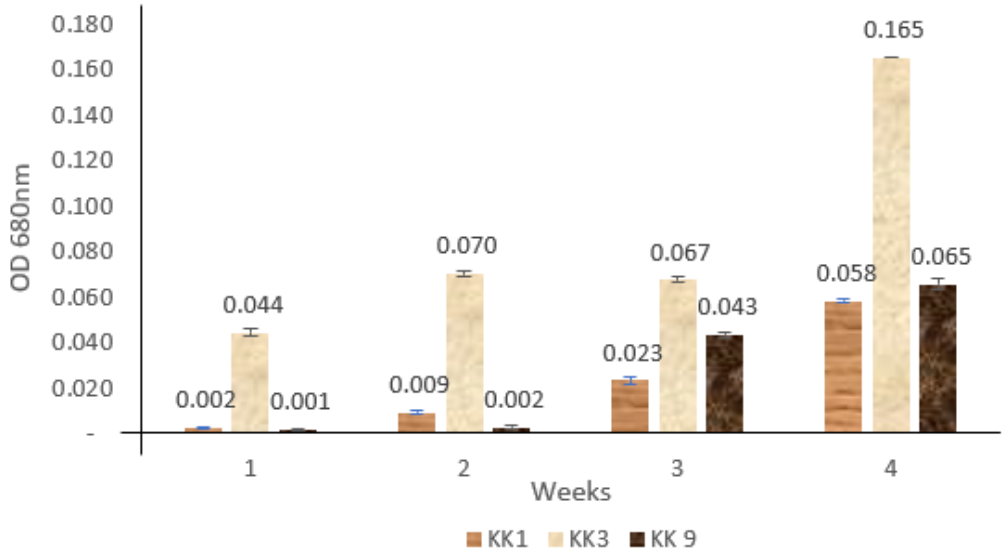

Fig. 5. Cell density of microalgae isolates cultured in the laboratory

The exponential phase was reached on the 28th day with the highest OD value in the KK3 isolate of 0.165nm, higher than the KK9 isolate with an OD value of 0.065nm and the lowest KK1 only reaching 0.058nm. Previous studies reported that cell growth from 3 microalgae strains from Brunei Darussalam Water obtained the highest cell growth value based on OD at 750nm; the highest OD value was obtained in the Micractiniumsp. Strain culture, achieved on the 14th day at 0.12 day $^{-1}$ **(Roseli *et al.*, 2023)**.

Diatoms, especially from the *Bacillarophyceae* class in this study, have not been successfully isolated using the isolation method from solid media to liquid media. It is suspected that the kind of nutrients used, namely KW21, is more suitable for supporting microalgae growth from the green algae group.

5. Specific growth rate (SGR)

The specific growth rate of three isolates in culture medium is shown in Fig. (6).

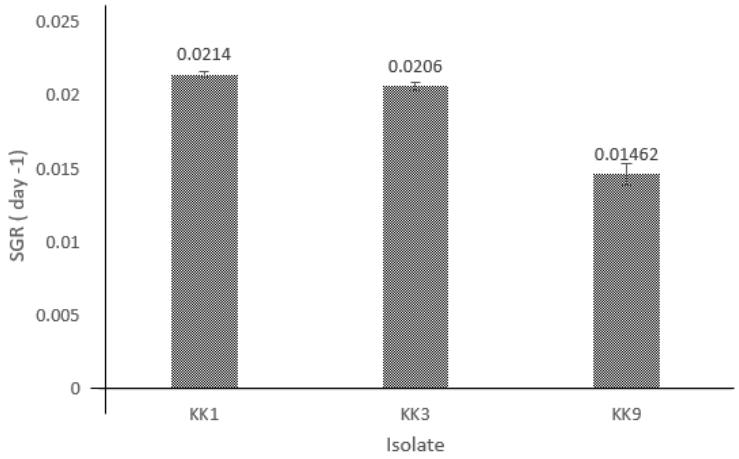

Fig. 6. Specific growth rate of three isolates succesfully adapted in laboratory

The specific growth rate measures the rate at which a population of cells grows over a particular period. Isolate KK1 and KK3 showed better SGR values than isolate KK9 grown on KW21 nutrients (Fig. 6). These results indicate that KK1 and KK3 have better adaptability than isolate KK9. Using the same nutrients can provide different responses to each species of microalgae.

The best SGR value was shown in isolate KK1 of 0.0214 day^{-1} and the lowest in isolate KK9, with an SGR value of 0.0146 day-1. Other studies reported that the highest SGR value in local microalgae isolates from the waters of Nambo Beach, Kendari, was achieved in salinity treatments of 3 and 4ppt reaching 0.18 day^{-1}, namely from the *Melosira* sp. isolate, where the exponential phase was achieved on day 5 **(Indrayani *et al.*, 2018)**. *Desmodesmus* sp. and *Micractinium* sp. isolates from Brunei Darussalam water had their optimum salinity at 5, with the growth constant of 0.592 day^{-1} and 0.464 day^{-1} **(Roseli *et al.*, 2023)**. Faster growth was noticied compared to the results obtained from this research. Numerous factors influence microalgae cell growth. Adapting isolates from nature requires time and adjustment of appropriate environmental factors to obtain high cell growth and biomass.

This study is still limited to using one kind of media, and the cell growth that occurs is still relatively low, and thus comparative data on using the best media for each isolate need to be studied further.

CONCLUSION

In conclusion, there were 46 species of microalgae from 10 classes that were successfully identified and dominated by the diatom group from the Bacillarophyceae class (63,04%), green algae (15,2%), dinoflagellata (13,04%), golden algae (6,52%) and euglenophyta (2,17%). Three types of microalgae have been successfully isolated and can grow in the laboratory. Of the three isolates, the highest specific growth rate value was

obtained in isolate KK1, which was 0.0214 day-1, while in KK3, the SGR value was 0.0206 day-1, and isolate KK9 with an SGR value of 0.0146 day-1 and has the potential to be developed on a large culture scale.

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