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Algal Organic Matter Fluorescence Analysis of *Chlorella* sp. for Biomass Estimation †

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Abstract: Algal Organic Matter (AOM) is derived from the dissolved organic matter composition of the algal species being observed. In this study, excitation–emission fluorescence spectroscopy was used to determine *Chlorella* sp.'s AOM and pigment characteristics in varying algal biomass concentrations. The AOM and pigment characteristics were observed at 400–600 nm and 600–800 nm fluorescence emission, respectively, with an excitation spectrum of 300–450 nm. F450/680 was computed based on the ratio between the dissolved organic matter contribution at 450 nm and chlorophyll-a at 680 nm. F450/680 positively correlated with algal biomass (r = 0.96) at an excitation wavelength of 405 nm. This study is a good reference for those interested in algal biomass estimation and production in natural waters.

Keywords: algal organic matter; *Chlorella* sp.; biomass estimation; fluorescence

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1. Introduction

Algae are mostly abundant in rivers and reservoirs connected to drinking water facilities and factories [1,2]. With rapid urbanization and industrialization, effluents can severely affect the environment. This may result in an increase in algal organic matter (AOM) in surface waters. Organic substances produced by algae lead to water discoloration, odor and toxicity problems, and algal blooms [3–5].

Different techniques have been used to characterize organic matter in aquatic systems, which can be used to understand the composition of AOM in natural waters. These include specific ultraviolet absorption (SUVA), excitation–emission matrix (EEM) fluorescence spectroscopy, and lidar systems [6–9]. These techniques are promising tools for understanding the behaviors and composition of AOM and the pigment chlorophyll-a in natural waters.

The behavior and composition of *Chlorella* sp. has been studied using spectroscopic techniques. We aimed to study excitation–emission (Ex/Em) pairs for algal organic matter and pigment measurement in natural waters. This EEM fluorescence analysis may improve the existing portable fluorescence lidar systems used in algal biomass estimation. A specific fluorescence ratio was used to understand the contributions of chlorophyll-a and AOM in the different growth phases of microalgae. The in situ and real-time monitoring technique was the focus of this study, as it provides new information with faster interpretation.

2. Materials and Methods

2.1. Algal Preparation

Chlorella sp. inoculum was provided by the Microalgae Systematics and Applied Phycology Research Unit of De La Salle University using a BG-11 culture medium. The initial optical density was 0.6, with a pH between 8 and 9. The light/dark cycle was controlled at 12 h/12 h, with a light intensity of 75 μmol/m^2/s.

The extracted AOM was obtained via the following processes. The algal supernatant was processed through centrifugation at 8000 r·min^{-1} for 5 min. It was then filtered with deionized water using 0.47 μm glass filter fiber (Whatman, Marlborough, MA, USA).

2.2. Spectral Characterization

2.2.1. Measuring UV-Vis Using Absorbance Spectroscopy

The aromaticity, size, and aromatic substances of algae can be interpreted by measuring $SUVA_{254}$. The protein-like structures of algae can also be characterized by measuring $SUVA_{280}$. The method performed was based on the characterization of water quality in rivers and estuaries [10,11].

2.2.2. Measuring Fluorescence Using Excitation–Emission Spectroscopy

The fluorescence measurements, expressed in normalized units, described in this paper are the EEM of AOM and their pigments at varying biomass concentrations. Excitation–emission pairs were obtained by changing the excitation wavelength at 5 nm intervals. A 3D EEM of varying algal biomass and pigments of *Chlorella* sp. was analyzed. A detailed discussion on the fluorescence set-up and data analysis is provided in our previous paper on algal growth and real-time monitoring in natural waters [12–14].

2.3. The Application of the EEM to Fluorescence Lidar Measurements

The fluorescence EEM provides preliminary guidelines for constructing a fluorescence lidar by identifying excitation–emission combinations. This study explores the possibility of using fluorescence intensity profiles with 380 nm and 405 nm excitation wavelengths. The region of interest for AOM is 400–600 nm, and the region of interest for pigment measurements is 600–800 nm. Developing a fluorescence lidar system for biomass estimation is recommended since it is an in situ and non-invasive technique.

2.4. Comparisons of the Fluorescence EEM

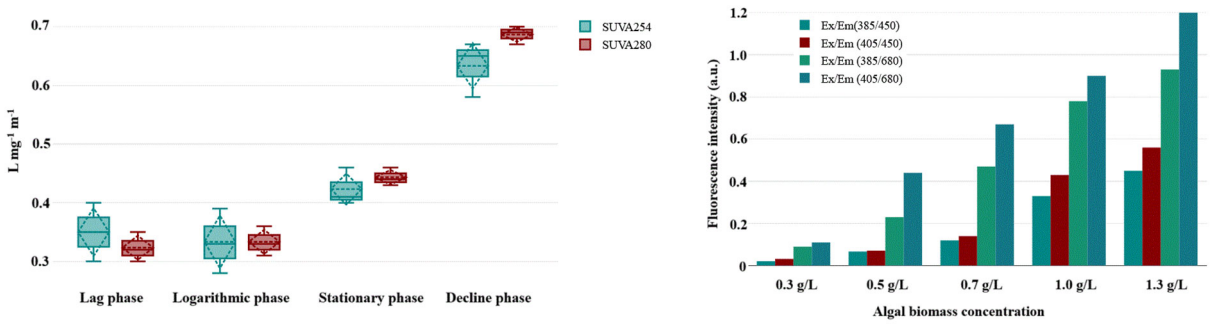
In recent studies, biomass measurements have been taken using indirect or direct techniques. The results from the introduced fluorescence ratio are correlated (Pearson's r) with optical density measurements, biomass estimation, and estimated chlorophyll-a concentrations.

3. Results and Discussion

The growth of *Chlorella* sp. in controlled culture media was analyzed for its AOM, and its pigments were analyzed for biomass estimation. The fluorescence EEM is crucial in developing a new fluorescence lidar system for biomass estimation.

3.1. The Spectral Characterization of Chlorella sp.

The variance in the $SUVA_{254}$ and $SUVA_{280}$ in varying biomass are shown in Figure 1a. The $SUVA_{254}$ measured in the AOM ranges from 0.3 to 1.4 L mg^{-1}·m^{-1}. This means that the aromaticity of AOM is low, indicating a smaller number of protein-like substances [15]. The same trend was observed between $SUVA_{254}$ and $SUVA_{280}$, which shows a positive correlation between aromaticity and protein-like structures ($r = 0.89$, $p < 0.05$). These changes in organic matter from the logarithmic phase to the decline phase are helpful in algal growth monitoring.



(**a**) (**b**)

Figure 1. (**a**) Whisker and box plot of AOM in different growth phases. This figure represents the absorbance at $SUVA_{254}$ and $SUVA_{280}$ (n = 6). (**b**) Fluorescence intensity from the 3D EEMs at varying algal biomass concentrations.

The fluorescence intensity results from the 3D EEMs at varying algal biomass are presented in Figure 1b. The fluorescence EEMs, based on Ex/Em peaks, are valuable for distinguishing different types of organic matter components and types of natural waters. All algal biomass showed similar fluorescence EEM trends for the AOM and pigments. The total organic matter contribution was measured at a fluorescence emission wavelength of 450 nm [16]. Fluorescence emission spectroscopy is commonly used for surface water and terrestrial systems [17]. The observed excitation/emission pairs for algal organic matter are 385 nm/405 nm and 405 nm/450 nm. Higher fluorescence intensity profiles were measured using 405 nm/450 nm, but no significant differences were shown at 385 nm/450 nm ($p < 0.05$).

The same trend was reflected in the 405 nm/680 nm and 385 nm/680 nm pairs for our measurement of chlorophyll-a ($p < 0.05$). The mean values were compared using Pearson's r correlation ($p < 0.05$), as shown in Figure 2. The Ex/Em 385 nm/450 nm pair showed a higher correlation compared to the Ex/Em 405 nm/450 nm with the algal biomass concentration. This was also the case when using a fluorescence emission wavelength of 680 nm. On the other hand, the fluorescence ratio showed opposite results. F450/680 at an excitation wavelength of 405 nm (r = 0.96) positively correlates with F450/680 at an excitation wavelength of 385 nm (r = 0.94) with algal biomass. This F-ratio is recommended to understand the dry-weight algal biomass in natural waters.

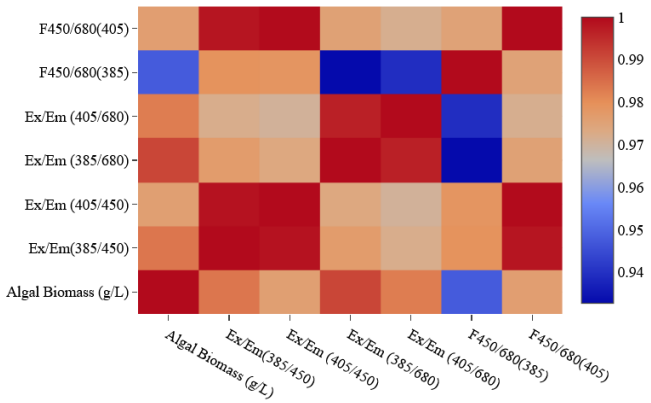
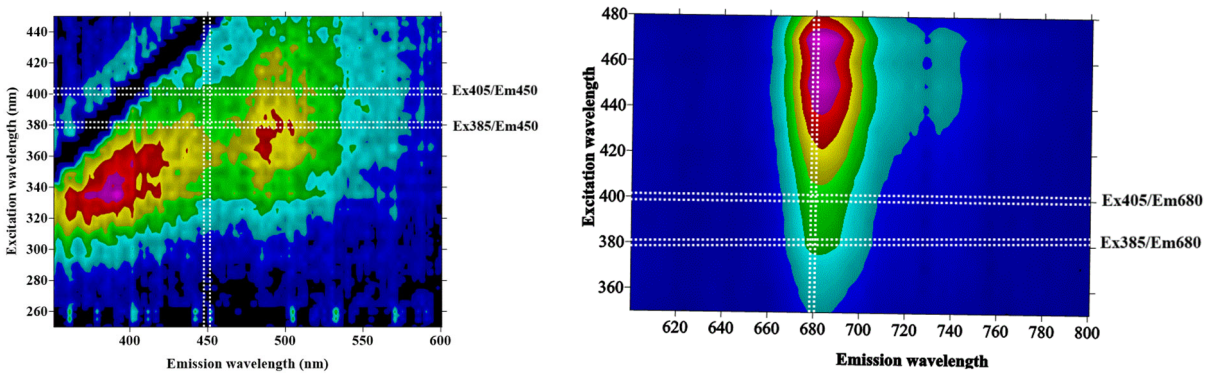


Figure 2. Heat map depicting the correlations between the Ex/Em pairs, F ratio, and algal biomass of *Chlorella* sp.

3.2. The Analysis of the Fluorescence EEM regarding the Development of the Lidar System

Developing a portable fluorescence lidar system is vital for the real-time monitoring and estimation of algal biomass in natural waters. With the guidance of the excitation–emission pairs used in this study, improving the existing fluorescence lidar system with a

new excitation wavelength at 405 nm is recommended. Simultaneous measurements at 450 and 680 nm are also suggested to understand the behavior of AOM and the chlorophyll concentrations of microalgae, respectively (Figure 3). F450/680 was used to calculate dry-weight measurements of algal biomass of *Chlorella* sp. for excitation wavelengths of 385 nm and 405 nm. Similar trends were observed, but a higher positive correlation was computed at an excitation wavelength of 405 nm.



(**a**) (**b**)

Figure 3. Fluorescence EEMs of *Chlorella* sp. at 0.3 g/L. (**a**) Algal organic matter EEM showing Ex/Em 405 nm/450 nm and Ex/Em 385 nm/450 nm. (**b**) Chlorophyll-a pigment EEM showing Ex/Em 405 nm/680 nm and Ex/Em 385 nm/680 nm.

The developed fluorescence lidar system discussed in our previous study showed its unique features and robustness [13,14]. It uses a pulsed LED circuit at an excitation wavelength of 385 nm, which serves as a transmitting system. With our new understanding of Ex/Em pairs, we suggest the development of a portable pulsed laser diode (LD) at an excitation wavelength of 405 nm. This study paves the way for the discovery of a new technique for developing a fluorescence lidar system using excitation–emission pairs [8].

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ebrahimzadeh, G.; Alimohammdi, M.; Kahkah, M.; Mahvi, A.H. Relationship between algae diversity and water quality-a case study: Chah Niemeh reservoir Southeast of Iran. *J. Environ. Health Sci. Eng.* **2021**, *19*, 437–443. [CrossRef] [PubMed]
2. Feng, J.; Zhou, L.; Zhao, X.; Chen, J.; Li, Z.; Liu, Y.; Ou, L.; Xie, Z.; Wang, M.; Yin, X.; et al. Evaluation of environmental factors and microbial community structure in an important drinking-water reservoir across seasons. *Front. Microbiol.* **2023**, *14*, 1091818. [CrossRef] [PubMed]
3. Caron, D.A.; Garneau, M.E.; Seubert, E.; Howard, M.D.A.; Darjany, L.; Schnetzer, A.; Cetinic, I.; Filteau, G.; Lauri, P.; Jones, B.; et al. Harmful algae and their potential impacts on desalination operations off southern California. *Water Res.* **2010**, *44*, 385–416. [CrossRef] [PubMed]
4. Villacorte, L.O.; Ekowati, Y.; Neu, T.R.; Kleijn, J.M.; Winters, H.; Amy, G.; Schippers, J.C.; Kennedy, M.D. Characterisation of algal organic matter by bloom-forming marine and freshwater algae. *Water Res.* **2015**, *73*, 216–230. [CrossRef] [PubMed]
5. Du, Y.; An, S.; He, H.; Wen, S.; Xing, P.; Duan, H. Production and transformation of organic matter driven by algal blooms in a shallow lake: Role of sediments. *Water Res.* **2022**, *219*, 118560. [CrossRef] [PubMed]
6. Chu, H.; Yu, H.; Tan, X.; Zhang, Y.; Zhou, X.; Yang, L.; Li, D. Extraction procedure optimization and the characteristics of dissolved extracellular organic matter (bEOM) from chlorella pyrenoidosa. *Coll. Surf. B Biointerfaces* **2015**, *125*, 238–246. [CrossRef] [PubMed]
7. Tan, L.; Zhang, Z.; Wang, Z.; Zhang, P.; Xiong, C.; Kuang, Y.; Peng, X.; Yu, M.; Qian, Y. Compositional variations in algal organic matter during distinct growth phases in karst water. *Front. Environ. Sci.* **2023**, *11*, 1112522.
8. Saito, Y.; Hosokawa, T.; Shiraishi, K. Collection of excitation-emission-matrix fluorescence of aerosol-candidate-substances and its application to fluorescence lidar monitoring. *Appl. Opt.* **2022**, *61*, 653–660. [CrossRef] [PubMed]
9. Cadondon, J.G.; Vallar, E.A.; Beltran, A.B.; Orbecido, A.H.; Galvez, M.C.D. Variation in Dissolved Organic Matter Using Absorbance and Fluorescence Measurements during Dry Season in St. Rosa and Cabuyao Rivers, Philippines. *Water* **2022**, *14*, 1444. [CrossRef]
10. Lal, P.P.; Juste-Poinapen, M.S.N.; Poinapen, J. Assessing the water quality of Suva foreshore for the establishment of estuary and marine recreational water guidelines in the Fiji Islands. *Water Sci. Technol.* **2021**, *84*, 3040–3054. [CrossRef] [PubMed]
11. Sanyal, P.; Ray, R.; Paul, M.; Gupta, V.K.; Acharya, A.; Bakshi, S.; Jana, T.K.; Mukhopadhyay, S.K. Assessing the Dynamics of Dissolved Organic Matter (DOM) in the Costal Environments Dominated by Mangroves, Indian Sundarbans. *Front. Earth Sci.* **2020**, *8*, 218. [CrossRef]
12. Cadondon, J.; Vallar, E.; Belo, L.; Orbecido, A.; Galvez, M.C. UV-Vis Absorbance and Fluorescence Characterization of Pasig River Surface Water Samples Towards the Development of an LED Fluorescence Lidar System. *Int. J. Adv. Sci. Eng. Inf. Technol.* **2021**, *11*, 968–980. [CrossRef]
13. Cadondon, J.G.; Ong, P.M.B.; Vallar, E.A.; Shiina, T.; Galvez, M.C.D. Chlorophyll-a pigment measurement of spirulina in algal growth monitoring using portable pulsed LED fluorescence lidar system. *Sensors* **2022**, *22*, 2940. [CrossRef]
14. Cadondon, J.G.; Vallar, E.A.; Shiina, T.; Galvez, M.C.D. Real-time Chlorophyll-a Pigment Monitoring of *Chlamydomonas reinhardtii* in a Controlled Environment Using Pulsed LED Fluorescence LiDAR System. *Photonics* **2023**, *10*, 144. [CrossRef]
15. Li, L.; Gao, N.; Deng, Y.; Yao, J.; Zhang, K. Characterization of intracellular & extracellular algae organic matters (AOM) of Microcystic aeruginosa and formation of AOM-associated disinfection byproducts and odor & taste compounds. *Water Res.* **2012**, *46*, 1233–1240. [PubMed]
16. Lee, S.; Park, J. Identification of Dissolved Organic Matter Origin Using Molecular Level Analysis Methods. *Water* **2022**, *14*, 1317. [CrossRef]
17. Harjung, A.; Schweichhart, J.; Rash, G.; Griebler, C. Large-scale study on groundwater dissolved organic matter reveals a strong heterogeneity and a complex microbial footprint. *Sci. Total Environ.* **2023**, *854*, 158542. [CrossRef] [PubMed]