ISSN: 1675-7785
eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 18(1) 2024, 39 – 49.

Science Letters

Comparative Analysis of Lipid Accumulation and Fatty Acid Composition in Microalgae *Chaetoceros* sp. and *Isochrysis* sp. Under Varied Light Intensities for Biodiesel Production

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ARTICLE INFO

Article history:
Received 6 September 2023
Revised 22 November 2023
Accepted 25 November 2023
Online first
Published 22 January 2024

Keywords:
Growth rate
Light intensity
Chaetoceros sp.,
Isochrysis sp.
Lipid
Fatty acid

DOI:
10.24191/sl.v18i1.23906

ABSTRACT

The escalating demand for renewable and sustainable energy sources has spurred research into innovative alternatives to conventional fossil fuels. Among these alternatives, microalgae have emerged as promising for biodiesel production due to their rapid growth rates, high lipid content, and ability to thrive in diverse environmental conditions. However, the efficient conversion of microalgae biomass into biodiesel hinges on optimizing lipid accumulation and ensuring desirable fatty acid compositions. Therefore, this study investigates the effects of different light intensities on lipid accumulation and fatty acid composition in two prevalent microalgae species, Chaetoceros sp. and Isochrysis sp., to optimize their biodiesel potential in seawater containing sodium metasilicate and Conway medium. The cultivation was carried out at three different light intensities (1000 lux, 2000 lux, and 3000 lux) with a photoperiod of 12:12 hours of light: dark cycle. The results showed that the samples achieved peak growth rates at a light intensity of 2000 lux, with Chaetoceros sp. reaching 12.32% growth and Isochrysis sp. achieving 9.72%. Chaetoceros sp. and Isochrysis sp. displayed their peak saturated fatty acid content, crucial for high-grade biodiesel, at the 2000 lux light intensity. Chaetoceros sp. reached 51.06%, while Isochrysis sp. recorded 44.05%. Additionally, both species contained notable proportions of C14, C16, and C18 fatty acids, essential components in selecting viable alternative biodiesel sources. Therefore, a light intensity of 2000 lux is recommended to be applied during the growing stage of this seaweed to produce quality biodiesel.

INTRODUCTION

The demand for renewable energy is increasing due to environmental issues such as global warming, climate change, and air pollution. Biofuel can be an excellent substitute to replace fossil fuels and reduce the greenhouse gas emissions that are responsible for global warming [1,2]. Generally, biofuel in biodiesel and bioethanol is formed from various non-edible and edible sources like animal fat, waste frying oil, corn oil, and palm oil. However, their production could be more to meet the energy demand while their large-scale production needs vast amounts of land, and it will compete with other production of food crops [3]. Algae are primary producers supplying cellular carbon and chemical energy for other organisms. Algae can

be grouped as macroalgae (seaweed) and microalgae (unicellular). Among these two communities, microalgae have become one of the concerns as feedstock for future biodiesel production due to their ability to produce high amounts of lipids and high productivity [4]. Commonly, microalgae are cultivated and processed to produce beneficial compounds, use as food, and act as water filters to remove excess nutrients and other pollutants and aquaculture. Microalgae can be cultivated under three major cultivation modes, namely photoautotrophic cultivation, heterotrophic cultivation, and mixotrophic cultivation [5]. Generally, microalgae can synthesize and accumulate a variety of high-energy molecules, such as fatty acids (FA) and triacylglycerides (TAG), which are the primary feedstock for biodiesel production [6]. Some species can produce up to 40% oil, an excellent alternative source because they accumulate triglyceride and rapid growth [7]. Environmental factors such as light intensity, temperature, photoperiod, and nutrient composition in the culture system can affect the microalgal growth rates [8]. As reported by [9], light and temperature are the critical factors for controlling lipid and biomass production in algae. Increasing the temperature up to certain limits will increase algal growth. Different light intensity also affects the composition of lipids, fatty acids, and pigments in the microalgae [10]. The Genus *Chaetoceros* is one of the largest marine phytoplankton genera [11] and the most important genus in marine planktonic diatoms. This species contributes to primary production in near-shore upwelling and coastal areas [12]. This genus is widely used in aquaculture for larvae fish feedings. It is also used in marine hatcheries as a food source as well as to maintain water quality [13].

However, much research has been conducted on multiple uses of this genus in different disciplines. Some research proposed that the genus can be used in pharmaceuticals, mainly in antimicrobial compounds [14]. The genus can also be used in lipid production for industrial purposes. Some industries focus on producing high-quality fatty acids, such as PUFA extracted from *Chaetoceros* sp., that benefit humans. It has been proven that other species under the same genus, such as *C. gracilis* [15], can produce high-quality fatty acids for the lipid industry. *Isochrysis* sp. has received increasing interest because of its ability to produce the polyunsaturated fatty acid docosahexaenoic acid (DHA), one of the n-3 fatty acids believed to provide health benefits associated with the consumption of certain marine fish and their oils [16]. *Isochrysis* sp. has been widely used as a mariculture feed due to its high long-chain polyunsaturated fatty acid (PUFA) content. Therefore, this study determined the growth rate, total lipid production, and fatty acids composition of *Chaetoceros sp.* and *Isochrysis* sp. cultured at three different light intensities (1000, 2000, 3000 lux) as a potential source for biodiesel.

EXPERIMENTAL

Preparation of microalgae culture and medium

The pure stock of microalgae Chaetoceros sp., Isochrysis sp., and seawater was obtained from Fish Research Institute (FRI) Pulau Sayak, Kedah. Filtered seawater and Erlenmeyer flasks were autoclaved at pressure 1 atm with a temperature of 1210 C for 15 minutes using a semi-manual autoclave machine. The composition of the Conway medium was autoclaved before preparing the medium. The medium was prepared based on the Conway media recipe by [17].

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Table 1: Composition of Conway Medium

Chemical Composition	Amount/ Litre
Stock Enrichment Solutions	
$NaNo_3$	100.00 g
$Na_2H_2PO_4.2H_20$	45.00 g
Na_2EDTA	33.60 g
H_3BO_3	1.30 g
$Fe.Cl_3.6H_20$	0.36 g
Trace metal	1 mL
Distilled water	1 L
Trace Metal Solutions	
$ZnCl_2$	2.10 g
$CoC_2.6H_2O$	2.00 g
$(NH4)_6MO_7O_{24}.4H_2O$	0.90 g
$CuSO_4.5H_2O$	2.00 g
Distilled water	100 mL
Vitamin Stock Solutions	
Vitamin B12 (Cynocobalanium)	10.00 mg
Vitamin B1 (Thiamine)	10.00 mg
Vitamin H (Biotin)	200.00 µg
Distilled water	200.00 mL
(0.1 mL is added to each liter of seawater)	

Microalgae cultivation

Microalgae cultivation was carried out in the Marine Mariculture Laboratory located in Universiti Teknologi MARA (UiTM) Perlis Branch. Microalgae *Chaetoceros sp.* and *Isochrysis sp.* were cultivated in 250 mL Erlenmeyer flasks. Subsequently, 30 mL of pure algae*, Chaetoceros* sp., and 30 mL of *Isochrysis* sp. were introduced into 90 mL of seawater containing sodium metasilicate and Conway medium. The cultivation was carried out at three different light intensities, which were 1000 lux, 2000 lux, and 3000 lux, with a photoperiod of 12:12 hours light: dark cycle. The temperature and pH were maintained at 23±0.5^{0}C and eight, respectively, throughout the 14 days of the experiment.

Specific growth rate

The specific growth rate and division time were measured by using the formula below:

$$\text{Specific Growth Rate } (\mu) = \frac{\ln(N_2 - N_1)}{T}$$

N_2 = initial microalgae density
N_1 = microalgae density at day T
T = culture period in days

$$\text{Division rate } (\mu) = \frac{\mu}{\ln 2}$$

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Lipid extraction

The total lipid was determined by using the method of [18]. A 10 mL sample of microalgae of each species was filtered using a vacuum pump. The filtered microalgae were kept in the oven for 12 hours at 60. 2 mL of methanol and 1 mL of chloroform (2:1) were added into the centrifuge tube and shaken carefully to homogenize the content. Then, 1 mL of chloroform was added to the mixture for 1 minute, and 1 mL of distilled water was added for another 1 minute. The mixture was centrifuged for 10 minutes at 27 and 2000 rpm. The bottom chloroform layer containing lipids was collected using a Pasteur pipette and transferred into a pre-weighted centrifuge tube. Then, the centrifuge tube containing the lipid was re-weighted again. Total lipid was calculated by using the following formula:

$$\text{Total lipid (\%)} = \frac{\text{Lipid extract (g)}}{\text{Lipid in chloroform solvent (g)}} \times 100$$

Fatty acid analysis

Fatty acid analysis was determined by using the method of [19]. The fatty acids profiles were determined using an Agilent Gas Chromatograph, Model 6890N, fitted with an Agilent Mass Selective Detector, 5973 series. Separation was performed in a capillary column (30 x 0.25 mm id x 0.25µm DB wax). The starting temperature was maintained at 150 for 2 minutes at a heating rate of 10 minutes. The total running time was 22 minutes. Helium gas was used as the carrier gas, and the injection volume was 1 µL. The fatty acids peaks were identified using Agilent Technology software 5988-5871EN by comparing their retention time against the authentic standard Supelco 37 Component FAMEs Mix.

Statistical analysis

The lipid and fatty acid content of microalgae, *Chaetoceros sp.*, and *Isochrysis sp*. on different light intensities was analyzed using two-way ANOVA. The SPSS statistical software was used, and a $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Specific growth rate

Table 2 shows the result of the cell density, specific growth rate, and division time of *Chaetoceros sp*. and *Isochrysis sp*. Both microalgae show the highest maximum cell density, specific growth rate, and division time at a light intensity of 2000 lux and the lowest growth at a light intensity of 3000 lux. This finding agrees with [20], who stated that the optimum light intensity starts with an intensity of around 2500 lux as too high of a light intensity will result in photoinhibition. The result also aligns with the finding reported by [21], who found that increasing light intensity from 2000 lux will increase the growth of marine *Chlorella sp.*

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Table 2. The maximum cell density, specific growth rate (μ), and division time (μ) of microalgae, *Chaetoceros sp.*, and *Isochrysis sp*., are cultivated in different light intensities.

Microalgae species	*Chaeteceros* sp.		
Light intensity	Max cell density (x10^6 cell mL^{-1}	μ (day^{-1})	K (day^{-1})
1000 lux	9.83	1.55	2.24
2000 lux	10.58	1.74	2.51
3000 lux	8.10	1.68	2.42
	Isochrysis sp.		
1000 lux	10.22	1.71	2.47
2000 lux	12.47	1.75	2.52
3000 lux	9.97	1.70	2.45

The light or dark regime is needed in microalgae for productive photosynthesis, where the light is needed for the photochemical phase to produce ATP and NADPH. In contrast, the biochemical phase needs the dark to synthesize essential molecules for growth [22]. Light is one of the most critical factors affecting microalgae growth and photoperiod, which plays a vital role in microalgae growth. However, these two parameters vary significantly with the species, culture condition, and depth of the cultivation [23].

Lipid Composition in Chaetoceros sp. and Isochrysis sp.

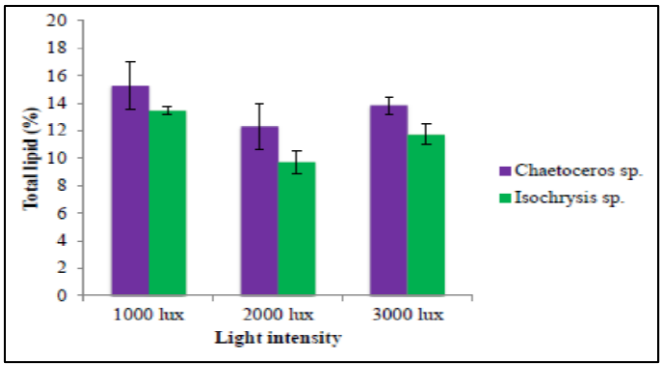

Fig. 1. Lipid content in *Chaetoceros sp.* and *Isochrysis sp*. in 1000 lux, 2000 lux and 3000 lux.

In the three different light intensities, lipid contents in *Chaetoceros sp.* and *Isochrysis sp.* range from 9.72% to 15.28%. The highest lipid content in *Chaetoceros sp.* is 15.28% at 1000 lux, followed by 13.85% at 3000 lux and 12.32% at 2000 lux. The lipid content in this study was higher than the previous study reported by [24] but in line with the result reported by [25], which found that the percentage of lipids in *Chaetoceros sp.* is 15.40%. The variations in total lipid content in the species are affected by different conditions such as culture conditions, cultivation type, nutrient concentration, growth rate, life cycle phase, environmental conditions, and the state of cells in the culture period [26]. The lipid content in *Isochrysis sp* ranges from 9.72% to 13.47%. The highest lipid percentage in *Isochrysis sp*. is 13.47% at 1000 lux, followed by 11.74% at 3000 lux and 9.72% at 2000 lux. The percentage of lipids in the present study is lower than the previous study reported by [25,27], which found that lipids in *Isochrysis* sp are 17.00%. The results show significant differences between microalgae species and light intensity in lipid production (two-way ANOVA, $p<0.05$). Among these two microalgae, *Chaetoceros sp.* has the highest lipid composition in all treatments compared to *Isochrysis sp*. The lipid content, lipid class composition, and the proportions of

https://doi.org/10.24191/sl.v18i1.23906

the various fatty acids in microalgae also vary according to the environmental or culturing variables such as light intensity, temperature, CO_2 concentration, nitrogen, and phosphorus concentration. The result shows that both microalgae have the highest lipid production at 1000 lux. As stated by [28], the type and intensity of light did not cause variations in protein content yet showed an essential decrease in carbohydrates and an increase in lipids. This result aligns with the previous study by [23], which found that increased light intensity enhances microalgae growth. However, it reduces the lipid content**.** The microalgae might have used the synthesized energy from the light to divide themselves rather than accumulate lipids [21]. A previous study reported by [23] showed that *Ankistrodesmus falcatus* has the highest lipid content (37.12%) in the culture grown under a light intensity of 60 µmol m^{-2} s^{-1}; however, in light intensities of 30 µmol m^{-2} s^{-1} and 150 µmol $m^{-2}s^{-1}$ lipid content which was found were 30.99% and 31.89% respectively. Low light intensity was more suitable for lipid accumulation in *Ankistrodesmus falcatus* than high light intensity.

The optimal light intensity levels for supporting cell growth and lipid accumulation differed. Moreover, different light intensities and light-dark cycles have been reported to alter microalgae's lipid metabolism and lipid profile. Optimal light intensity is required for favorable growth and productivity of the algae in the cultivation. The light intensity needed also may vary for each species of microalgae. The higher lipid production at high light intensity is due to the storage of excess light energy converted to chemical energy to overcome cell damage [29]. As stated by [7], in their study, *Isochrysis galbana* has the highest number of cells (8.56 x 107) compared to *Dunaliella tertiolecta* (0.94 x 106). However, the lipid production of *Dunaliella tertiolecta* (11.64%) has shown a high percentage of lipids compared to *Isochrysis galbana* (10.54%). Thus, it shows that a more significant number of cells does not necessarily mean it has a high amount of lipid. Besides, the morphological change in marine microalgae will affect the overall lipid contents [8]. Usually, lipid compounds are synthesized by microalgae as phospholipids, triacylglycerol in cell membranes, and intracellular energy storage components [30]. Under suitable environmental conditions, microalgae will synthesize fatty acids to produce membrane glycerolipids, such as glycolipids and phospholipids. However, many microalgae change their lipid under unfavorable growth conditions to produce large amounts of neutral lipids [31]. Glycolipids are rich in stearidonic acid, while saturated fatty acids such as myristic and palmitic acids are abundant in phospholipids. Meanwhile, oleic acid is dominant in neutral lipids [32].

Fatty acid composition in *Chaetoceros* sp. and *Isochrysis* sp.

Fig. 2 and Fig. 3 below show the composition of Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA), and Polyunsaturated Fatty Acid (PUFA) at 1000, 2000, and 3000 lux in *Chaetoceros sp.* and *Isochrysis* sp.

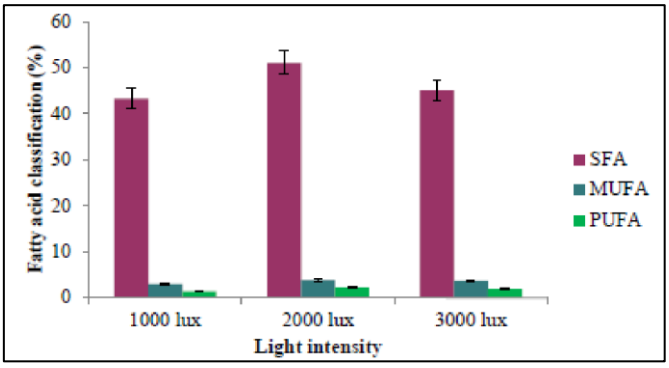

Fig. 2**.** Fatty acid composition of *Chaetoceros sp.* cultivated in light intensity 1000 lux, 2000 lux, and 3000 lux.

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The SFA is the primary fatty acid found in both *Chaetoceros sp.* and *Isochrysis sp.*, followed by MUFA and PUFA in all light intensity studied. SFA plays a significant role in fuel properties. The cetane number increases in fuels with high amounts of SFA [33]. Both species studied show the highest percentage of fatty acid at 2000 lux.

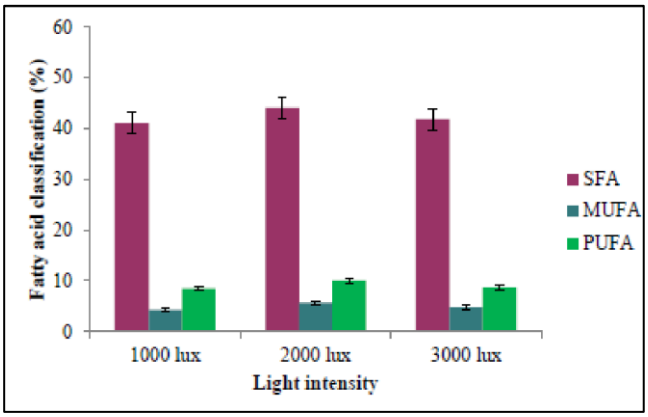

Fig. 3. Fatty acid composition of *Isochrysis* sp. cultivated in light intensity 1000 lux, 2000 lux, and 3000 lux.

The percentage of SFA in *Chaetoceros sp.* is 43.28% at 1000 lux, 51.06% at 2000 lux, and 45.04% at 3000 lux, while in *Isochrysis sp*. is 41.07% at 1000 lux, 44.05% at 2000 lux, and 41.76% at 3000 lux. Among the three fatty acids, PUFA represents the lowest fatty acid composition among the two species studied. Microalgae usually produce large amounts of polyunsaturated fatty acids (PUFA) that benefit aquaculture. However, many PUFA is useless for biodiesel production because it reduces biofuel oxidative stability. The level of oxidation stability of biodiesel increases as polyunsaturated fatty acid composition decreases [19]. The synthesis of biodiesel fuel with algal oils that contain more saturated fatty acid and monounsaturated fatty acid will result in a higher number of cetane, lower emissions of hydrocarbon, lower emissions of nitrogen monoxide, smoke, and carbon monoxide as well as shorter ignition delay [34]. Biodiesel from microalgae has advantages over petroleum diesel. This is because biodiesel is derived from biomass. Thus, it is renewable, biodegradable, non-toxic, and contains reduced particulates such as carbon monoxide, soot, and hydrocarbons. Besides, the significant advantage is that biodiesel from microalgae can reduce carbon dioxide emissions by up to 78% compared to emissions from petroleum diesel [35]. The cetane number of the biodiesel increases with the increase in the chain length of the fatty acids and saturation. The high content of unsaturated components such as linoleic acid and linolenic acid results in a low cetane number of biodiesels [36]. Fig. 4. below shows the fatty acid composition of *Chaetoceros sp.* and *Isochrysis sp.* cultivated in different light intensities.

https://doi.org/10.24191/sl.v18i1.23906

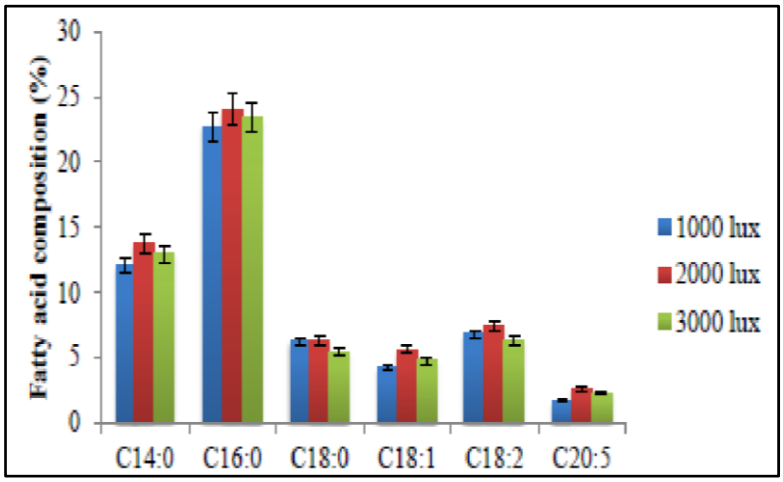

Fig. 4. Fatty acid composition of *Chaetoceros sp.* and *Isochrysis sp.* cultivated in different light intensities 1000 lux, 2000 lux, and 3000 lux.

The primary fatty acids found in both *Chaetoceros sp.* and *Isochrysis sp.* are C16:0, C14:0, C18:0, C18:1, C20:5 and C18:2. Saturated Fatty Acid (SFA) which consists of C16:0, C14:0 and C18:0, Monounsaturated fatty acid (MUFA) is C18:1. At the same time, Polyunsaturated Fatty Acid (PUFA) includes C20:5 and C18:2. From the result, it shows that the dominant fatty acid found in all treatments is C16:0 with 21.95%, 26.47% and 23.16% at light intensity 1000 lux, 2000 lux and 3000 lux respectively. Their results show that a high amount of fatty acids was produced at 2000 lux, followed by 3000 lux and 1000 x. The present study agrees with the previous study reported by [37] which found that the most common fatty acids of microalgae are-C16:0, C18.0, C18:1, C18:2 and C18:3. Most algae have only small amounts of eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6). However, in some species of genera, these PUFAs can accumulate in appreciable quantities depending on cultivation conditions. Generally, SFA contains high cetane numbers (C12:0, C16:0, and C18:0), and this is considered a massive advantage because they improve the oxidative stability of the biodiesel, whereas C18:1 acts as a controller to biodiesel with low-temperature properties and becomes a balance between oxidative stability. Its proportion is considered an important index to assess the biodiesel quality of microalgae oil [38]. The result of fatty acids reveals that both of these species have the potential of biodiesel as they are the main components in biodiesel which are palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), palmitoliec acid (16:1) and myristic acid (14:0). All the microalgal lipids are mainly composed of 40-50% saturated and 50-60% unsaturated fatty acids [33]. For biodiesel production, algae with a high proportion of saturated fatty acids are preferred because these lead to higher oxidative stability and ignition quality (cetane number) and produce an overall higher quality product [34,39]. Furthermore, the fatty acid methyl ester profile is critical in determining any feedstock's suitability for biodiesel fuel production [39].

CONCLUSION

The results indicated that the highest growth rate for these samples was obtained at 2000 lux, which resulted in 12.32% and 9.72% for *Chaetoceros sp.* and *Isochrysis sp.*, respectively. The highest composition of saturated fatty acids, which could produce high-quality biodiesel, was also obtained at this light intensity for both species *Chaetoceros sp.* (51.06%) and *Isochrysis sp.* (44.05%). Both species also contain a high composition of C14, C16, and C18 fatty acids, which are essential components for the selection of alternative sources of biodiesel. Therefore, a light intensity of 2000 lux is recommended to be applied during the growing stage of this seaweed to produce quality biodiesel.

https://doi.org/10.24191/sl.v18i1.23906

ACKNOWLEDGEMENT

Upon completing this paper, I would like to thank and appreciate Siti Nur Rohimah Jamaluddin and all the lecturers of Marine Technology for their hard work, opinions, comments, and suggestions and for sharing their experiences.

AUTHOR'S CONSTRIBUTION

Zamzila Erdawati had carried out the research and wrote the article. Aziani and Khairul Naim had conceptualized the central research idea, provided the theoretical framework and analysis the data. Sharir Aizat and Zamzila Erdawati had designed the research, monitor the research progress and revised the article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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