SUSTAINABLE TREATMENT OF SHRIMP AQUACULTURE WASTEWATER USING ATS–HRAP SYSTEM WITH CHLORELLA VULGARIS: A NATURE-BASED APPROACH FOR ENVIRONMENTAL PROTECTION

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Abstract

This study evaluates the effect of ATS substrate mesh size (0.2 mm, 0.6 mm, and 1 mm) and biomass retention time (7, 14, and 20 days) on nutrient removal efficiency and biomass development in an integrated High Rate Algal Pond (HRAP) system using Chlorella vulgaris for shrimp aquaculture wastewater treatment. A synthetic saline wastewater (7 ppt) simulating shrimp effluent was treated under controlled conditions. The results show that phosphate was removed most effectively, with average removal efficiencies reaching 86% under the 0.6 mm mesh and 7-day BRT. Total nitrogen removal peaked at 71.4% under the same condition. COD removal was modest, with a maximum of 22.1%, observed with the 1 mm mesh and 7-day BRT. Biomass accumulation was influenced by both substrate mesh size and retention time, with the highest MLVSS to MLSS ratios (above 0.75) found under mid-range mesh configurations. The findings highlight the role of substrate design and harvesting strategy in optimizing microalgae-based wastewater treatment systems and support the use of C. vulgaris as a feasible option for nutrient recovery and biomass production in saline aquaculture settings.

Keywords: Algal Turf Scrubber; Biomass retention time; Chlorella vulgaris; Nutrient removal; Shrimp aquaculture wastewater.

JEL Classifications: Q53, Q55,Q57.

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1. INTRODUCTION

Intensive shrimp aquaculture is currently one of the fastest-growing sectors in global aquaculture, particularly in Asia. However, this rapid expansion poses serious environmental challenges. Shrimp farming generates large volumes of wastewater with high concentrations of organic matter, nitrogen, phosphorus, and persistent compounds that contribute to eutrophication, water quality degradation, and adverse impacts on coastal ecosystems if inadequately treated (Hang et al., 2024). It is estimated that only 20–25% of dietary nitrogen is assimilated by shrimp, with the remainder accumulating in the water column as ammonium, nitrate, and other nitrogenous compounds (Iber &Kasan, 2021). The direct discharge or insufficient treatment of shrimp effluent increases the risk of harmful algal blooms, hypoxia, biodiversity loss, and disease outbreaks (Dauda et al., 2019). Therefore, there is a critical need to develop effective and sustainable wastewater treatment systems that enable both pollution control and resource recovery.

In this context, microalgae-based treatment systems have emerged as promising solutions that simultaneously remove nutrients and produce biomass with economic value (Kusuma et al., 2024). Chlorella vulgaris, a fast-growing unicellular microalga, is known for its high nitrogen and phosphorus uptake capacity and its adaptability to saline environments typical of shrimp wastewater (Ahmad et al., 2020; Borowitzka, 2013). Under optimal cultivation conditions, C. vulgaris has been shown to remove over 90% of total nitrogen and phosphorus, while producing protein-rich biomass suitable for aquafeed or organic fertilizer applications (Safi et al., 2014).

The High Rate Algal Pond (HRAP) is one of the most widely adopted systems for microalgae-based wastewater treatment (Butterworth et al., 2024). HRAPs are shallow, mixed ponds designed to enhance light and CO_2 availability, thereby promoting algal growth and nutrient removal (Fallowfield et al., 2018). In contrast, Algal Turf Scrubber (ATS) systems involve wastewater flowing over inclined surfaces with attached substrates, where a mixed biofilm of algae and bacteria efficiently assimilates nutrients. ATS systems offer advantages such as high biomass retention, low washout rates, ease of harvesting, and minimal aeration requirements (Adey et al., 2011; Leong et al., 2021). Experimental studies have reported removal efficiencies of up to 99% for phosphorus and 84% for total nitrogen in aquaculture wastewater using ATS (Gan et al., 2022; Kishi et al., 2018).

However, most existing ATS studies focus on indigenous filamentous algae. The application of C. vulgaris in ATS systems remains limited due to its unicellular nature and low adhesion capacity, which increases its susceptibility to washout under continuous flow. Recent studies have indicated that C. vulgaris can produce extracellular polymeric substances (EPS) that enhance adhesion, especially when combined with appropriately sized mesh substrates (Shen et al., 2017; Wang et al., 2014). Additionally, design parameters such as ATS mesh size and biomass retention time (BRT) are critical determinants of treatment efficiency and biomass accumulation (Sutherland et al., 2020).

Based on these premises, this study aims to evaluate the effects of ATS mesh size (0.2 mm, 0.6 mm, and 1 mm) and biomass retention time (7, 14, and 20 days) on the removal efficiency of COD, total nitrogen, and phosphate, as well as on the biomass development of Chlorella vulgaris in an HRAP system integrated with ATS. The findings are expected to identify optimal operational conditions and contribute to the advancement of circular, scalable, and sustainable wastewater treatment technologies for intensive shrimp aquaculture.

2. MATERIALS AND METHODS

2.1. Materials and research subjects

The experimental system was established based on a High Rate Algal Pond (HRAP) model integrated with mesh substrates functioning as an Algal Turf Scrubber (ATS). Chlorella vulgaris was cultured under laboratory conditions at the Environmental and Sustainable Development Laboratory, Interdisciplinary Institute of Science, Nguyen Tat Thanh University. Prior to the treatment experiments, the algal culture was grown to a biomass concentration of approximately 106 cells/L.

Synthetic shrimp aquaculture wastewater was prepared to simulate typical effluent characteristics based on previously analyzed data. The formulated wastewater contained 120 mg/L of chemical oxygen demand (COD), 12.2 mg/L of total nitrogen (TN), 10.2 mg/L of ammonium (N–NH$_4^+$), 0.2 mg/L of nitrite (N–NO$_2$-), 1.6 mg/L of nitrate (N–NO$_3$-), and 1.0 mg/L of total phosphorus (TP), with salinity adjusted to approximately 7 parts per thousand (ppt).

2.2. Operating parameters of the model

The wastewater treatment system implemented in this study aimed to evaluate the growth performance of microalgae and the removal efficiency of pollutants through the integration of a High Rate Algal Pond (HRAP) and Algal Turf Scrubber (ATS) substrates.

The HRAP unit was constructed with dimensions of 0.7 × 0.5 × 0.3 meters, resulting in a total volume of 96 liters and an effective operating volume of 90 liters. Within this configuration, the ATS substrates were suspended 5 centimeters above the water surface, serving as a biological attachment surface for Chlorella vulgaris. This design facilitated expanded light exposure zones and enhanced photosynthetic efficiency as well as nutrient uptake. Additionally, the ATS provided a supportive structure for microbial colonization, promoting nutrient assimilation and the biodegradation of organic and inorganic contaminants present in the wastewater.

The system operated under continuous low-speed mixing using a paddle wheel rotating at 20 revolutions per minute, ensuring stable flow, uniform light distribution, and optimal contact between microalgae and dissolved nutrients. An initial volume of 9 liters of microalgal culture was introduced, followed by the addition of synthetic wastewater to reach a total volume of 90 liters. Daily, 10 liters of synthetic shrimp wastewater were added and discharged at an equal volume, corresponding to a hydraulic retention time (HRT) of 9 days, while biomass retention time (BRT) was maintained at 10 days. The system was exposed to natural light with a 12:12 hour light–dark photoperiod, and ambient temperature ranged from 25 – 32°C. The pH was maintained at 7.0 ± 0.49 to support optimal physiological activity of both microalgae and associated microbial communities.

2.3. Experimental setup, sampling methods and frequency

2.3.1. Experimental setup

The experiment was designed to investigate the effects of key operational factors, specifically ATS mesh size and biomass retention time (BRT), on the treatment performance of the system. A series of continuous-flow trials were conducted under different combinations of these two variables. The ATS mesh sizes tested included 0.2 mm, 0.6 mm, and 1 mm, while the BRTs were set at 7, 14, and 20 days, respectively. Each treatment condition was operated independently under steady-state conditions, and system performance was monitored accordingly. The objective was to evaluate how variations in mesh size and biomass harvesting frequency influenced the removal efficiency of pollutants in shrimp aquaculture wastewater. The findings aimed to determine the most effective combination of ATS mesh size and retention time to optimize system performance.
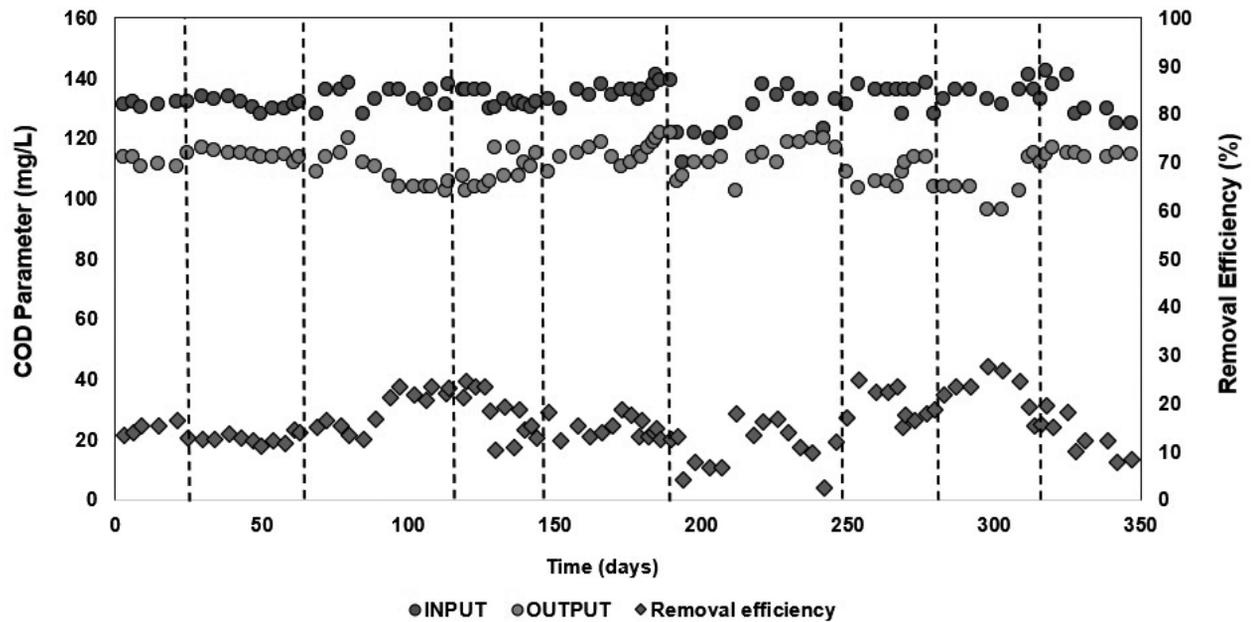
Figure 1. COD removal efficiency of the HRAP model integrated with ATS media under different treatments.

2.3.2. Sampling methods and frequency

To evaluate treatment performance, both influent and effluent samples were analyzed with a focus on specific environmental parameters. Chemical oxygen demand (COD) was measured following the Vietnamese standard TCVN 6186:1996, equivalent to ISO 8467:1993 (E), ensuring analytical accuracy and comparability. Total nitrogen (TN) was determined using the Standard Methods for the Examination of Water and Wastewater (SMEWW) 4500-N B and C. Orthophosphate ($P-PO_4^{3-}$), representing total phosphorus, was analyzed according to SMEWW 4500-P B and E (2012). For biomass characterization, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were quantified based on SMEWW 2540 D (2012).

All samples were collected and analyzed three times per week across nine experimental treatments. The entire system was operated over a 30 to 40-day period to ensure the reliability and representativeness of the results.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the pollutant removal efficiency of the HRAP Model Integrated with ATS Media

The microalga Chlorella vulgaris, when integrated into wastewater treatment systems, utilizes photosynthesis to degrade organic pollutants, thereby contributing to a reduction in chemical oxygen demand (COD) levels in the aquatic environment. This process parallels the assimilation of CO_2 during photosynthesis, wherein microalgae metabolize available organic substrates such as glucose, fructose, amino acids, and fatty acids. The consumption of these compounds by C. vulgaris leads to a concurrent decrease in COD concentrations, thus enhancing the treatment performance of photobioreactor-based systems such as High-Rate Algal Ponds (HRAPs).

As illustrated in Figure 1, the influent COD concentration remained relatively stable, with an average value of 132.7 mg/L, peaking at 142.4 mg/L and dropping to a minimum of 112 mg/L. These values indicate a controlled and consistent synthetic wastewater composition during the experimental period. In contrast, effluent COD values exhibited greater variability, with an average of 111.5 mg/L, ranging from a low of 96 mg/L to a high of 121.6 mg/L. This fluctuation reflects the influence of operational conditions on treatment efficiency.

The system's average COD removal efficiency was approximately 15.85%, with a maximum observed removal of 29.41% and a minimum of only 4.29%. Although the overall COD removal performance was modest, the experimental conditions had a marked impact on treatment outcomes. In treatments employing finer ATS mesh sizes (0.2 mm) and shorter biomass retention times (BRT) of 7 days, COD removal efficiency ranged between 13% and 16%, likely due to limited algal contact time and retention. However, increasing the BRT to 14 or 20 days particularly in the treatment condition with a 0.6 mm mesh size and 20-day BRT resulted in significantly improved removal rates, with values frequently ranging from 22% to 27%. The
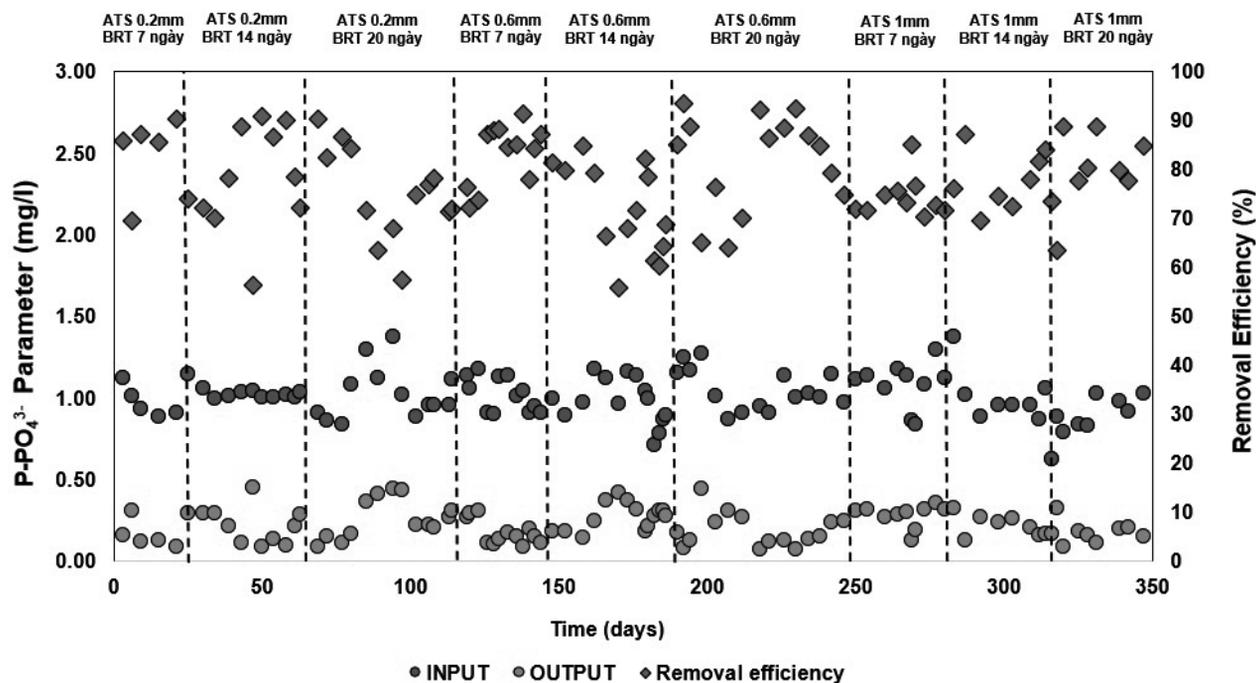
Figure 2. P-PO$_4^{3-}$ removal efficiency of the HRAP model integrated with ATS media under different treatments.

highest efficiency (29.41%) was recorded on days 298 and 303 under the condition with a 1 mm mesh size and 20-day BRT.

These findings suggest that prolonged biomass retention combined with larger ATS mesh sizes favors algal growth, enhances organic matter uptake, and extends algal residence time within the system, thereby improving overall treatment performance. Such biofilms promote a balanced bacterial community and facilitate synergistic interactions, reducing biomass washout and promoting microbial oxidation processes (Craggs & Technology, 2001; Villar-Navarro et al., 2018). Moreover, shorter retention times involve more frequent harvesting, which helps remove older algal and microbial biomass, thereby maintaining a younger and metabolically active biomass population that contributes to consistent COD removal efficiency (Magalhães et al., 2024).

After removing outlier values, phosphate data analysis revealed that the HRAP system integrated with an Algal Turf Scrubber (ATS) achieved consistently high and stable removal efficiencies across most experimental conditions, as illustrated in Figure 2. The application of microalgae for nutrient removal from wastewater while simultaneously recovering biomass has been widely documented in the literature (Wang et al., 2012). Notably, certain microalgal strains are capable of uptaking phosphorus in quantities

exceeding their immediate growth requirements, particularly under suboptimal nutrient conditions. Recent findings indicate that phosphorus assimilated by microalgae is not solely allocated to biosynthesis processes but can also be stored intracellularly in the form of polyphosphate (Poly-P), a distinctive cellular storage compound (Harold, 1966).

The influent phosphate (PO$_4^{3-}$) concentrations ranged from 0.72 to 1.38 mg/L, with an average value of approximately 1.02 mg/L. In contrast, the effluent concentrations ranged from 0.07 to 0.46 mg/L, with an average reduced to 0.22 mg/L. This substantial difference between influent and effluent concentrations indicates the system's high phosphorus removal capacity under various operational scenarios. Phosphorus removal efficiencies, after excluding outlier data, ranged from 55.77% to 93.60%, with an average efficiency of 78.31%, which surpasses the 71% reported by Picot et al. (1991). These results demonstrate excellent treatment performance, particularly considering the system utilizes a consortium of Chlorella vulgaris and heterotrophic bacteria for wastewater remediation.

When analyzed by experimental phase, the configurations employing ATS mesh sizes of 0.2 mm and 0.6 mm combined with biomass retention times (BRT) of 7 to 14 days exhibited high and stable phosphorus removal efficiencies, with several
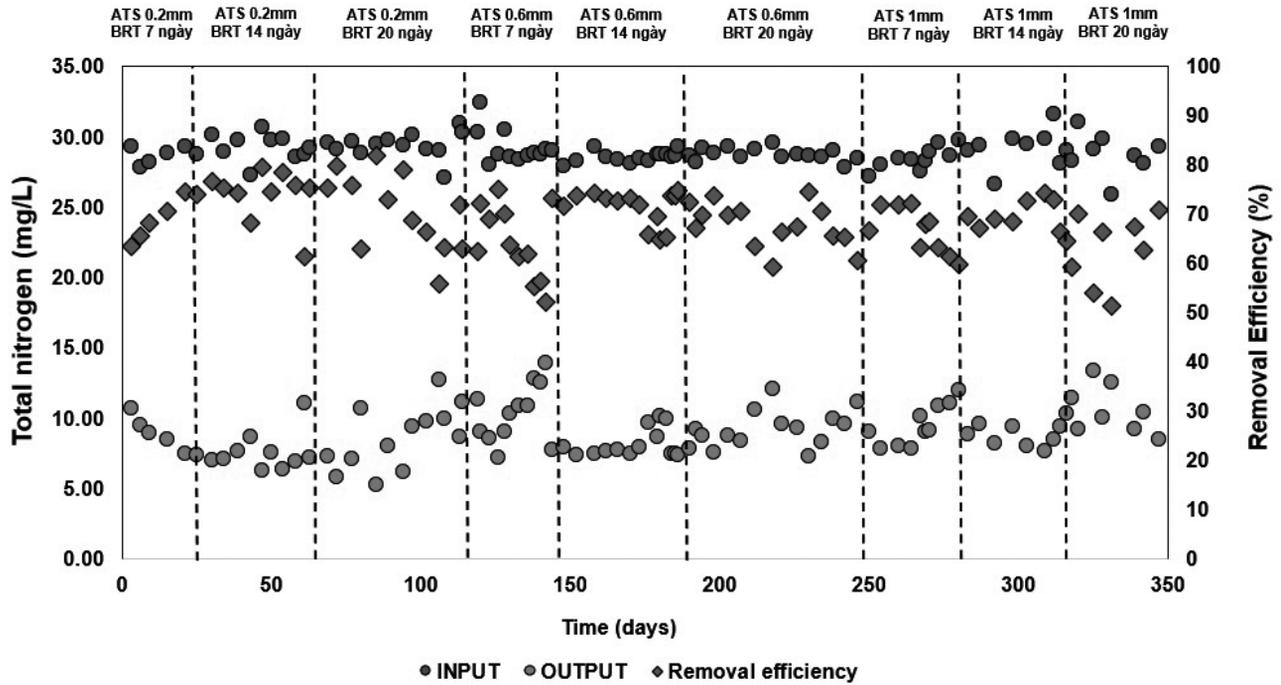
Figure 3. Total nitrogen removal efficiency of the HRAP model integrated with ATS media.

measurements exceeding 85%. This suggests that smaller mesh sizes provide enhanced surface area for microalgal attachment and optimal light exposure, which facilitate nutrient assimilation. Conversely, treatments using 1 mm ATS mesh and shorter BRTs showed slightly lower removal efficiencies, ranging between 65% and 75%. This reduction may be attributed to the decreased effective contact area due to the larger mesh size, which in turn reduces algal attachment density and phosphorus uptake. Moreover, shorter BRTs may not allow sufficient time for biomass development and phosphorus accumulation, thereby limiting overall treatment efficiency.

Chlorella vulgaris is a photosynthetic microalga capable of nitrogen uptake from wastewater through assimilation during photosynthesis. In addition to microalgae, bacteria play a crucial role in nitrogen removal, particularly through the transformation of organic nitrogen compounds into oxidized forms such as nitrate (NO_3^-) and nitrite (NO_2^-) via nitrification processes. While bacteria can also assimilate total nitrogen, they predominantly utilize ammonia as their primary nitrogen source for biomass synthesis, including the production of proteins and cellular components. The integration of Chlorella vulgaris with nitrifying bacteria in wastewater treatment systems enhances nitrogen removal efficiency by coupling algal assimilation with microbial nitrification and denitrification.

Total nitrogen removal efficiency is illustrated in Figure 3. The average removal efficiency was 68.55%, with a maximum value of 82.05% and a minimum of 51.51%. Compared to other treatment indicators, this is a relatively high performance, especially considering that the system operated without any chemical coagulants or external support technologies, relying solely on microalgae and the ATS structure. The nitrogen removal mechanism is primarily driven by the assimilative capacity of Chlorella vulgaris and its symbiotic interactions with associated bacteria. Under optimal light conditions and biomass retention times (BRT), the microalgae absorb ammonium and nitrate for growth, while simultaneously supplying oxygen to support bacterial nitrification and subsequent denitrification within the system.

Performance analysis across different experimental treatments indicated that configurations using smaller ATS mesh sizes (0.2 mm and 0.6 mm) combined with longer BRTs (14 to 20 days) achieved higher nitrogen removal efficiencies, with many measurements ranging from 70% to over 75%. In contrast, treatments utilizing 1 mm mesh and short BRTs (7 days) tended to exhibit slightly reduced removal efficiency, with values falling below 65% in some instances. This reduction may be attributed to a decrease in effective surface area and algal biomass density due to the larger mesh size,
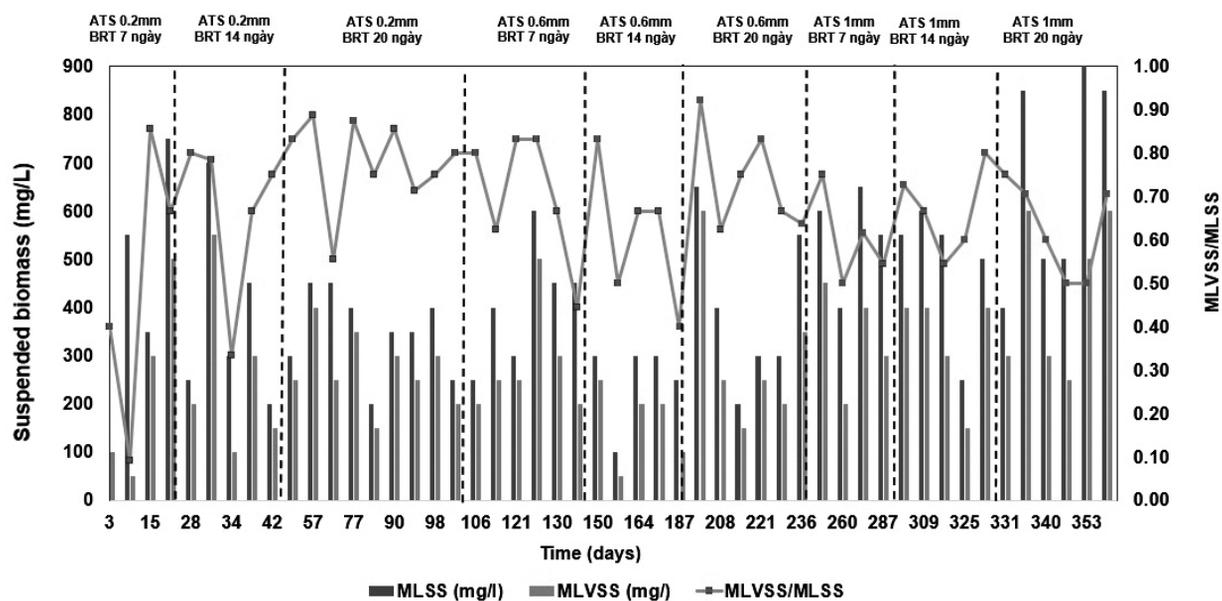
Figure 4. MLSS and MLVSS biomass in the HRAP model integrated with ATS media

along with insufficient biomass accumulation under shorter retention periods, which collectively limited the system's nitrogen removal capacity.

3.2. Evaluation of biomass development in the system

MLSS (Mixed Liquor Suspended Solids) and MLVSS (Mixed Liquor Volatile Suspended Solids) are critical indicators used to evaluate biomass transformation efficiency in wastewater treatment systems employing both bacteria and microalgae. MLSS represents the total concentration of suspended solids in the mixed liquor, comprising bacterial and algal biomass along with organic and inorganic particulates present in the treatment process. This parameter reflects the density of microbial and algal populations within the system. Monitoring MLSS provides insight into the pollutant load and treatment performance, enabling appropriate adjustments in nutrient supply and hydraulic loading. MLVSS, a subset of MLSS, represents the volatile fraction primarily attributed to active biomass. Collectively, MLSS and MLVSS monitoring serves as a valuable tool for assessing system performance, optimizing operational conditions, and maintaining biological balance for effective wastewater remediation.

As illustrated in Figure 4, the biomass accumulation within the treatment system was generally favorable, with total MLSS reaching approximately 376g and average MLVSS values around 258g. This indicates robust growth of both microalgae and microbial communities. Notably, during the phase utilizing an ATS mesh size of 0.6 mm, MLSS and MLVSS values

exhibited significant variation, with MLSS peaking at approximately 750g and MLVSS reaching 500g. The MLVSS/MLSS ratio ranged from 0.6 to 0.8, suggesting a high proportion of active biomass and well-maintained algal and microbial growth dynamics. In contrast, during the phase with a 1 mm mesh size, biomass formation remained stable, yet the larger pore size appeared to limit algal and microbial development. Consequently, total biomass values ranged from 200 to 450g, and the MLVSS/MLSS ratio decreased slightly to 0.45-0.8. These observations indicate that both mesh size and biomass retention time (BRT) on the ATS influence biomass accumulation. Specifically, smaller mesh sizes provide more favorable conditions for organic biomass growth due to enhanced surface area for attachment and optimal light exposure.

4. CONCLUSION

The results demonstrate that ATS mesh size and biomass retention time are key factors influencing the pollutant removal efficiency of the HRAP–ATS system. The use of Chlorella vulgaris enabled stable biofilm formation and effective treatment under simulated saline wastewater conditions (7 ppt). The highest COD removal (22.1%) was achieved using a 1 mm mesh with a 7-day retention time, while phosphate and total nitrogen removal reached 86.1% and 71.4%, respectively, under a 0.6 mm mesh and 7-day retention. These findings confirm that optimizing substrate configuration and harvesting intervals enhances both nutrient removal and biomass productivity. This study provides a practical foundation for designing sustainable and biologically integrated wastewater

treatment systems for application in aquaculture and similar saline environments.

REFERENCES

1. Adey, W. H., Kangas, P. C., & Mulbry, W. (2011). Algal turf scrubbing: cleaning surface waters with solar energy while producing a biofuel. BioScience, 61(6), 434-441.

2. Ahmad, M. T., Shariff, M., Md. Yusoff, F., Goh, Y. M., & Banerjee, S. (2020). Applications of microalga Chlorella vulgaris in aquaculture. Reviews in Aquaculture, 12(1), 328-346.

3. Borowitzka, M. A. (2013). Dunaliella: biology, production, and markets. Handbook of microalgal culture: applied phycology and biotechnology, 359-368.

4. Butterworth, S., Fallowfield, H. J. W. S., & Technology. (2024). Comparison of the wastewater treatment performance of continuously and discontinuously mixed high-rate algal ponds at Kingston on Murray. 89(3), 505-512.

5. Craggs, R. J. W. S., & Technology. (2001). Wastewater treatment by algal turf scrubbing. 44(11-12), 427-433.

6. Dauda, A. B., Ajadi, A., Tola-Fabunmi, A. S., & Akinwole, A. O. (2019). Waste production in aquaculture: Sources, components and managements in different culture systems. Aquaculture and Fisheries, 4(3), 81-88.

7. Fallowfield, H. J., Young, P., Taylor, M. J., Buchanan, N., Cromar, N., Keegan, A., . . . Technology. (2018). Independent validation and regulatory agency approval for high rate algal ponds to treat wastewater from rural communities. 4(2), 195-205.

8. Gan, X., Klose, H., & Reinecke, D. (2022). Optimizing nutrient removal and biomass production of the Algal Turf Scrubber (ATS) under variable cultivation conditions by using Response Surface Methodology. Frontiers in bioengineering and biotechnology, 10, 962719.

9. Hang, D. T. T., Dat, T. T., Nghi, D. N., Nhu, N. H., Nam, L. H., Phuong, T. T. B., . . . Tran, T. (2024). Biomass recovery and wastewater treatment in shrimp aquaculture: A comparative study on the performance of HRAPS and PMBR. IOP Conference Series: Earth and Environmental Science,

10. Iber, B. T., & Kasan, N. A. J. H. (2021). Recent advances in Shrimp aquaculture wastewater management. 7(11).

11. Kishi, M., Takee, H., Kawai, M., Nagao, N., & Toda, T. J. J. o. E. B. (2018). Sequential high rate algal ponds operation for enhanced treatment of organic wastewater. 39(5), 835-842.

12. Kusuma, H. S., Illiyanasafa, N., Jaya, D. E. C., Darmokoesoemo, H., Putra, N. R. J. S. C., & Pharmacy. (2024). Utilization of the microalga Chlorella vulgaris for mercury bioremediation from wastewater and biomass production. 37, 101346.

13. Leong, Y. K., Huang, C.-Y., & Chang, J.-S. (2021). Pollution prevention and waste phycoremediation by algal-based wastewater treatment technologies: The applications of high-rate algal ponds (HRAPs) and algal turf scrubber (ATS). Journal of Environmental Management, 296, 113193.

14. Magalhães, I. B., de Paula Pereira, A. S. A., Silva, T. A., Ferreira, J., Braga, M. Q., Couto, E. A., . . . Calijuri, M. L. J. J. o. W. P. E. (2024). Advancements in high-rate algal pond technology for enhanced wastewater treatment and biomass production: A review. 66, 105929.

15. Safi, C., Zebib, B., Merah, O., Pontalier, P.-Y., & Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. Renewable and sustainable energy reviews, 35, 265-278.

16. Shen, Y., Yang, T., Zhu, W., & Zhao, Y. (2017). Wastewater treatment and biofuel production through attached culture of Chlorella vulgaris in a porous substratum biofilm reactor. Journal of Applied Phycology, 29, 833-841.

17. Sutherland, D. L., Burke, J., & Ralph, P. J. (2020). Increased harvest frequency improves biomass yields and nutrient removal on a filamentous algae nutrient scrubber. Algal research, 51, 102073.

18. Villar-Navarro, E., Baena-Nogueras, R. M., Paniw, M., Perales, J. A., & Lara-Martín, P. A. J. W. r. (2018). Removal of pharmaceuticals in urban wastewater: High rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes.139, 19-29.

19. Wang, H., Xiong, H., Hui, Z., & Zeng, X. J. B. T. (2012). Mixotrophic cultivation of Chlorella pyrenoidosa with diluted primary piggery wastewater to produce lipids.104, 215-220.

20. Wang, M., Kuo-Dahab, W. C., Dolan, S., & Park, C. (2014). Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, Chlorella sp. and Micractinium sp., in wastewater treatment. Bioresource technology, 154, 131-137.