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Research Article

ISOLATION OF *Micractinium Pusillum* FRESENIUS FROM FISH PONDS USING SDE TECHNIQUES: SEDIMENTATION, DILUTION, AND ENRICHMENT

Chaidir Adam[1,2], Agus Haryono[1,2,*], Titin Purnaningsih[1], Elga Araina[1], Sugeng Mashabhi[1], Yessy Velina[3], Awalul Fatiqin[4]

[1] Biology Education Program, Faculty of Teacher Training and Education, University of Palangka Raya, Central Kalimantan, Indonesia
[2] Center for Development of Science, Technology and Peatland Innovation (PPIIG), University of Palangka Raya, Central Kalimantan, Indonesia
[3] Interdisciplinary Environments Department, Kyoto University, Japan
[4] Department of Biology, Faculty of Mathematics and Natural Sciences, University of Palangka Raya, Central Kalimantan, Indonesia
Corresponding author email: agus.haryono@fkip.upr.ac.id

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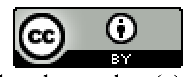
Abstract
Green water in fish ponds, caused by algal blooms, harbors a diverse array of microalgae species and is commonly observed in aquaculture settings. This resource-rich water source holds promise for research focused on microalgae cultivation at a laboratory scale, serving as a valuable starter sample for such investigations. Preliminary observations suggested that the predominant species in such green water habitat belonged to the genus *Micractinium* Fresenius 1858. An effective isolation technique of this microalgae species is necessary, not only to reduce the contamination of the rotifers but also to purify the starter cultures. Although automated microalgae isolation techniques have been developed recently, such as using *Flow Cytometry via* Cell Sorting, traditional isolation techniques are still relevant. One of the traditional microalgae isolation techniques that has been widely used for many years is the dilution technique. This study aims to isolate *Micractinium pusillum* Fresenius from fish ponds using the modified dilution technique: SDE (sedimentation, dilution, and enrichment). The dilution results showed that rotifer contamination was reduced at a dilution of 10^{-3} and the density of microalgae was also reduced. At this dilution level, only one type of microalgae was observed, i.e., *Micractinium pusillum* Fresenius. which was then cultured for enrichment using a simple photobioreactor. This 10-3 culture was observed to grow well during the enrichment stage for 10 days. These results indicate that the SDE isolation technique can be effectively used to isolate microalgae from green water, especially for *Micractinium pusillum* which is the most abundant microalgae species in green water in this study.

Keywords: Fish Ponds, Isolation, Microalgae, Serial Dilution.



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INTRODUCTION

Microalgae are photosynthetic microorganisms, widely distributed in marine, freshwater, or terrestrial environments, that convert sunlight, water, and carbon dioxide into algal biomass, and play an important role in ecosystems as primary producers. Microalgae have benefits for the sustainability of human needs in various fields, such as nutritional sources (Adam & Haryono, 2022; Koyande et al., 2019); renewable energy sources (Suali & Sarbatly, 2012; Medipally et al., 2015; Simas-Rodrigues et al., 2015; Milano et al., 2016); cosmetics (Mourelle et al., 2017); and bioremediation agents (Priyadarshani et al., 2012; Dewi & Nuravivah, 2018; Viswanaathan & Sudhakar, 2019; Zohoorian et al., 2020). Because of this potential, microalgae are widely cultivated for various purposes, both for laboratory or industrial research purposes with mass production.

In addition, the occurrences of microalgae are also commonly used as biological indicators of environmental quality (Holt & Miller, 2010; Omar, 2010; Dell'Aglio et al., 2017; Kadam et al., 2020). One of the most known freshwater microalgae occurrences in aquatic environments as a quality bioindicator is algal blooms caused by eutrophication which is indicated by excessive algal growth because of the nutrient enrichment required for photosynthesis (Holt & Miller, 2010). An algal bloom is a very rapid increase in the growth of algae in aquatic systems that usually form mats, in both natural and artificial aquatic systems (Kazmi et al., 2022; Das Sarkar et al., 2024). Various species of microalgae can be part of an algal bloom, both eukaryotic microalgae and cyanobacteria (Williamson et al., 2023). Cyanobacteria in algal blooms produce toxins that worsen water quality (HABs; harmful algal blooms). Basically, algal blooms are a natural phenomenon, but their frequency, intensity, and duration can increase rapidly due to nutrient pollution which is common in artificial aquatic systems such as fish ponds. In certain conditions, e.g., excess nutrients in the pond, the water turns green caused by an algal bloom suspended in the pond water.

Green water in fish ponds due to algal blooms is rich in microalgae species and is easily found in fish farming areas that have the potential to be utilized in research related to microalgae cultivation on a laboratory scale as a starter sample. The preliminary observation showed that the green water contains various microalgal species including the following genera: *Askistrodesmus* Corda 1838, *Chlorella* M.Beijerinck 1890, *Golenkinia* Chodat 1894, *Micractinium* Fresenius 1858, *Pediastrum* Meyen 1829, and *Scenedesmus* Meyen 1829. *Micractinium* was the most abundant among all observed microalgae species. *Micractinium* is a genus of green algae in the class Trebouxiophyceae and is commonly found in freshwater habitats with round to ellipsoidal cell shapes and forms long, narrow bristles that taper from base to tip. This genus is closely related to *Chlorella* with similar potency in biofuel production.

The challenge faced is that green water from fish ponds still contains many rotifers as predatory zooplankton which can inhibit the growth of microalgae and consists of large microalgae communities with a large number of species. An effective isolation technique is necessary to overcome this, not only to reduce the contamination of the rotifers but also to purify the starter cultures. Although automated microalgae isolation techniques have been developed recently, such as Flow Cytometry with Cell Sorting (Sieracki et al., 2005), the traditional techniques are still widely used. One of the traditional microalgae isolation techniques that has been widely used for many years is the dilution technique. According to (Andersen & Kawachi, 2005), the dilution technique is effective for organisms that are rather abundant in the sample such as microalgae samples in green water from fish ponds. This study aims to isolate microalgae from fish ponds using the sedimentation, dilution, and enrichment (SDE) technique.

RESEARCH METHOD

Green water samples were collected directly from the fish ponds of the farmers in Palangkaraya, Central Kalimantan, Indonesia, in the following Figure 1.

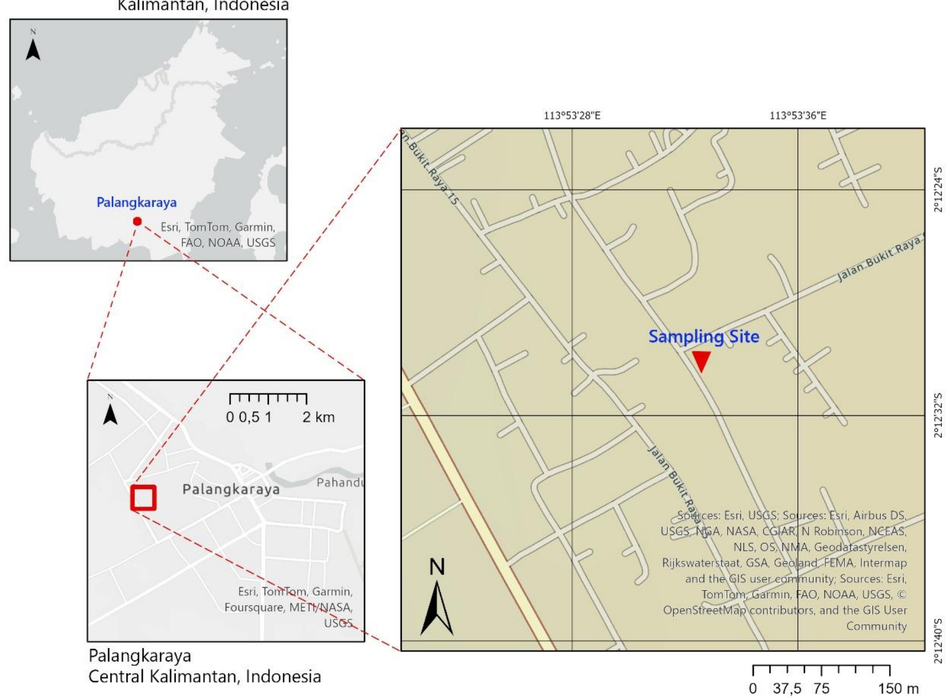

Figure 1. Map of sampling location Microalgae from the fish ponds of the farmers in Palangkaraya

Isolation of *Micractinium pusillum* Fresenius from water samples used three main techniques, i.e., sedimentation, dilution, and enrichment (SDE). The isolation procedure was carried out in 2 phases as presented in the following Figure 2.

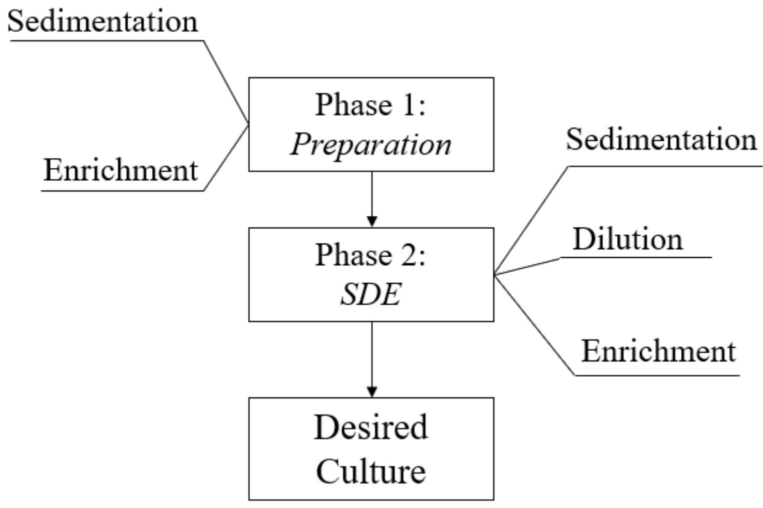

Figure 2. SDE techniques for microalgae isolation

Phase 1: Preparation

Sedimentation of green water samples taken directly from fish ponds is carried out as a sample preparation stage for isolation. In this process, microalgae particles settle to the bottom of the suspension to obtain a higher concentration of cells. Sedimentation was carried out for 24 hours until the microalgae particles completely settled (Figure 3). Then enrichment is carried out with the aim of obtaining a sufficient amount of suspension for phase 2. The enrichment process uses a simple laboratory-scale photobioreactor designed by Adam et al. (2020) for 7 days.

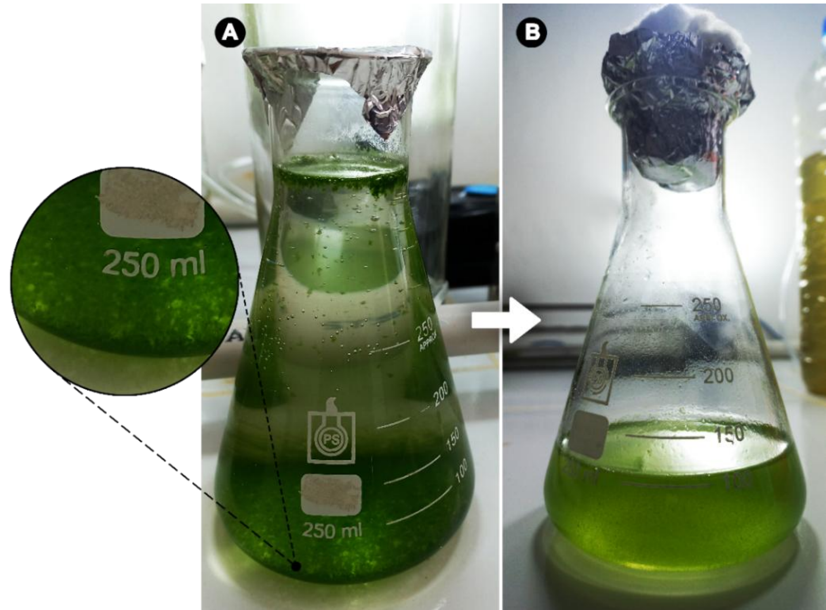

Figure 3. Sedimentation in an Erlenmeyer flask

Phase 2: SDE

Phase 2 consists of three steps, e.g., *sedimentation, dilution,* and *enrichment.* The three steps are explained as follows:

1) *Sedimentation.* Right after Phase 1, the second sedimentation was carried out on the samples using 10 test tubes for 24 hours. Furthermore, each precipitate was collected to obtain a more concentrated sample (Figure 4).

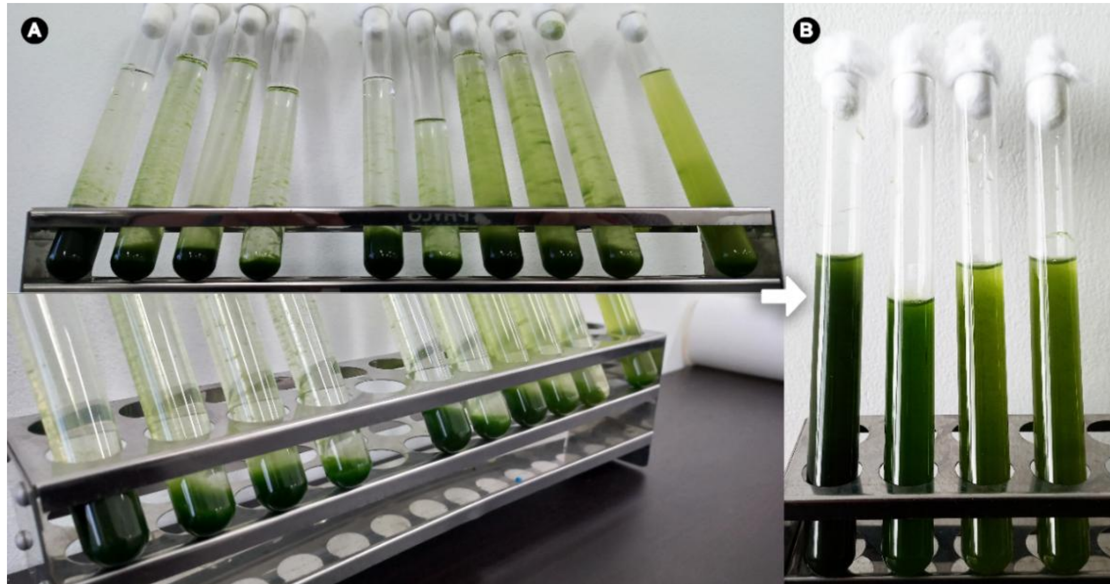

Figure 4. Sedimentation in test tubes

2) *Dilution.* At this stage, 1 ml of concentrated sample was added to a test tube containing 9 ml of distilled water. The dilution was carried out 4 times (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) continuously and observations were made of each dilution using a microscope until only one microalga isolate could be observed (Figure 5).

3) *Enrichment.* Microalgae that had been isolated at the dilution stage were enriched for 10 days to obtain pure cultures. The enrichment uses a photobioreactor and a 250ml Erlenmeyer flask as the reactor.

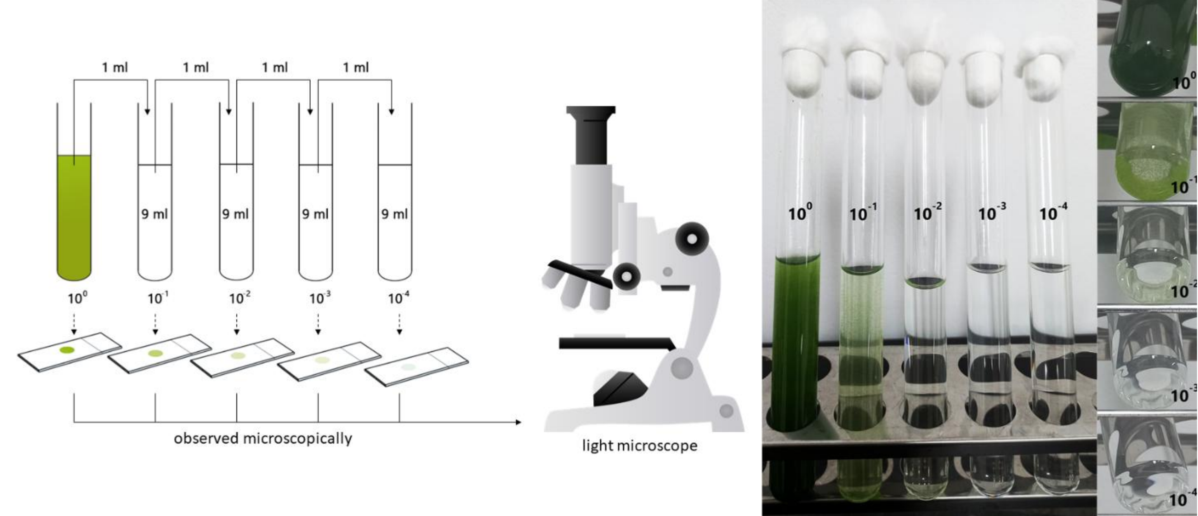

Figure 5. Dilution

RESULTS AND DISCUSSION

Microscopic Screening of Microalgal Species in Each Dilution Level

Microscopic screening was performed at each dilution level to recheck and determine which dilutions will be enriched at the next stage. The parameter observed was the density of the microalgae community with the lowest density (at least one microalgal isolate) being the selected condition. Detailed microscopic screening results are presented in the Figure 6 and Table 1. The highest density was observed at the 100 dilution which consisted of various species of microalgae and appeared so congested that the microscope's field of view was filled with almost no empty space. Decreased density was observed at dilutions 10^{-1} and 10^{-2}, but still contained more than one species of microalgae. At the 10^{-3} dilution, only one microalgae species was found, i.e., *Micractinium*, and it was the only one that met the requirements for the enrichment stage, because at the 10^{-4} dilution not a single microalgae species was observed. These results confirm that the dilution technique is effectively used to isolate microalgae from water samples as stated by Andersen & Kawachi (2005).

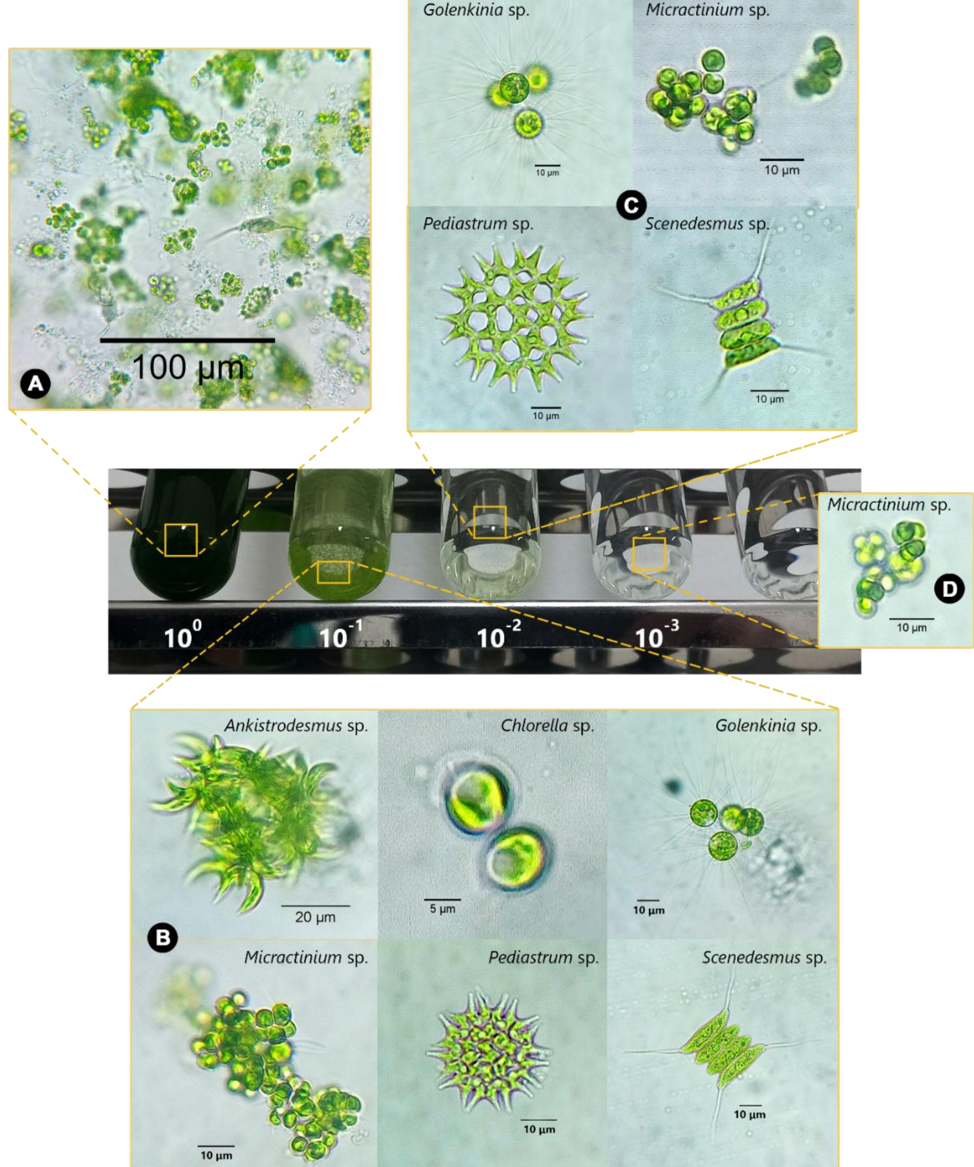

Figure 6. Microalgae found in each dilution level: (A) 10^0; (B) 10^{-1}; (C) 10^{-2}; (D) 10^{-3}

Verma et al. (2021) in their research used dilution as a microalgae isolation technique with slightly different modifications but basically used the same principle, i.e., Microscope Assisted Uni-algal isolation through Dilution (MAU-D). MAU-D provides a fast and simple method for the isolation and purification of microalgae, especially those cyanobacteria that are difficult to isolate through routine sub-culturing (Verma et al., 2021). In addition, based on these results, serial dilution is also effective in reducing the presence of rotifers. The presence of rotifers decreased at higher dilutions and almost no presence was found at the 10^{-3} dilution which was the selected dilution to be enriched in this study. This means that serial dilution can be used to reduce the risk of contamination of microalgae cultures, especially contamination of rotifers and other algivore zooplankton.

Rotifers are common predators in microalgae cultures. The presence of rotifers as predators will inhibit the growth of microalgae and reduce their productivity (Huang et al., 2014; Montemezzani et al., 2016; H. Wang et al., 2013; L. Wang et al., 2016; Yuan et al., 2018). Furthermore, algivore zooplankton contamination, including rotifers, is a major management challenge in industrial-scale microalgae cultivation (H. Wang et al., 2013), causing serious economic losses (Huang et al., 2014). Fernandez-Valenzuela et al. (2021) stated that as a quality control in isolating microalgae using dilution, a contamination test and microscope observation are required to detect other unwanted microorganisms present in the culture.

Table 1. Microscopic Screening Results

No.	Dilution Level	Color	Microalgal Species Observed	Rotifer Contamination
1	10^0	Dark Green	Numerous microalgal species (highest density)	++
2	10^{-1}	Green	*Ankistrodesmus* sp. *Chlorella* sp. *Golenkinia* sp. *Micractinium* sp. *Pediastrum* sp. *Scenedesmus* sp.	++
3	10^{-2}	Light Green	*Golenkinia* sp. *Micractinium* sp. *Pediastrum* sp. *Scenedesmus* sp.	+
4	10^{-3}	Colourless with a few small green particles scattered around	*Micractinium pusillum*	-
5	10^{-4}	Colourless	None	-

Enrichment of Isolated *Micractinium pusillum* Fresenius 1858 from 10^{-3} dilution level

Micractinium pusillum which had been isolated at a dilution of 10^{-3} was then enriched using a simple photobioreactor for 10 days. Enrichment used general principles in microalgae cultivation, namely by adding Walne as a fertilizer with a concentration of 1:10. The *M. pusillum* culture was observed to start growing on day 4. This means that days 1 to 3 are the lag phase, where the microalgae undergo acclimatization to the new environment, generally referred to as the adaptation period. Lag phase The lag phase commonly occurs in the growth of microorganisms including microalgae, which is followed by the log phase and stationary phase (Adam, 2022; Forde et al., 2014; Lee et al., 2015). Krishnan et al. (2015) stated that lag phase is the initial phase of cultivation in which the microalgae adapts to the surround environment such as nutrients, pH, temperature, and lighting.

Figure 7. Enrichment of the isolated *Micractinium pusillum* Fresenius 1858 at 10^{-3} dilution

Exponential growth was observed starting on day 4 to day 8 gradually as seen from the culture color becoming increasingly intense and the stationary phase was thought to occur on days 9 dan 10 because the color of the culture remained the same, no color changes were observed (Figure 7). The growth of the culture is visually presented as a curve in Figure 7. According to Price & Farag (2013), exponential growth (log phase) provides a maximum microalgae population compared to the stationary phase because in the stationary phase microalgae cell division becomes slow due to reduction of the nutrients that are necessary for cell growth. Therefore, the microscopic examination was carried out on day 10 which had entered the stationary phase to check whether the isolation and enrichment of *M. pusillum* was successful.

As seen in Figure 8, *M. pusillum* has grown well by forming coenobia and larger colonies. The cells are green and spherical in shape with spiny hairs (or bristles) that point outward.

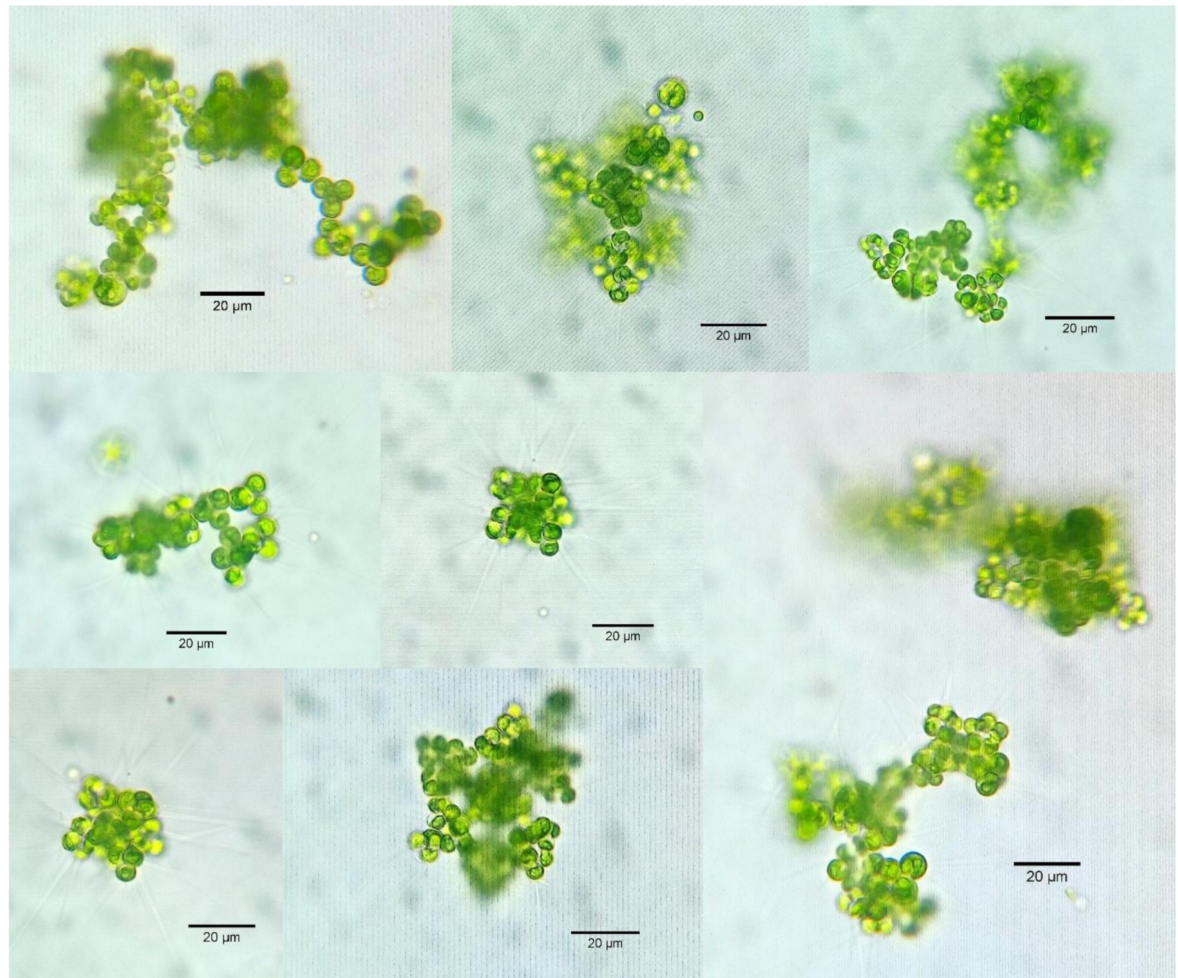

Figure 8. Isolated *Micractinium pusillum* Fresenius 1858

CONCLUSION

The dilution results showed that rotifer contamination was reduced at a dilution of 10^{-3} and the density of microalgae was also reduced. At this dilution level, only one type of microalgae was observed, i.e., *Micractinium pusillum* Fresenius which was then cultured for enrichment using a simple photobioreactor. This 10^{-3} culture was observed to grow well during the enrichment stage for 10 days. These results indicate that the SDE isolation technique can be effectively used to isolate microalgae from green water, especially *M. pusillum* which is the most abundant microalgae species in green water in this study.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.A.; Methodology, C.A.; Software, C.A.; Validation, C.A. and A.H.; Formal Analysis, C.A.; Investigation, C.A., A.F., and S.M.; Resources, C.A. and A.H.; Data Curation, C.A.; Writing – Original Draft Preparation, C.A.; Writing – Review & Editing, C.A. and A.F.; Visualization, C.A.; Supervision, A.H.; Funding Acquisition, A.H.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and

manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

REFERENCES

Adam, C. (2022). Variety of Cell Size of Cosmarium spp. And Euastrum spp. (Desmidiaceae, Charophyte) from the Aquatic Environment around Palangka Raya, Central Kalimantan, Indonesia. *Jurnal Biota*, *8*(1), 1–10. https://doi.org/10.19109/Biota.v8i1.8002

Adam, C., & Haryono, A. (2022). Identification of Euglenids (Euglenophyceae, Euglenophyta) from the Peat Waters of Palangka Raya, Indonesia. *Journal of Multidisciplinary Applied Natural Science*, *3*(1), 81–89. https://doi.org/10.47352/jmans.2774-3047.145

Adam, C., Neneng, L., & Haryono, A. (2020). Penggunaan Pupuk Hidroponik AB-Mix sebagai Fertiliser Alternatif dalam Kultivasi Chlorella sp. *Seminar Nasional Sains Dan Teknologi FMIPA UPR*.

Andersen, R. A., & Kawachi, M. (2005). Traditional Microalgae Isolation Techniques. In *Algal Culturing Techniques*. Elsvier Academic Press.

Das Sarkar, S., Bera, A. K., & Kumari, S. (2024). Algal bloom in inland open water and their prospective management strategies. *Brazilian Journal of Development*, *10*(2), e67269. https://doi.org/10.34117/bjdv10n2-028

Dell'Aglio, E., Cosentino, F., & Campanella, L. (2017). Use of Algae Scenedesmus as Bioindicators of Water Pollution from Active Ingredients. *Journal of Analytical & Pharmaceutical Research*, *6*(5). https://doi.org/10.15406/japlr.2017.06.00189

Dewi, E. R. S., & Nuravivah, R. (2018). Potential of Microalgae Chlorella vulgaris As Bioremediation Agents of Heavy Metal Pb (Lead) On Culture Media. *E3S Web of Conferences*, *31*, 3–6. https://doi.org/10.1051/e3sconf/20183105010

Fernandez-Valenzuela, S., Chávez-Ruvalcaba, F., Beltran-Rocha, J. C., San Claudio, P. M., & Reyna-Martínez, R. (2021). Isolation and Culturing Axenic Microalgae: Mini–Review. *The Open Microbiology Journal*, *15*(1). https://doi.org/10.2174/1874285802115010111

Forde, C. J., Meaney, M., Carrigan, J. B., Mills, C., Boland, S., & Hernon, A. (2014). Chapter 12—Biobased Fats (Lipids) and Oils from Biomass as a Source of Bioenergy. In V. K. Gupta, M. G. Tuohy, C. P. Kubicek, J. Saddler, & F. Xu (Eds.), *Bioenergy Research: Advances and Applications* (pp. 185–201). Elsevier. https://doi.org/10.1016/B978-0-444-59561-4.00012-7

Holt, E. A., & Miller, S. W. (2010). Bioindicators: Using Organisms to Measure Environmental Impacts. *Nature Education Knowledge*, *3*(10), 8.

Huang, Y., Liu, J., Wang, H., & Gao, Z. (2014). Treatment potential of a synergistic botanical pesticide combination for rotifer extermination during outdoor mass cultivation of Spirulina platensis. *Algal Research*, *6*, 139–144.

Kadam, A. D., Kishore, G., Mishra, D. K., & Arunachalam, K. (2020). Microalgal diversity as an indicator of the state of the environment of water bodies of Doon valley in Western Himalaya, India. *Ecological Indicators*, *112*, 106077. https://doi.org/10.1016/j.ecolind.2020.106077

Kazmi, S. S. U. H., Yapa, N., Karunarathna, S. C., & Suwannarach, N. (2022). Perceived Intensification in Harmful Algal Blooms Is a Wave of Cumulative Threat to the Aquatic Ecosystems. *Biology*, *11*(6), 852. https://doi.org/10.3390/biology11060852

Koyande, A. K., Chew, K. W., Rambabu, K., Tao, Y., Chu, D.-T., & Show, P.-L. (2019). Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness*, *8*(1), 16–24. https://doi.org/10.1016/j.fshw.2019.03.001

Krishnan, V., Uemura, Y., Thanh, N. T., Khalid, N. A., Osman, N., & Mansor, N. (2015). Three types of Marine microalgae and Nannocholoropsis oculata cultivation for potential source of biomass production. *Journal of Physics: Conference Series*, *622*(1), 012034. https://doi.org/10.1088/1742-6596/622/1/012034

Lee, E., Jalalizadeh, M., & Zhang, Q. (2015). Growth kinetic models for microalgae cultivation: A review. *Algal Research*, *12*, 497–512. https://doi.org/10.1016/j.algal.2015.10.004

Medipally, S. R., Yusoff, F. Md., Banerjee, S., & Shariff, M. (2015). Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production. *BioMed Research International*, *2015*, 519513. https://doi.org/10.1155/2015/519513

Milano, J., Ong, H. C., Masjuki, H. H., Chong, W. T., Lam, M. K., Loh, P. K., & Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews*, *58*, 180–197. https://doi.org/10.1016/j.rser.2015.12.150

Montemezzani, V., Duggan, I. C., Hogg, I. D., & Craggs, R. J. (2016). Zooplankton community influence on seasonal performance and microalgal dominance in wastewater treatment High Rate Algal Ponds. *Algal Research*, *17*, 168–184. https://doi.org/10.1016/j.algal.2016.04.014

Mourelle, M. L., Gómez, C. P., & Legido, J. L. (2017). The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. *Cosmetics*, *4*(4). https://doi.org/10.3390/cosmetics4040046

Omar, W. M. W. (2010). Perspectives on the use of algae as biological indicators for monitoring and protecting aquatic environments, with special reference to malaysian freshwater ecosystems. *Tropical Life Sciences Research*, *21*(2), 51–67.

Price, K., & Farag, I. (2013). Resources Conservation in Microalgae Biodiesel Production. *International Journal of Engineering and Technical Research (IJETR)*, *1*, 49–56.

Priyadarshani, I., Sahu, D., & Rath, B. (2012). Microalgal bioremediation: Current practices and perspectives. *Journal of Biochemical Technology*, *3*(3), 299–304.

Sieracki, M., Poulton, N., & Crosbie, N. (2005). Automated Isolation Techniques for Microalgae. In *Algal Culturing Techniques*. Elsvier Academic Press.

Simas-Rodrigues, C., Villela, H. D. M., Martins, A. P., Marques, L. G., Colepicolo, P., & Tonon, A. P. (2015). Microalgae for economic applications: Advantages and perspectives for bioethanol. *Journal of Experimental Botany*, *66*(14), 4097–4108. https://doi.org/10.1093/jxb/erv130

Suali, E., & Sarbatly, R. (2012). Conversion of microalgae to biofuel. *Renewable and Sustainable Energy Reviews*, *16*(6), 4316–4342. https://doi.org/10.1016/j.rser.2012.03.047

Verma, S., Bagul, S. Y., Choudhary, P., Chakdar, H., Das, S., Siddiqui, N., & Saxena, A. K. (2021). Microscope Assisted Uni-algal isolation through Dilution (MAU-D): A simple modified technique for tapping diverse cyanobacteria. *3 Biotech*, *11*(7), 343. https://doi.org/10.1007/s13205-021-02890-w

Viswanaathan, S., & Sudhakar, M. P. (2019). *Chapter 8—Microalgae: Potential agents for CO2 mitigation and bioremediation of wastewaters* (J. S. B. T.-N. Singh, F. D. in M. Biotechnology, & Bioengineering, Eds.; pp. 129–148). Elsevier. https://doi.org/10.1016/B978-0-12-818258-1.00008-X

Wang, H., Zhang, W., Chen, L., Wang, J., & Liu, T. (2013). The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresource Technology*, *128*, 745–750.

Wang, L., Yuan, D., Li, Y., Ma, M., Hu, Q., & Gong, Y. (2016). Contaminating microzooplankton in outdoor microalgal mass culture systems: An ecological viewpoint. *Algal Research*, *20*, 258–266.

Williamson, D., Fragoso, G., Majaneva, S., Dallolio, A., Halvorsen, D., Hasler, O., Oudijk, A., Langer, D., Johansen, T., Johnsen, G., Stahl, A., Ludvigsen, M., & Garrett, J. (2023). Monitoring Algal Blooms with Complementary Sensors on Multiple Spatial and Temporal Scales. *Oceanography*. https://doi.org/10.5670/oceanog.2023.s1.11

Yuan, D., Zhan, X., Wang, M., Wang, X., Feng, W., Gong, Y., & Hu, Q. (2018). Biodiversity and distribution of microzooplankton in Spirulina (Arthrospira) platensis mass cultures throughout China. *Algal Research*, *30*, 38–49. https://doi.org/10.1016/j.algal.2017.12.009

Zohoorian, H., Ahmadzadeh, H., Molazadeh, M., Shourian, M., & Lyon, S. (2020). *Chapter 41—Microalgal bioremediation of heavy metals and dyes* (O. B. T.-H. of A. S. Konur Technology and Medicine, Ed.; pp. 659–674). Academic Press. https://doi.org/10.1016/B978-0-12-818305-2.00041-3