engineering proceedings

MDPI

Proceeding Paper

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

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† Presented at the 10th International Electronic Conference on Sensors and Applications (ECSA-10), 15–30 November 2023; Available online: https://ecsa-10.sciforum.net/.

Abstract: Algal Organic Matter (AOM) is derived from the dissolved organic matter composition of the algal species observed. In this study, excitation-emission fluorescence spectroscopy has been 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. F680/450 is computed based on the ratio between the chlorophyll-a at 680 nm and dissolved organic matter contribution at 450 nm. F450/680 positively correlated with algal biomass (r = 0.96) at 405 nm excitation wavelength of *Chlorella* sp. This study is a good reference in algal biomass estimation and production in natural waters.

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

Citation: Cadondon, J.; Lesidan, J.R.; Bulan, J.; Vallar, E.; Shiina, T.; Galvez, M.C. Algal Organic Matter Fluorescence Analysis of *Chlorella* sp. for Biomass Estimation. *Eng. Proc.* **2023**, *56*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2023

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 discussed 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 in understanding the behaviors and composition of AOM and chlorophyll-a pigment in natural waters.

The behavior and composition of *Chlorella sp.* has been studied using spectroscopic techniques. Our interest aims to study excitation-emission (Ex/Em) pairs for algal organic matter and pigment measurement in natural waters. This EEM fluorescence analysis may improve our existing portable fluorescence lidar systems used in algal biomass estimation. 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 is currently being studied 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 is 0.6, with a pH between 8 and 9. The light/dark duration was controlled at 12 h/12 h with a light intensity of 75 μmol/m^2/s.

The extracted AOM was obtained with 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, USA).

2.2. Spectral Characterization

2.2.1. UV-Vis Measurement by Absorbance Spectroscopy

Aromaticity, size, and aromatic substances of algae can be interpreted by measuring $SUVA_{254}$. The protein-like structures 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. Fluorescence Measurement by Excitation-Emission Spectroscopy

The fluorescence measurements expressed in normalized units are EEM of AOM and pigment at varying biomass concentrations. The excitation-emission pair is 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 setup and data analysis is provided in our previous paper on algal growth and real-time monitoring in natural waters [12–14].

2.3. Application of 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 fluorescence intensity profiles using 380 nm and 405 nm excitation wavelengths. The region of interest for AOM is 400–600 nm and 600–800 nm for pigment measurements. Developing a fluorescence lidar system for biomass estimation is recommended since it is an *in-situ* and non-invasive technique.

2.4. Comparison of Fluorescence EEM

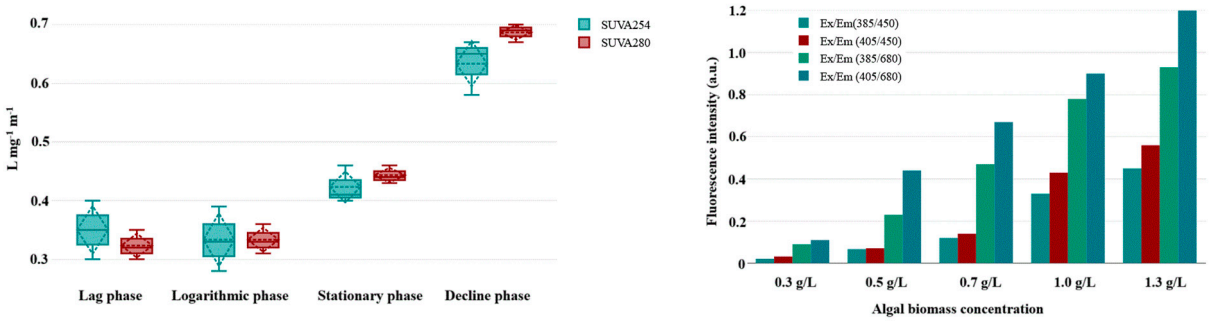
In recent studies, Biomass measurements use indirect or direct techniques. The results from the introduced fluorescence ratio are correlated (Pearson's r) with optical density measurements, biomass estimation, and estimated chlorophyll-a concentrations.

3. Results and Discussion

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

3.1. Spectral Characterization of Chlorella sp.

The variance in $SUVA_{254}$ and $SUVA_{280}$ in varying biomass are shown in Figure 1a. The $SUVA_{254}$ measured in 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. It represents the absorbance at $SUVA_{254}$ and $SUVA_{280}$ (n = 6). (**b**) Fluorescence intensity from 3D EEM at varying algal biomass concentration.

The fluorescence intensity results from 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 AOM and pigments. The total organic matter contribution is measured at 450 nm fluorescence emission [16]. The fluorescence emission is commonly used for surface water and terrestrial systems [17]. The studied excitation/emission pair 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 showed no significant difference at 385 nm/450 nm ($p < 0.05$).

The same trend was reflected in the 405 nm/680 nm and 385nm/680 nm pairs for chlorophyll-a measurement ($p < 0.05$). 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 as compared to Ex/Em 405 nm/450 nm with the algal biomass concentration. This is also identical to using 680 nm fluorescence emission. On the other hand, the fluorescence ratio showed opposite results. F450/680 at 405 nm excitation wavelength (r = 0.96) positively correlates to F450/680 at 385 nm excitation wavelength (r = 0.94) with algal biomass. This F-ratio is recommended to understand the dry-weight algal biomass in natural waters.

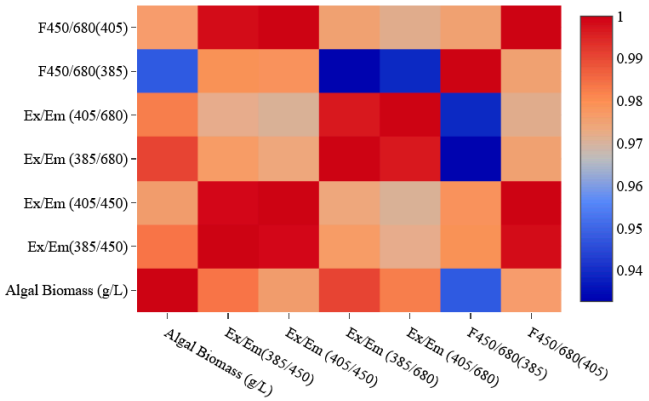
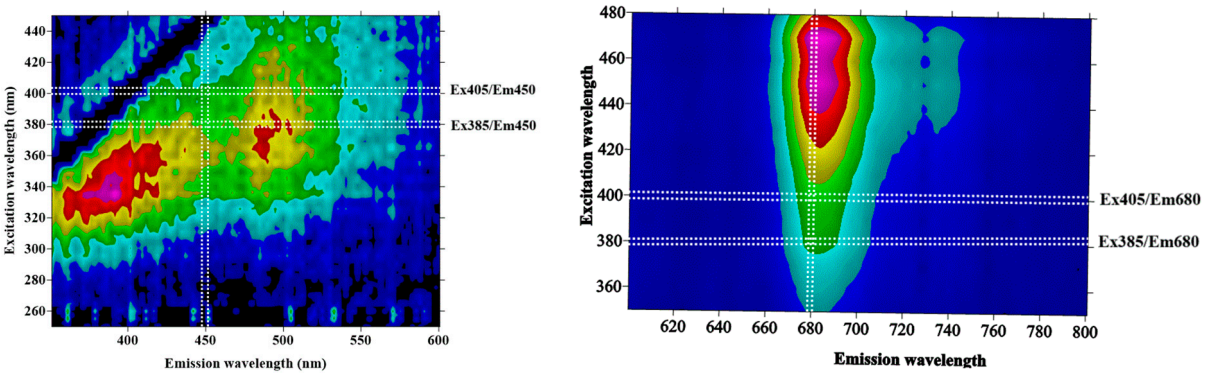


Figure 2. Correlation heat map of Ex/Em pairs, F ratio, and algal biomass of *Chlorella* sp.

3.2. Analysis of Fluorescence EEM to the Development of the Lidar System

Developing a portable fluorescence lidar system is vital for real-time monitoring and estimating algal biomass in natural waters. With the guidance of 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 chlorophyll concentration of microalgae, respectively (Figure 3). F450/680 is used to calculate dry weight measurements of algal biomass of *Chlorella* sp. for 385 nm and 405 nm excitation wavelengths. Similar trends were observed, but a higher positive correlation was computed at 405 nm excitation wavelength.



(**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 385 nm excitation wavelength, which serves as a transmitting system. With the new understanding of Ex/Em pairs shown, we suggest the development of a portable pulsed laser diode (LD) at 405 nm excitation wavelength. This study paves the discovery of a new technique for developing a fluorescence lidar system using excitation-emission pairs [8].

Author Contributions: Conceptualization, J.C., J.R.L., and J.B.; methodology, J.C.; validation, formal analysis, J.C., J.R.L., J.B. and T.S.; investigation, J.C.; writing—original draft preparation, J.C., J.R.L., and J.B.; writing—review and editing, J.C., M.C.G., E.V., and T.S.; supervision, M.C.G., E.V., and T.S.; funding acquisition, M.C.G. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Not Applicable.

Acknowledgments: The authors would like to acknowledge support from the University of the Philippines Visayas, Department of Science and Technology-Accelerated Science and Technology Human Resource Development Program, and Visayas State University. We thank DLSU-URCO project No. 16 IR2TAY19-2TAY21 for supporting this project.

Conflicts of Interest: The authors declare no conflict 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.
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.
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.
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.

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.
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.
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.
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.
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.
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.
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. *IJASEIT* **2021**, *11*, 968–980.
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.
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.
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.
16. Lee, S.; Park, J. Identification of Dissolved Organic Matter Origin Using Molecular Level Analysis Methods. *Water* **2022**, *14*, 1317.
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**.**