Revelation and Science
Vol. 14, No. 01 (1445H/2024) 11-27

Biomass and Lipid Production by Microalgal Strain Isolated from Freshwater Lake in Kuantan, Pahang in Response to Nutrient Stress and Its Phycoremediation Potential

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ABSTRACT

Microalgae is a potential feedstock for biofuel production due to high biomass production and lipid content. It is a treatment agent for pollution as it can remove excess nutrient from water using a technique called phycoremediation. In this study, freshwater microalgae are isolated and purified with the aim to screen for high biomass yield and lipid content. The microalgal strain *Scenedesmus* sp. and *Oscillatoria* sp. were successfully isolated with preliminary screening of biomass yield of 0.0502 ± 0.005 g dwt L^{-1} by *Oscillatoria* sp. compared to the yield of *Scenedesmus* sp. which was 0.0873 ± 0.004 g dwt L^{-1}. *Scenedesmus* sp. showed the high value of lipid content which was 87% while *Oscillatoria sp.* constitutes 49% of lipid content. The conditions of nutrient stress or starvation particularly for nitrate and phosphate were established to determine whether the biomass yield and lipid content of *Scenedesmus* sp. could be further increased but it was proved to be vice versa. This study has able to find potential locally isolated candidate for biofuel production with the additional potential in remediation of pollutants such as nitrate and phosphate and can be further manipulated to increase the biomass yield and lipid content.
Keywords: *Microalgae, Scenedesmus sp., biofuel, lipid content, phycoremediation*

ABSTRAK

Microalgae adalah bahan mentah yang berpotensi untuk pengeluaran biofuel kerana pengeluaran biojisim yang tinggi dan kandungan lipid. Ia adalah agen rawatan untuk pencemaran kerana ia boleh mengeluarkan nutrien yang berlebihan dari air menggunakan teknik yang dipanggil phycoremediation. Dalam kajian ini, mikroalga air tawar diasingkan dan disucikan dengan tujuan untuk menyaring hasil biojisim yang tinggi dan kandungan lipid. Strain mikroalgal Scenedesmus sp. dan Oscillatoria sp. berjaya diasingkan dengan pemeriksaan awal hasil biojisim 0.0502 ± 0.005 g dwt L-1 oleh Oscillatoria sp. berbanding hasil Scenedesmus sp. iaitu 0.0873 ± 0.004 g dwt L-1. Scenedesmus sp. menunjukkan nilai kandungan lipid yang tinggi iaitu 87% manakala Oscillatoria sp. membentuk 49% kandungan lipid. Keadaan tekanan nutrien atau kelaparan terutamanya untuk nitrat dan fosfat telah ditubuhkan untuk menentukan sama ada hasil biojisim dan kandungan lipid Scenedesmus sp. boleh ditingkatkan lagi tetapi ia terbukti sebaliknya. Kajian ini telah dapat mencari calon terpencil tempatan yang berpotensi untuk pengeluaran biofuel dengan potensi tambahan dalam pemulihan bahan pencemar seperti nitrat dan fosfat dan boleh dimanipulasi lagi untuk meningkatkan hasil biojisim dan kandungan lipid.
Kata kunci: Microalgae, Scenedesmus sp., biofuel, kandungan lipid, phycoremediation

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1. INTRODUCTION

Microalgae have become the attention in biotechnology research due to their potential in biofuel productions. Depletion of fossil fuels has increased the awareness to explore alternative fuels such as biofuel which are more economical, renewable, and environmentally friendly. Microalgae has been suggested as a good candidate for biofuel production as they have higher productivities of biomass and a faster growth rate than higher plants with cell doubling time of one to four days. They are one of the sources of lipid to produce biofuels (Griffiths and Harrison, 2009) and their high lipid productivity is one of the characteristics of microalgae essential to produce biodiesel. Moreover, this third-generation biofuel production from microalgae has emerged as a feasible option compared to the first-generation biofuel (i.e generated from edible crops biomass) and second-generation biofuel (i.e generated from non-food crops). This makes microalgal biofuel production moves to a more sustainable manner, fulfilling all the criteria of economic, environmental and social aspects of sustainable biofuels (Patnaik and Malick, 2021).

Microalgae can grow practically anywhere with enough light, has high photosynthetic ability and short regeneration time (Ahmad et al., 2011). Microalgae are more efficient to perform photosynthesis and are able to remove carbon dioxide up to 50 folds more than higher plant (Li et al., 2008). In addition, freshwater microalgae are a group of organisms that are extremely heterogenous with only minimum nutrient requirements and therefore is a suitable candidate for biofuel production. They include strains such as *Chlamydomonas*, *Chlorella*, *Microcystis*, *Oscillatoria*, *Scenedesmus*, *Spirogyra* and many more (Azeez et al., 2021, Sinha et al., 2016). Phosphorus and nitrogen are essential nutrients that play a significant role in microalgal growth and their biochemical compositions. Microalgae can uptake phosphorus in the form of polyphosphate or orthophosphate and can assimilate nitrogen in the form of nitrate, nitrite, urea and ammonium although the most stable one with less likelihood of pH shift would be the use of nitrate for microalgae culture (Yaakob et al., 2021).

Different microalgae produce different types of biofuels as suggested by Maity et al. (2014). Green algae like *Chlorella* sp., *Scenedesmus* sp. and *Spirulina* sp. can produce hydrogen, biodiesel ethanol and methanol. Blue-green algae (*Cyanobacteria* sp.) can produce biodiesel, ethanol and methane while red algae such as *Haematococcus pluvialis* can produce biodiesel. Large quantities of oil are produced by some species of algae, regularly achieving 50% to 60% of their lipid contents (Chisti, 2008). Production of lipid content by microalgae such as *Scenedesmus* sp. can be enhanced by starvation of nitrogen, phosphates and by applying stress on physical stimuli such as light intensity, pH, and temperature (Pancha et al., 2014, Pushpakumari Kudahettige et al., 2018). Chu et al. (2014) mentioned that sufficient number of phosphates in nitrogen deficient condition may enhance the lipid production for biodiesels. Despite of very high accumulation of lipid content up until more than 70% in some microalgae species which under special nutrient starvation conditions, the overall lipid productivity under nutrient shortage was somehow affected (Gour et al., 2016). Lipid productivity which is defined by lipid content and growth rate would be optimum by further bioprospecting process of isolating, screening and selecting the fast-growing oleaginous microalgal species (Lim and Schenk, 2017). This is considered as optimization of upstream part of biofuel production while the downstream part such as harvesting and after harvesting also can be manipulated to increase lipid productivity (Peng et al., 2020). Latest development in microalgal biofuel production even involves the addition of various forms of nano-additives to enhance production (Hossain et al., 2019).

In addition, the use of microalgae in the process of remediation of pollutants known as phycoremediation also further exploit their ability. It is one of the alternative strategies for wastewater treatments and is more sustainable, feasible, more environmentally friendly and cost-effective as compared to the conventional treatment (Koul et al., 2022). Extensively used microalgae for wastewater treatment includes species of the genera Chlamydomonas, Chlorella and Scenedesmus (Priyadharshini et al., 2021). Another application of microalgae in bioremediation is as potential biosorbents which sequester heavy metal ions in wastewater and assisting in removing these pollutants (Ankit et al., 2022). Pollutants such as nitrogen and phosphorus which are the two major compounds in agricultural industries might leach into groundwater or transferred to other water bodies through surface runoff. These two chemicals are widely used in agriculture industry as pesticides and also exist in detergents, manures of animals and other daily products. In phycoremediation, microalgae convert solar energy into useful product such as oxygen for the usage of other organisms and at the same time reducing the nutrients contents in the water preventing the formation of eutrophication (El-Sheekh et al., 2016).

All the obstacles and challenges in biofuel production by microalgae such low growth rate as well as biomass output, low lipid content and productivity can be further resolved with optimizing the processes involved in biofuel production while at the same time identifying other species of microalgae to discover new isolates with desirable properties for biofuel production. Therefore, this study aims to isolate and identify new local freshwater microalgal strains which could produce high lipid productivity with environmental stressors such as the nitrogen and phosphorus pollutants. These microalgal isolates can be further exploited as potential candidates for phycoremediation with an added value of having high biomass production to be turned into biofuel feedstocks.

2. MATERIALS AND METHODS

2.1 Isolation and Purification of Microalgal Strains

Water samples were collected from sites that appeared green in the freshwater lake at Taman Bandar, Kuantan, Pahang. Isolation and cultivation of microalgae was done using the bold basal medium (BBM) in which 10 mL of water samples from the lake were inoculated into 200 mL of the media. Incubation was done with 150 $\mu mol.m^{-2}.s^{-1}$ light intensity, light/dark cycle 12:12 hours and at room temperature (23-25 ^{o}C). Serial dilutions were prepared, and further purification of the microalgae was done using a capillary glass pipette under the light inverted microscope. The microalgal colony were placed in the 24-well plate filled up with the media and incubated for 21 days with periodical observation of every 2 days. Selected isolated strains were streaked on agar plate containing solidified media with bacteriological agar. The cultures were incubated under 150 $\mu mol.m^{-2}.s^{-1}$light intensity, light/dark cycle 12:12 hours and at room temperature until a single colony was formed. This process was repeated a few times to obtain pure strain. Microscopic observation was performed to further identify the isolates.

2.2 Microalgal Strains Growth and Biomass

Microalgal cells inoculum ($OD_{680} \approx 0.05$) were inoculated in 800 ml of BBM at room temperature with shaking at 150 rpm under continuous fluorescence illumination for 21 days. Algal growth was monitored by measuring optical density at 680 nm for every 48h. Microalgal cells were harvested by centrifugation and washed twice with deionized water after 21 days of incubation. Microalgal pellets were freeze-dried and the dry weight per litre (g L-1) was measured. Experiments were carried out in triplicates, and data are expressed as mean ± SD.

2.3 Extraction of Total Lipid

Total lipids were extracted from fresh microalgal biomass using a modified Folch method (Iverson *et al.,* 2001). The biomass was homogenised with chloroform-methanol (2:1, v/v) and the whole mixture was agitated for 30 minutes at room temperature. The homogenate was centrifuged to recover the watery phase and was washed with 0.2 volume of water. The mixture was vortexed followed by the centrifugation at low speed (2000 rpm) to separate the two phases. The upper phase was removed, and the interface was rinsed twice with methanol-water (1:1, v/v) without mixing the whole preparation. After centrifugation and removal of the upper phase, the lower chloroform, phase containing lipids was evaporated in the water bath for 4 hours. Thereafter, the weight of the crude lipid obtained from each sample was measured by weighing the crude lipid. Experiments were carried out in triplicate, and data are expressed as mean ± SD.

2.4 Biomass Yield, Lipid Content and Nutrient Removal Analysis of Selected Microalgae

Further analysis of biomass yield, lipid content and nutrient removal by selected microalgae were conducted to determine its potential for phycoremediation. Biomass yield and lipid content in different phosphates and nitrates concentration were measured as described in section 2.2 and 2.3 respectively. In order to measure nutrient removal, absorbance at 890 nm and 410 nm were recorded for microalgae grown in different phosphate and nitrate concentration respectively. Concentration of phosphate and nitrate in the media at specific days of sampling were determined based on the standard curve constructed prior to the experiment.

3. RESULTS AND DISCUSSIONS

3.1 Identification of Microalgal Strains

There were two isolates that have been successfully identified. These isolates were identified based on the morphological characteristics which were the size and shape of the isolates viewed under the light microscope at magnification of 40x.The first one is blue greenish in colour; cells are simple filament with cylindrical shape or slightly tapering with small diameter cells. This isolate is identified as *Oscillatoria* sp. under the phylum of Cyanophyta (Figure 1 (a)). They do not have heterocyst and have very long filament up to 100 µm (van Vuuren et al., 2006). Another microalgae isolate is identified as *Scenedesmus* sp. (Figure 1 (b)) in which they are small green, colonial microalgae, usually 4 or 8 single-celled attached and the cells were aligned in a flat plate with oblong shape. Their cells have nucleus, and the colonies are non-motile (van Vuuren et al., 2006). The average size recorded was 17-13 µm in length and 16-12 µm in width.

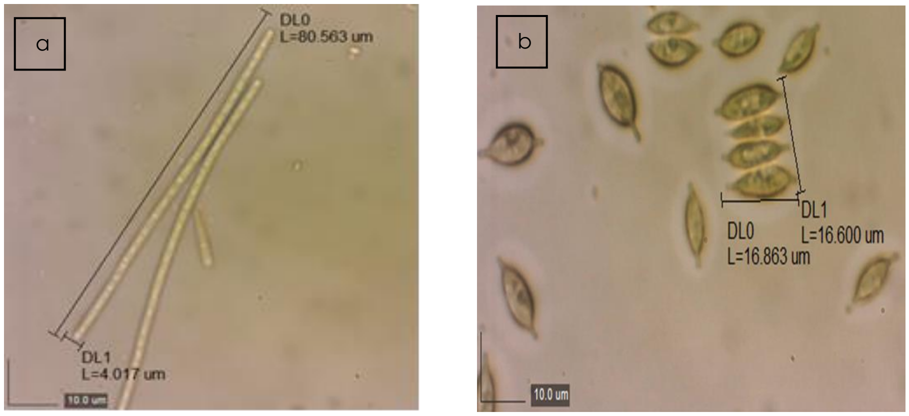

Figure 1 Identification of microalgae isolates by morphological observation. a) *Oscillatoria* sp. and b) *Scenedesmus* sp. under the light microscope at 40x magnification.

3.2

Growth, Biomass and Lipid Content of Microalgal Strains

In terms of growth in BBM, the green colour was seen at day 7 of culture and based on the growth curve plotted, both microalgal strains grew exponentially and reached the stationary phase at about day 17 (Figure 2). The two microalgal strains were measured for the lipid productivity by monitoring the biomass yield and lipid content in cultures under the similar conditions after 21 days of incubation (Table 1). Biomass yield (g dwt L^{-1}) recorded for *Oscillatoria* sp. and *Scenedesmus* sp. was 0.0502 ± 0.005 g dwt L^{-1} and 0.0873 ± 0.004 g dwt L^{-1} respectively. Meanwhile, the total lipid contents obtained from *Oscillatoria* sp. was 49% and *Scenedesmus* sp. contained about 87%. *Scenedesmus* sp. showed a higher value of biomass yield and lipid content compared to *Oscillatoria* sp. which makes its lipid productivity also higher which is approximately 0.0756 ± 0.003 g L-1.

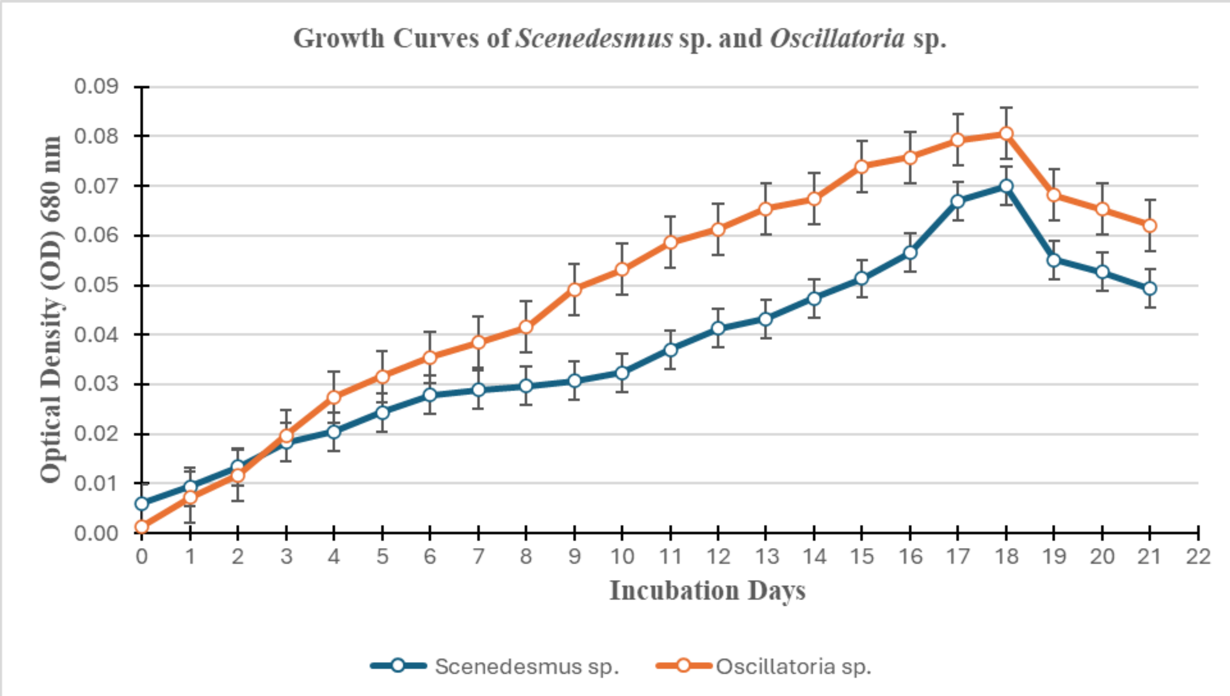

Figure 2 Growth curve of both isolates cultivated in BBM with 150 μmol.m^{-2}s^{-1} light intensity, light/dark cycle 12:12 hours and at room temperature (23-25 ^{o}C) for 21 days.

Table 1 Biomass yield, lipid content and lipid productivity of the isolates. Experiments were carried out in triplicates and data are expressed as mean ± SD.

Isolates	***Scenedesmus* sp.**	***Oscillatoria* sp.**
Biomass yield (g dwt L^{-1})	0.0873 ± 0.004	0.0502 ± 0.005
Lipid productivity (g L^{-1})	0.0756 ± 0.003	0.0244 ± 0.002
Lipid content (% biomass)	87	49

Many microalgae species including the blue-green algae can be induced to accumulate substantial quantities of lipids, thus leading to higher oil content including those isolated from freshwater environment (Prihanto et al., 2022; Sharma et al., 2012). The lipid productivity of microalgae also might be increased when microalgae was induced by nutrient limitation (Shokravi et al., 2020). For example, under nitrogen deprivation, green microalgae were able to yield 30% lipid content which was high compared to normal conditions (Suparmaniam et al., 2023; Breuer et al., 2012). This is because, under unfavorable conditions, microalgae will accumulate neutral lipid due to reprogramming of their lipid biosynthesis pathway to encounter adverse environmental conditions. Nutrient stress environment such as involving phosphate and nitrate starvation has been suggested to benefit the synthesis and accumulation of lipids and therefore can be manipulated for biofuel production (Song et al., 2022; An et al., 2020; Chen et al., 2017). Further analysis of the effect of nutrient stress to the potential biofuel producing microalgal strain with the highest lipid content among the two, *Scenedesmus* sp. was done to investigate whether the yield and lipid content could be more inducible with the stressors.

3.3 Biomass Production and Lipid Content of *Scenedesmus* sp. in Nutrient Stress Environment

3.3.1 Growth of Scenedesmus sp. in different phosphate and nitrate concentrations

Growth pattern of *Scenedesmus* sp. in modified BBM with different concentrations of phosphate were shown in Figure 3. All samples showed increment in growth over time throughout the 24-days cultivation except the culture amended with 0.2 mg/L phosphate as a slight decrement could be seen after Day 8 but the growth increased after Day 10. Sample which showed the highest reading of optical density at Day 24 was from the culture amended with 0.5 mg/L phosphate with the reading of 0.056 and sample from the culture amended with 0.2 mg/L phosphate showed the lowest reading with absorbance of 0.036.

Meanwhile, growth pattern of *Scenedesmus* sp. in modified BBM with different concentrations of nitrate is shown in Figure 4. In average, all cultures showed increment in growth over time. By Day 22, only culture amended with 2.5 mg/L and 5.0 mg/L nitrate showed reduction in growth while culture amended with 10.0 mg/L, 15.0 mg/L and 25.0 mg/L nitrate still showed increment in growth. Sample which showed the highest reading of optical density at Day 24 was from the culture amended with 15 mg/L nitrate and sample from the culture amended with 2.5 mg/L nitrate showed the lowest reading of absorbance.

In the phosphate modified BBM, the absorbance reading for cultures were almost comparable to the absorbance readings recorded for growth of *Scenedesmus* sp. in unmodified BBM (Figure 2). In contrast, cultures in nitrate modified BBM recorded lower absorbance readings compared to culture of *Scenedesmus* sp. in unmodified BBM. From the result obtained, it was shown that the addition of different concentrations of either phosphate or nitrate did not increase the *Scenedesmus* sp. growth. It was reported previously that the limitation of nitrogen in the culture medium may lower biomass production but would enhance lipid production and it was also reported that microalgae growth and phosphate uptake are linearly proportional to the biomass yield (Yaakob et al., 2021).

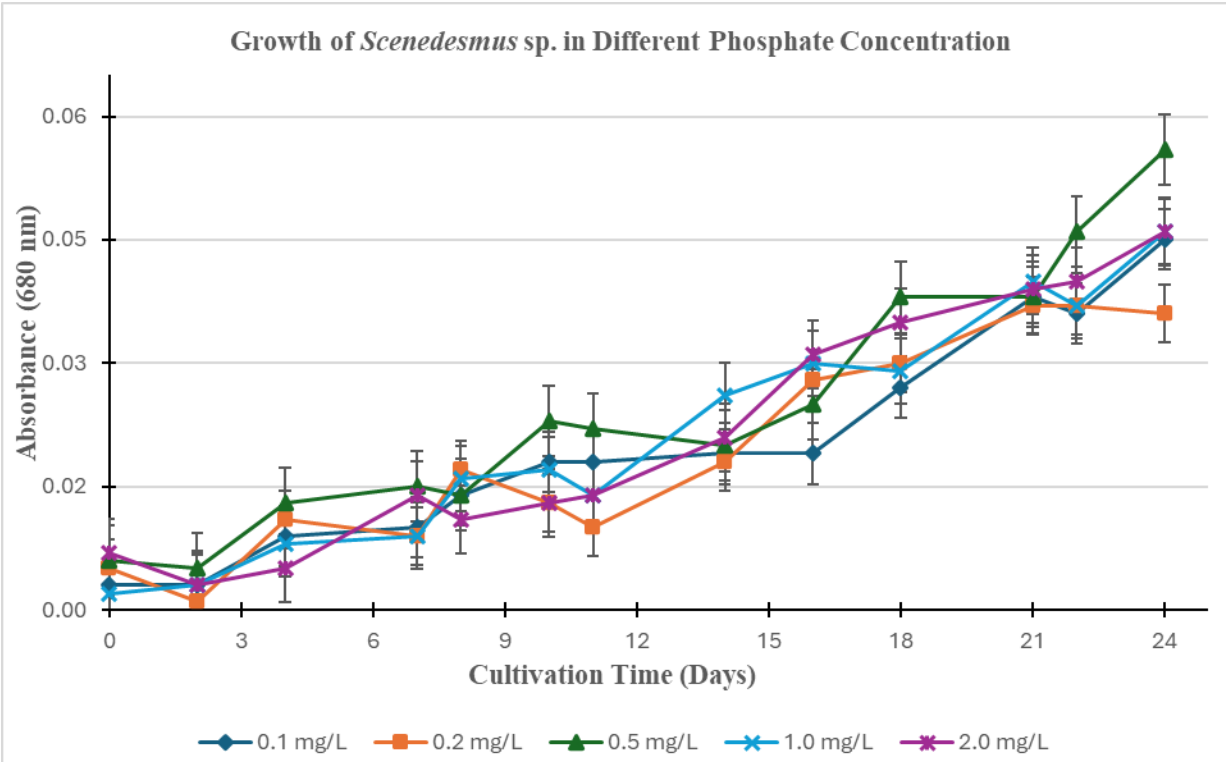

Figure 3 Growth of *Scenedesmus sp.* in BBM with modified concentration of phosphate.

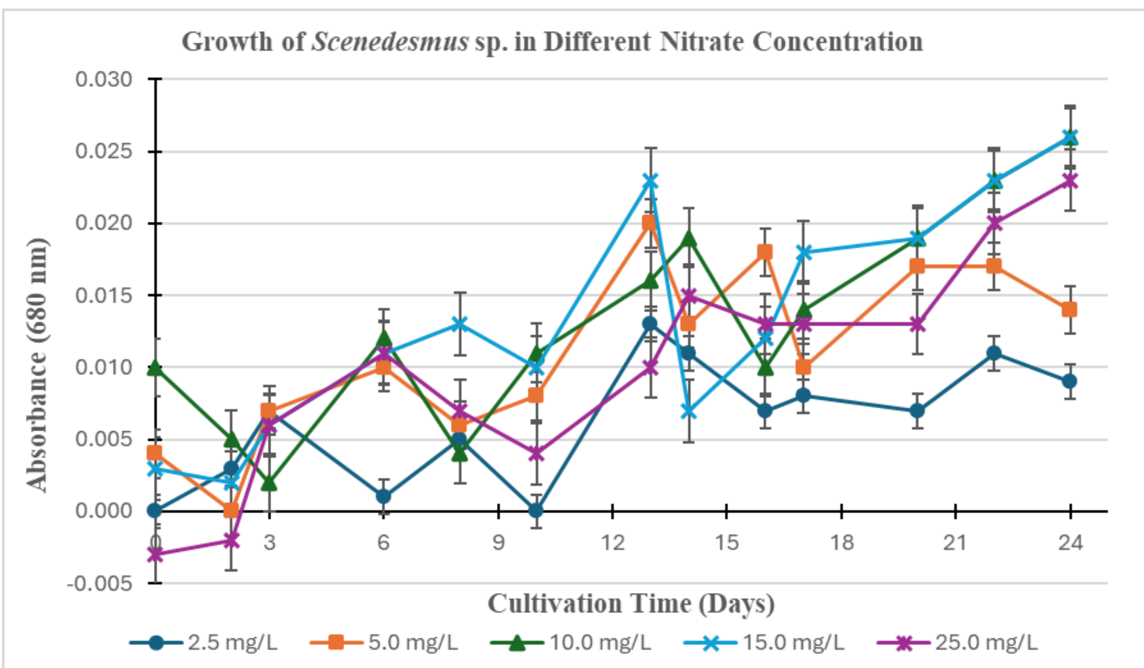

Figure 4 Growth of *Scenedesmus sp.* in BBM with modified concentration of nitrate.

3.3.2 Biomass Yield of Scenedesmus sp. in different phosphate and nitrate concentrations

In terms of biomass yield in different phosphate concentrations, the culture of *Scenedesmus* sp. amended with 0.5 mg/L phosphate produced the highest value of biomass which is 0.0210 g L^{-1} while the lowest production of biomass was recorded from the culture of *Scenedesmus* sp. amended with 0.2 mg/L phosphate which yielded about 0.0153 g L^{-1} cell biomass (Figure 5). This is proportional to the absorbance readings recorded in the growth analysis of *Scenedesmus sp.* in BBM with modified concentration of phosphate. Meanwhile, for the biomass yield of *Scenedesmus* sp. in different nitrate concentrations, culture amended with 25.0 mg/L nitrate showed highest biomass production with the weight of 0.0215 g L^{-1} and culture amended with 2.5 mg/L of nitrates concentration produced the lowest biomass of 0.0110 g L^{-1} (Figure 6). There is a little difference in comparison to absorbance reading during growth analysis of *Scenedesmus sp.* in BBM with modified concentration of nitrate since the highest absorbance recorded was from culture amended with 15 mg/L nitrate but the highest biomass was from culture amended with 25.0 mg/L nitrate. Nevertheless, the microalgae growth rate and biomass yield are expected to be low under the nutrient deficiency conditions as reported by previous studies (Khan et al., 2018) as P, N, and C are the primary inorganic nutrients that are essential for microalgal growth.

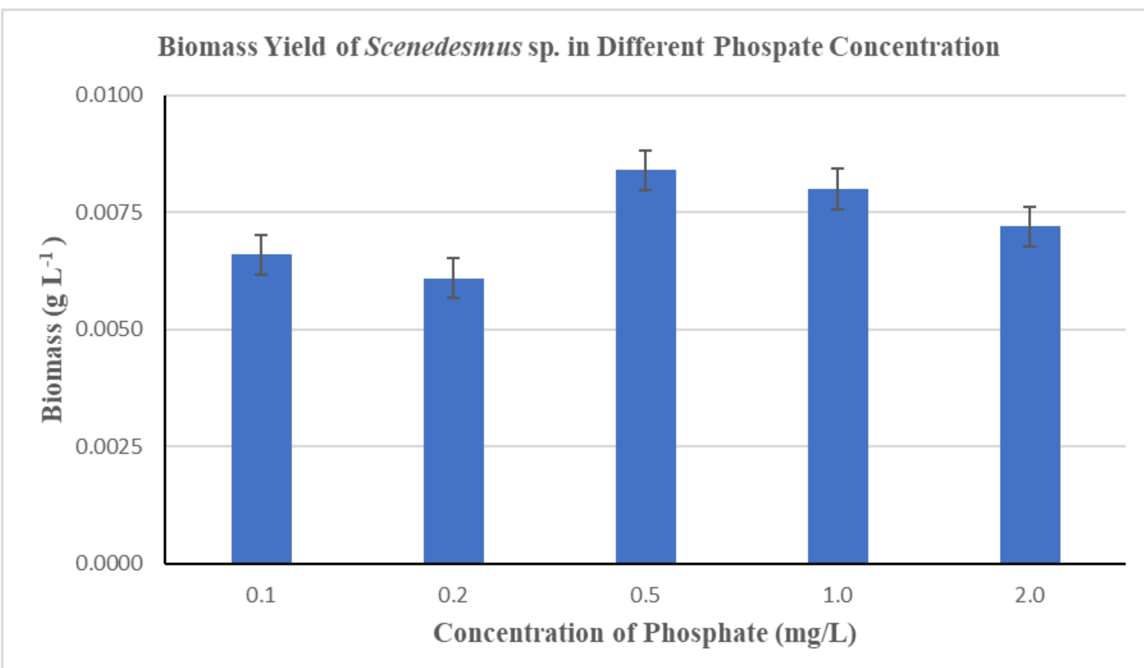

Figure 5 Biomass produced by *Scenedesmus sp.* cultivated in BBM with modified concentration of phosphate.

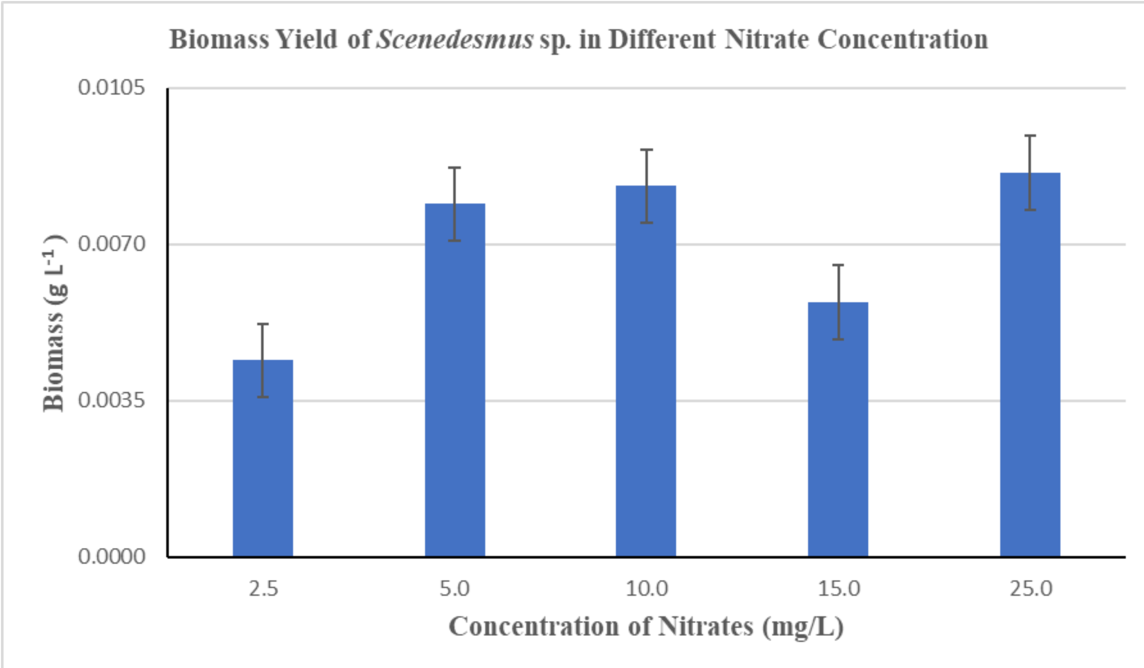

Figure 6 Biomass produced by *Scenedesmus sp.* cultivated in BBM with modified concentration of nitrate.

3.3.2 Lipid Content of Scenedesmus sp. in different phosphate and nitrate concentrations

For lipid production, culture of *Scenedesmus* sp. amended with 0.2 mg/L phosphate produced the highest percent of dry weight which is about 34.3%. The least amount of lipid produced was by culture of *Scenedesmus* sp. when the culture media was amended with the highest phosphate concentration which is 2.0 mg/L (Figure 7). It can be observed that culture of *Scenedesmus* sp. amended with 0.2 mg/L phosphate which produced the least biomass showed the highest amount of lipid production. In terms of lipid production by *Scenedesmus* sp. cultivated in BBM with modifications in nitrate concentrations, the lipid content increased as the nitrate concentration increased (Figure 8). Cultures of *Scenedesmus* sp. amended with the highest nitrates concentration, 25.0 mg/L produced highest lipid content with 59% of dry weight of lipid. Lowest lipid content was produced by both cultures of *Scenedesmus* sp. amended with 2.5 mg/L and 5.0 mg/L nitrate with 27% of dry weight of lipid respectively.

Phosphorus which is available to microalgae as soluble phosphate has been reported to increase lipid production when being restricted in microalgae culture (Maltsev et al., 2023). However, the effect of phosphorus deficiency on microalgae growth and lipid accumulation still needs to be further researched since the requirements varies for different microalgae species. It can be observed in this research too that the lipid production by culture of *Scenedesmus* sp. appeared inconsistent across amendment with different phosphate concentrations. On the other hand, nitrogen deficiency disrupts physiological processes in microalgae, a decrease in the content of the main photosynthesis pigments and finally the disruption of photosynthesis (Dixit et al., 2020). The lack of nitrogen in microalgal cells which are experiencing nutritional stress changes carbon flow from protein synthesis to carbohydrate or lipid synthesis (Zheng et al., 2020). However, in this research the lipid content increase with the increment of nitrate concentrations in the culture of *Scenedesmus* sp. which is the opposite trend for nitrogen starvation and lipid content.

Previous research involving *Scenedesmus* sp. LX1 culture by Xin and co-workers (2010) reported that it could accumulate lipids to as high as 30% of its biomass in conditions of nitrogen limitation (2.5 mg L^{-1}) or 53% of its biomass in conditions of phosphorus limitation (0.1 mg L^{-1}) respectively. In another research by Yang and co-workers (2018), the culture of *Scenedesmus* sp. grown in 50 mg/L phosphorus achieved 22.3% lipid content, whereas lipid yield reached 42.5% in 1 mg/L phosphorus. However, it has been reviewed that the optimum phosphorus concentration for microalgae in general is in the range of 0.001 g/L to 0.179 g/L (Yaakob et al., 2021) and therefore limiting the phosphate concentrations to a very low extent might be a better strategy for increasing lipid production. Meanwhile, nitrogen-deficient culture conditions for the growth of *Scenedesmus* sp. has been reported to produce two-fold lipids compared to nitrogen-sufficient medium as reported by Yaakob and co-workers (2021). However, nitrogen limitation in culture conditions of *Scenedesmus* sp. can also result in the reduction of microalgae biomass productivity which might as well proportionally influence the lipid production (An et al., 2020). As seen in this research, the increment of nitrate concentrations proportionally increases the biomass yield and also lipid content for the culture of *Scenedesmus* sp. and therefore might be a good strategy for enhancing lipid production.

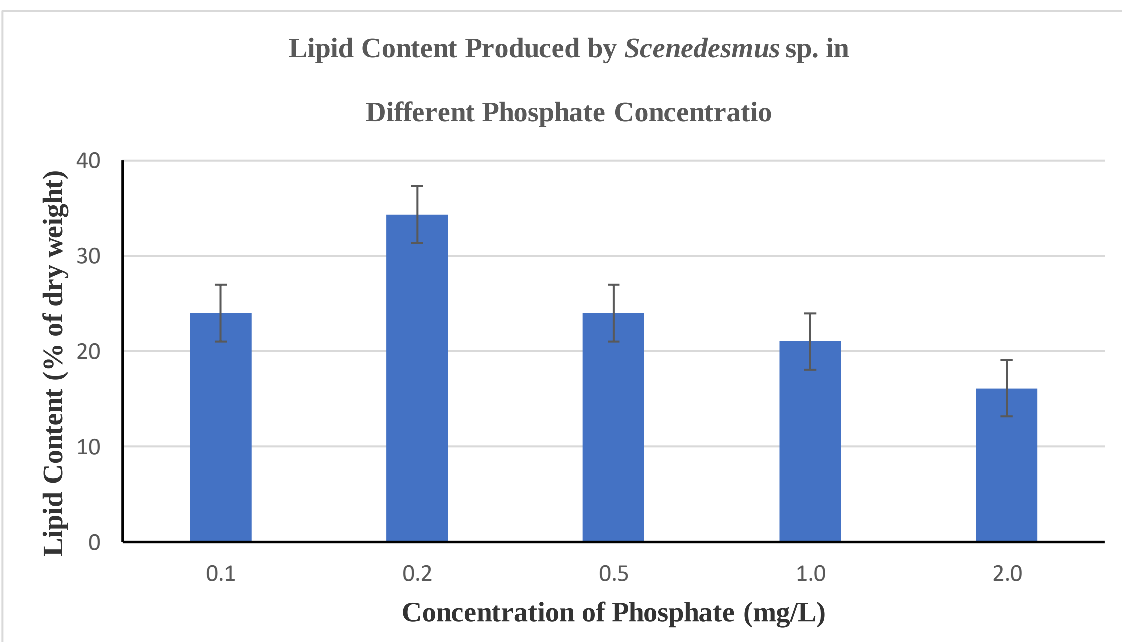

Figure 7 Lipid content (% of dry weight) produced by *Scenedesmus* sp. cultivated in BBM with modifications of phosphate concentration.

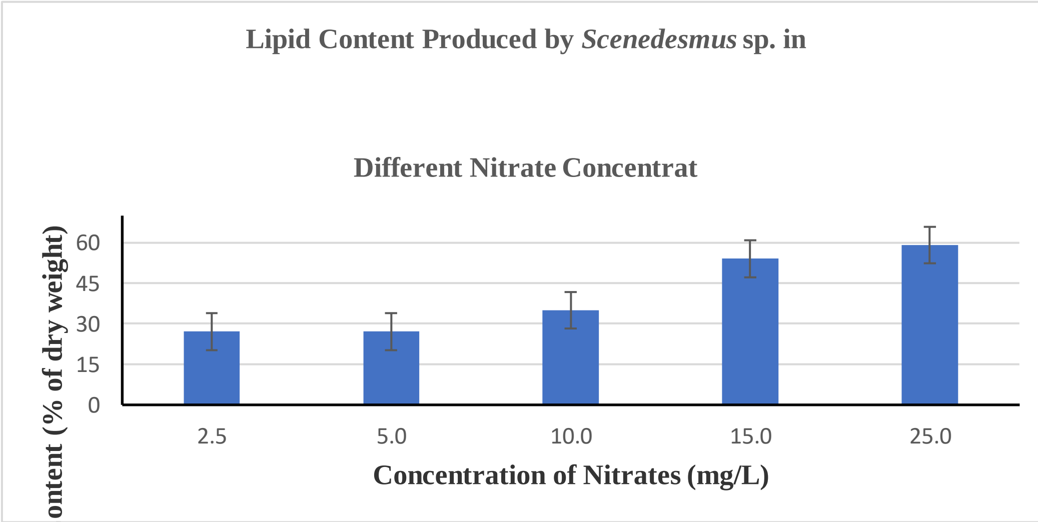

Figure 8 Lipid content (% of dry weight) produced by *Scenedesmus* sp. cultivated in BBM with modifications of nitrate concentration.

3.4 Phycoremediation Potential of *Scenedesmus* sp. in Reducing Phosphate and Nitrate Concentrations

The ability of *Scenedesmus* sp. in reducing phosphate and nitrate concentrations were observed to identify their potential in remediation of pollutants such as nitrogen and phosphorus which are the two major compounds in agricultural industries. Phosphate and nitrate removal analysis by *Scenedesmus* sp. was done across different phosphate and nitrate concentrations; similar to the modifications made in the culture media in biomass and lipid content analysis. Prior to analysis, standard curves were constructed for known concentrations of phosphates and nitrates at two different wavelengths. Culture of *Scenedesmus* sp. in modified BBM with concentration of 0.1 mg/L of phosphate was found to remove 0.1436 mg/L of phosphate which was the highest removal compared to cultures with other modification of phosphate concentrations (Figure 9). Overall, the phosphate concentration measurement showed negligible amount across the cultivation days of *Scenedesmus* sp. microalgae. Therefore, reduction of phosphate concentration shown by *Scenedesmus* sp. grown in cultures with modification of phosphate concentrations is considered insignificant and this might be due to the low growth of *Scenedesmus* sp. too. The same trend was observed for reduction of nitrate by *Scenedesmus* sp. Overall, only a slight reduction of nitrate concentration was recorded by culture of *Scenedesmus* sp. in modified BBM supplemented with different nitrate concentrations (Figure 10). Therefore, there was not much reduction of nitrogen and phosphorus in general from the assimilation process done by *Scenedesmus* sp. in this study which might be due to the low growth rate and biomass of the culture too.

Eutrophication of water as a result of high phosphate and nitrate concentrations is one of the environmental concerns that needs to be addressed and therefore this study was also done to look at the potential of microalgae not only to produce biofuel but also to be a potential candidate for phycoremediation. In an experiment conducted by Pham et al. (2020), it was shown that *Scenedesmus* sp. was able to remove phosphate and nitrate from fertilizer plant wastewater with the removal rates up to 97% and 84% respectively. In another study involving *Scenedesmus* sp. grown in domestic wastewater by Tan and co-workers (2023) resulted in well grown *Scenedesmus* sp. in which it simultaneously removes nitrogen and phosphorus effectively from the wastewater with removal efficiency of more than 90%. The removal efficiency of these pollutants in this study were not well established and it might be as a result to low growth and biomass of the isolated *Scenedesmus* sp.

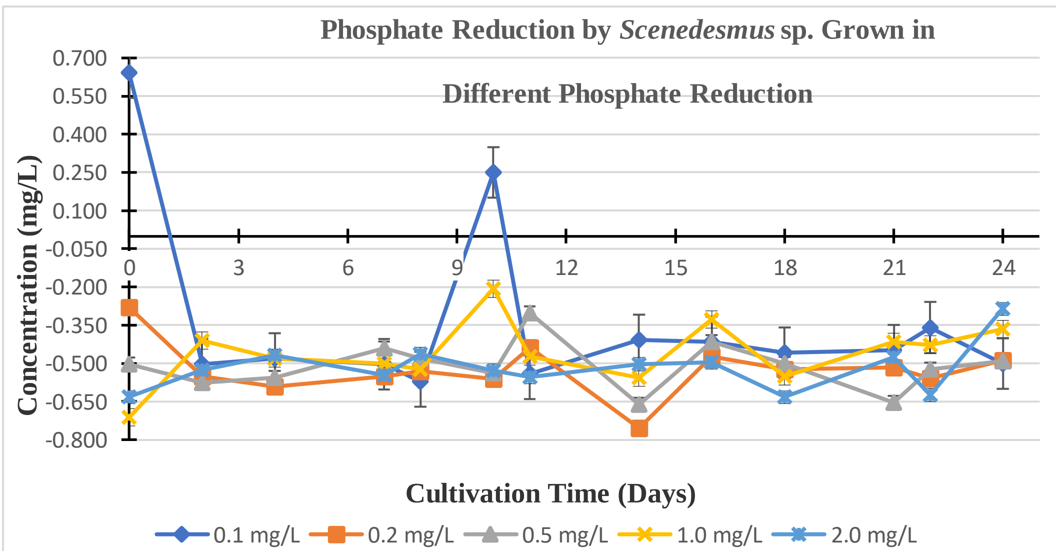

Figure 9 Graph for phosphates reduction by *Scenedesmus* sp. grown in BBM with different concentrations of phosphates. Error bars represent standard error data of triplicates.

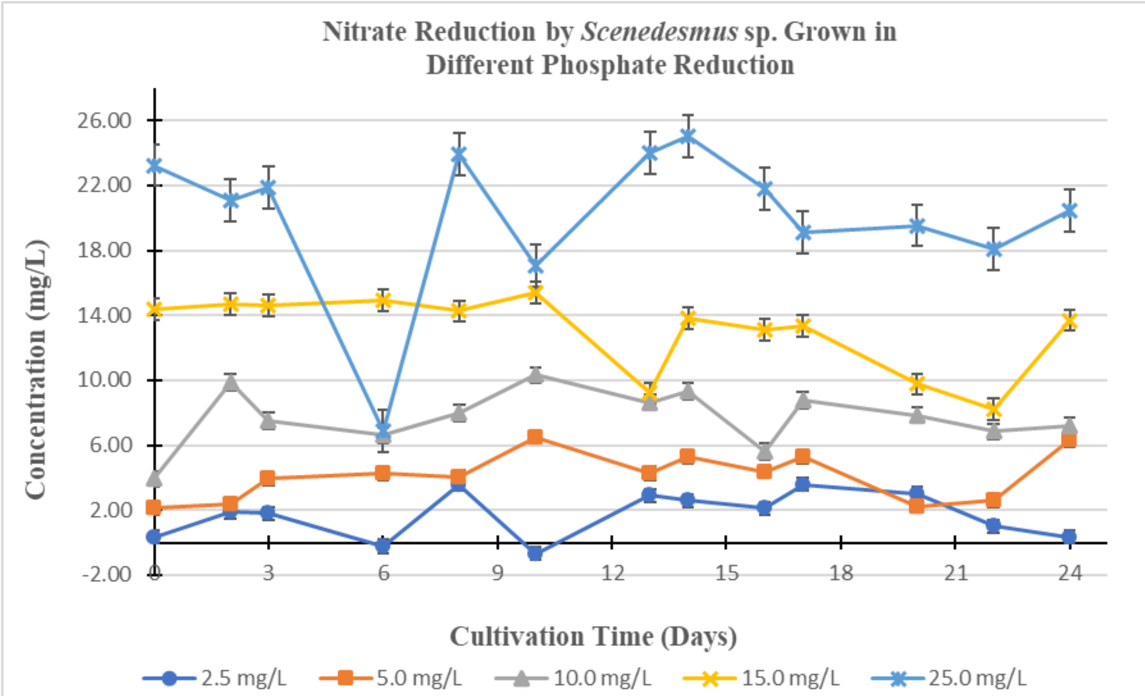

Figure 10 Graph for nitrates reduction by *Scenedesmus* sp. grown in BBM with different concentrations of phosphates. Error bars represent standard error data of triplicates.

4. CONCLUSION

Biomass productivity, lipid content, and overall lipid productivity are some of the parameters that determined the possibility of microalgae for biofuel production. Screening of new strains of microalgae for optimum yield of biofuel production has been continuously carried out. This study was able to find potential candidate for biofuel production which is the local isolate of freshwater microalgae *Scenedesmus* sp. with high lipid content. However, the lipid content did not increase in the condition of nitrogen and phosphorus starvation as described in previous research. In addition, the phycoremediation potential for the microalgae to simultaneously reduce nitrate and phosphate also was not clearly established. Therefore, further research could be done to optimize both the biomass yield and lipid content of the locally isolated *Scenedesmus* sp. as a potential candidate for biofuel production and at the same time can be manipulated to be used in phycoremediation process.

Recommendation for future research

Further isolation and screening of potential microalgae for biofuel production can be done to search for more promising microalgae strains. In addition, the yield and biomass of the potential microalgae can be further optimized to produce high lipid content. Furthermore, integrated approach for phycoremediation and sustainable biofuel production by microalgae can be further explored using the real wastewater which contains the pollutants of interest.

Acknowledgments

We would like to acknowledge the Ministry of Education, Malaysia, and International Islamic University Malaysia for their financial support through a research grant FRGS/1/2015/SG05/UIAM/03/3.

Ethical Statement

There are no ethical issues in this study.

Conflict of interest

The authors have no conflict of interest.

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Received: 31 January 2024
Accepted: 4 June 2024