

Evaluation of The Efficiency of Shrimp Farming Wastewater Treatment Using a Revolving Algal Biofilm Reactor: a Case Study in Can Gio, Ho Chi Minh City

Vo Thi Dieu Hien^{1,*}, Huynh Do Dat¹, Le Thi Ngoc Chau¹, Le Anh Vu¹, Nguyen Van Truc²

¹Faculty of Applied Science and Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam

²Faculty of Engineering and Technology, Saigon University, Ho Chi Minh City, Viet Nam

*vtdhien@ntt.edu.vn

Abstract

Untreated aquaculture wastewater discharged directly into the environment poses a significant potential risk to the ecosystem. This study applies a rotating algal biofilm system (RAB) to investigate the biomass growth and pollutant removal capabilities of *C. Vulgaris* in this type of wastewater. Results show that the TKN and $\text{NH}_4^+\text{-N}$ removal reached approx. 90% in the steady state, with the highest removal rates of 4.5 mg/L.day and 2.7 mg/L.day, respectively. The TP removal rate reached 1.4 mg/L.day, achieving a maximum efficiency of 86.8%. The COD removal was improved, reaching 84.6% with a highest rate of 67.67 mg/L.day. Attached biomass increased from 2.53 g/m².day to 3.85 g/m².day, while suspended biomass increased to 2.40 g/m².day. This demonstrates the high potential of the RAB system in nutrient recovery and biomass productivity in sustainable aquaculture.

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Keywords

Aquacultural wastewater; revolving algal biofilm; *C. Vulgaris*; nutrient recovery.

1 Introduction

The growth of Viet Nam's aquaculture industry is increasing rapidly, contributing more than 7% to the global market [1]. This means creating significant challenges for the aquatic environment. Shrimp farming, one of the most popular aquaculture activities in Vietnam, discharges large amounts of wastewater containing high concentrations of nutrients and organic matters into river systems. Notably, most of this wastewater is not or has not been properly treated [2]. This has contributed to eutrophication in water sources, causing human health and ecosystem risks. Faced with these challenges, many studies have developed algae-based technologies to recover nutrients and convert waste into valuable products. Some notable technologies used for

this purpose include high-speed algal ponds, airlift reactors, flat-plate photobioreactors, and biofloc technology [3].

Among the algae-based technologies mentioned above, revolving algal biofilm (RAB) has emerged as a particularly promising solution and has been the focus of much research in recent years [4]. The RAB system has a unique rotating belt that facilitates algal adhesion and allows direct contact with both sunlight and air. The continuous rotation of this belt helps maintain optimal growth conditions and prevents damage to the biofilm. Once formed, the algal biofilm becomes the dominant microbial community responsible for wastewater treatment. Furthermore, unlike other systems that require algal harvesting by sedimentation or centrifugation, the RAB system allows for efficient biomass collection by a

simple scraping mechanism directly on the belt, significantly reducing energy requirements.

Although many studies have previously investigated RAB systems, most of these have focused on synthetic wastewater and have not yet been conducted on shrimp pond wastewater. Therefore, this study aims to recover biomass and remove nutrients from actual shrimp pond wastewater. This work proposes a sustainable wastewater treatment method and contributes to promoting green technologies towards circular approaches aimed at achieving sustainable development goals in aquaculture.

2 Material and Research Methodology

2.1 Algae Strain

The culture medium was constructed based on Bold's Basic Medium (BBM), as described by [4], including macronutrients such as, NaCl (10 mL of a 2.5 g/L solution), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (10 mL of a 7.5 g/L solution), KH_2PO_4 (10 mL of a 17.5 g/L solution), K_2HPO_4 (10 mL of a 7.5 g/L solution), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (10 mL of a 2.5 g/L solution), and NaNO_3 (10 mL of a 25 g/L solution). Additionally, the

BBM medium was supplemented with micronutrients, including 1 mL of H_3BO_3 (11.42 g/L), EDTA solution, FeCl_3 , and a trace element solution containing $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (8.82 g/L), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.44 g/L), MoO_3 (0.71 g/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.57 g/L), and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.49 g/L). After 5 to 7 days of cultivation, when the biomass reached a stable growth stage with a cell concentration of approximately 1.2 g/L, the microalgal culture medium was inoculated into the RAB system. After inoculation, the system was directly supplied with actual shrimp farming wastewater, collected from aquaculture ponds located in Can Gio District (or An Thoi Dong Commune, the address after merged), Ho Chi Minh City (Fig. 1). The wastewater exhibited the following physicochemical characteristics: pH ranged from 7 to 8, COD was (363 ± 31.4) mg/L, NH_4^+-N was (18.8 ± 0.9) mg/L, TKN was (28.8 ± 2.7) mg/L and TP was (7.96 ± 0.7) mg/L. These characteristics are considered representative of typical wastewater generated from intensive shrimp farming activities and are used as a basis for evaluating the treatment efficiency of the RAB system.

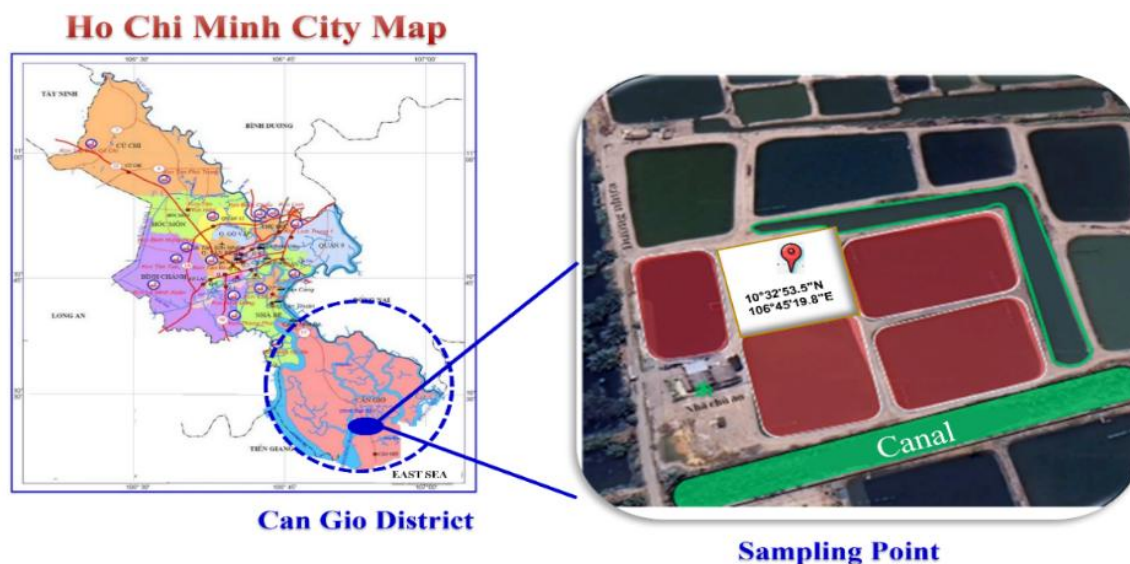


Figure 1 Location map of the studied area and sampling site in Can Gio District (or An Thoi Dong Commune, the address after merged), Ho Chi Minh City, Vietnam

2.2 RAB Design and Operation

As illustrated in Figure 2, the RAB system used in this study with a working volume of 90 L. The RAB is designed with a stainless-steel tank and a cotton belt

rotating around an axis arranged in a triangular configuration of three rotating cylinders. The belt with a rough surface is inexpensive, very durable, and durable in wastewater. The belt is rotated at a speed of

4 cm/s by the movement of the cylinders, which also function as mixers.

Wastewater was continuously added and drained at a rate of 15 L per day, corresponding to a hydraulic retention time (HRT) of 6 days. The pH of the reactor was not externally controlled but depended on the incoming wastewater. The system operated under natural light-dark cycles and ambient environmental conditions. Biomass was harvested weekly using a scraper mechanism, resulting in a biomass retention time of 7 days. DO is maintained at approximately (3-5) mg/L.

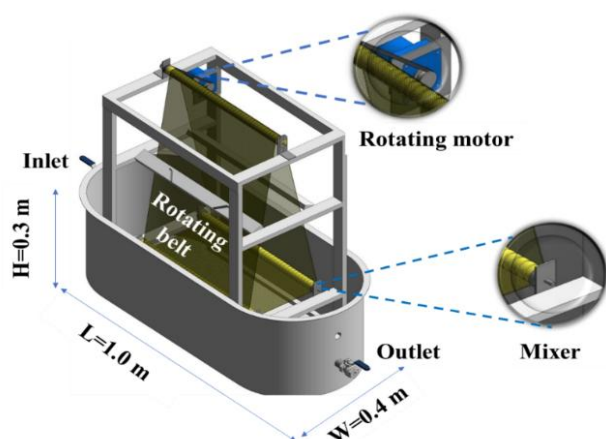


Figure 2 Schematic illustration of the RAB system

2.3 Analytical Method

2.3.1 Methods of Analyzing Water Quality Indicators

The parameters pH, DO, TDS, temperature, and light intensity were measured daily using a HANNA EDGE pH meter and a PEAKII-P512 handheld dissolved oxygen meter. COD was determined by the digestion and titration method (5220C:2017). TP was analyzed using the molybdate method with persulfate acid digestion (Method 4500-PD, Stannous Chloride Method). Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) was measured through titration using H_2SO_4 standard, following a preliminary distillation step (Method 4500- NH_3 , Titration Method), and the distillation was performed using the Kjeldahl UDK 129 apparatus. Nitrate nitrogen ($\text{NO}_3^-\text{-N}$) was determined by subtracting the absorbance at 275 nm from twice the absorbance at 220 nm (Method 4500- $\text{NO}_3\text{-B}$, UV Spectrophotometric Screening Method). Nitrite nitrogen ($\text{NO}_2^-\text{-N}$) was analyzed using the colorimetric method (Method 4500- $\text{NO}_2\text{-B}$, Colorimetric Method). The residual nutrient concentration in the outlet water was estimated using Eq. 1 and Eq. 2:

$$\text{Removal efficiency (RE, \%)} = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \times 100 \quad (1)$$

$$\text{Removal rate (RR, mg/L.day)} = \frac{(C_{\text{in}} \times F_{\text{in}}) - (C_{\text{out}} \times F_{\text{out}})}{V \times \Delta T} \quad (2)$$

Where C_{in} and C_{out} are the inlet and outlet concentrations wastewater, F_{in} and F_{out} are the volumes of inlet and outlet wastewater, V is the RAB working volume (90 L), and ΔT is the time interval between inflow and outflow wastewater (1 day).

2.3.2 Biomass Analysis Methods for Algae

The biomass concentration was determined through dry weight measurements. Specifically, 100 mL of the mixed liquid sample was collected daily for analysis. All samples were filtered using 0.45 μm pore-size membranes (Fisher Whatman Puradisc-25 mm), then dried at (103-105) $^{\circ}\text{C}$ for 2 hours to determine the mixed liquor suspended solids. Subsequently, the samples were ignited at (550 ± 50) $^{\circ}\text{C}$ for 20 minutes to determine the mixed liquor volatile suspended

solids. Biomass harvested on the conveyor belt was dried at 80 $^{\circ}\text{C}$ until a constant weight was achieved [5].

2.3.3 Nitrogen Mass Balance Calculation

Nitrogen transformation in the system can occur through four main pathways: nitrogen uptake by biomass (TN assimilation by biomass), denitrification process (TN denitrification), volatilization (TN stripping), and residual nitrogen after treatment (residual TN) [4]. Although the RAB system involves rotation and exposure to air, which may enhance gas exchange and potentially promote minor ammonia volatilization, its contribution is expected to be minimal under neutral pH conditions. In microalgae-based wastewater treatment systems operated at pH =

(7-8), nitrogen removal is generally dominated by biological processes such as algal assimilation and microbial nitrification-denitrification rather than ammonia volatilization [6]. Therefore, ammonia volatilization was not included as a major pathway in the TN balance of this study.

In the present study, *C. Vulgaris* was initially inoculated into the RAB system as the dominant microalgal species.

$$TN_{input} = TN_{output} + TN_{denitrification} + TN_{assimilation} \quad (3)$$

The uptake of TN by biomass represents nitrogen incorporated by both bacteria and microalgae. To estimate the nitrogen accumulated in the biomass, it is assumed that the combined bacterial-microalgae biomass contains 8.25% nitrogen [4].

3 Results and Discussion

3.1 Nitrogen Removal

3.1.1 TKN and NH_4^+ -N

As illustrated in Figure 3a and Fig. 3b, in the first few days, TKN and NH_4^+ -N in the wastewater decreased significantly from 18.10 to 12.80 mg/L and from 15.16 to 9.63 mg/L, respectively. This may be due to the rapid adaptation of algae to the change in nutrient supply from the BBM environment to the shrimp farming wastewater. From day 12 onwards, concentrations continued to decrease gradually, TKN from 12.30 to 5.70 mg/L and NH_4^+ -N from 9.01 to 4.08 mg/L, and then remained stable for the remainder of the period.

Figure 3c and Figure 3d illustrate NO_3^- -N and NO_2^- -N trends during the denitrification process. These trends

However, because the reactor is operated in open conditions using actual wastewater, a mixed microalgae-bacteria community is easily formed. The DO levels of (3-5) mg/L supports nitrification in the aerobic phase, while the oxygen gradient in the biofilm creates micro-regions that allow denitrification to occur [6]. Based on this assumption, the nitrogen balance is calculated using Eq. (3):

closely followed those of TKN and NH_4^+ -N. In the RAB system, microalgae primarily assimilated inorganic nitrogen such as NH_4^+ -N and NO_3^- -N for biomass growth, while the oxidation of ammonium to nitrite and nitrate was likely mediated by nitrifying bacteria associated with the algal biofilm. Through the combined activity of the microalgae-bacteria consortium, the system achieved over 80% removal of TN and its various forms (Fig. 3e). This finding is consistent with previous studies reporting an 87% TKN removal efficiency for municipal wastewater treatment and an efficiency exceeding 85% for aquaculture wastewater treatment [7,8].

Furthermore, Fig. 3f shows the highest TN removal rate of 8.70 mg/L.day, followed by 4.50 mg/L.day and 2.71 mg/L.day for TKN and NH_4^+ -N, respectively. The removal rates were 0.90 mg/L.day for NO_3^- -N and 0.60 mg/L.day for NO_2^- -N. Rapid denitrification helps prevent the accumulation of NO_2^- -N, which could otherwise be detrimental to the activity of microorganisms in the RAB system [9].

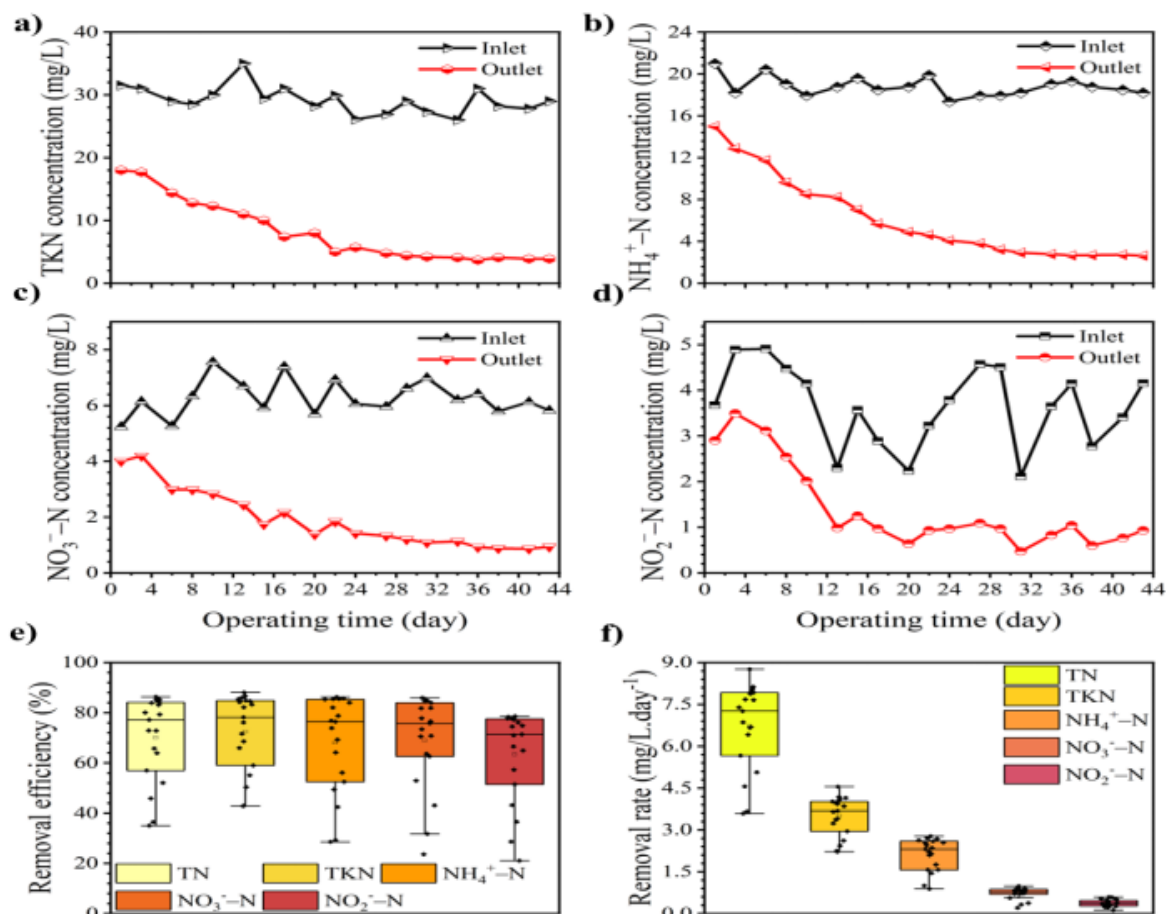


Figure 3 Nitrogen removal of the RAB system: a) TKN, b) $\text{NH}_4^+\text{-N}$, c) $\text{NO}_3^-\text{-N}$, and d) $\text{NO}_2^-\text{-N}$

3.1.2 Nitrogen Balance

Figure 4 illustrates the TN composition at two stages of operation. The results show a significant change in the nitrogen removal mechanism in the RAB system. During the first eight days, while biomass was still growing, denitrification remained limited at 48.93%, with a relatively high remaining TN content (39.91%). Simultaneously, 11.16% of TN was assimilated into the biomass, indicating that nitrogen was primarily used for biomass growth.

When the system reached the stable phase, the denitrification process increased significantly to

72.17%, leading to a decrease in initial TN amount to 22.19%. The nitrogen accumulation in the microalgal biomass also decreased and remained at approximately 5.64%. At this stage, nitrogen removal was mainly governed by the community activity of the microalgal-bacterial population. Microalgae accumulated nitrogen and provided oxygen through photosynthesis, supporting bacterial nitrification, while the oxygen-deficient zones within the biofilm facilitated denitrification. This result is consistent with previous studies [4, 7].

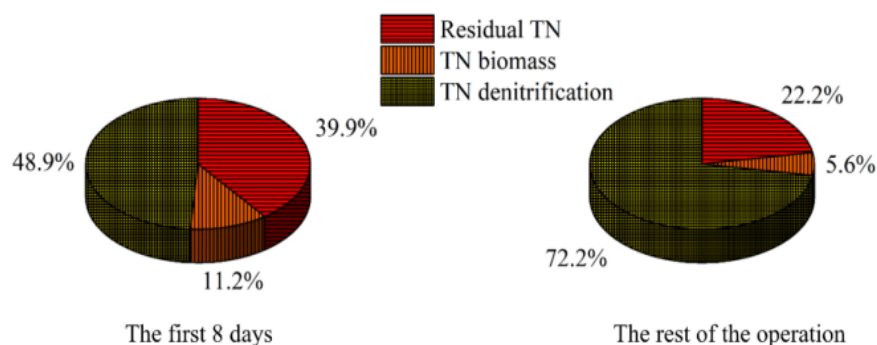


Figure 4 Nitrogen mass balance in the RAB system at the first and stable stages

3.2 Phosphorus Removal

Similar to nitrogen removal, the RAB system demonstrated efficient and stable TP removal, as shown in Figure 5. The input TP concentration remained relatively stable (7.96 ± 0.69 mg/L) during whole experiment. After adaptation, the output TP concentration ranged at (1.37 ± 0.30) mg/L, with a maximum efficiency of 86.8%. Although the input TP concentration fluctuated in the final days, the performance of the RAB system still maintained, achieving a removal efficiency of 85.2% and a maximum removal rate of 1.40 mg/L.day. Phosphorus removal in wastewater typically occurs through two main mechanisms: (i) bio-absorption, which involves active transport into cells for metabolic use and bioadsorption onto cell walls; and (ii) bioaccumulation, which involves intracellular storage or incorporation into complex phosphorus-containing molecules within algal cells [10].

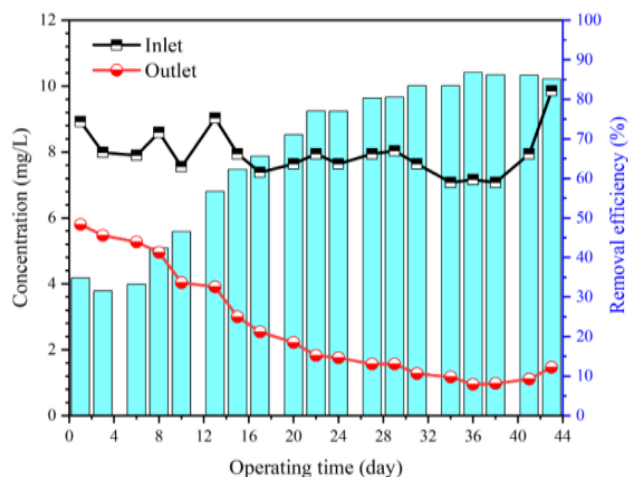


Figure 5 Trends of TP influent, effluent concentrations and removal efficiency in the RAB system

3.3 Organic Matter Removal Efficiency

As illustrated in Figure 6, the RAB system showed limited COD removal capacity at initial stage ((29.17-43.48) and (18.67-26.70) mg/L.day). From day 10 onwards, COD removal improved, exceeding 70% on day 22 and peaking at 84.58% on day 38, then stabilizing. The removal rate also increased significantly, peaking at 67.67 mg/L.day. These results demonstrate the system's robust ability to handle organic loads and its stability under fluctuating wastewater conditions. The stable performance observed in the later stages underscores the feasibility of applying RAB technology in practical aquaculture systems, consistent with previous findings showing COD removal efficiencies of up to 76.40% [11].

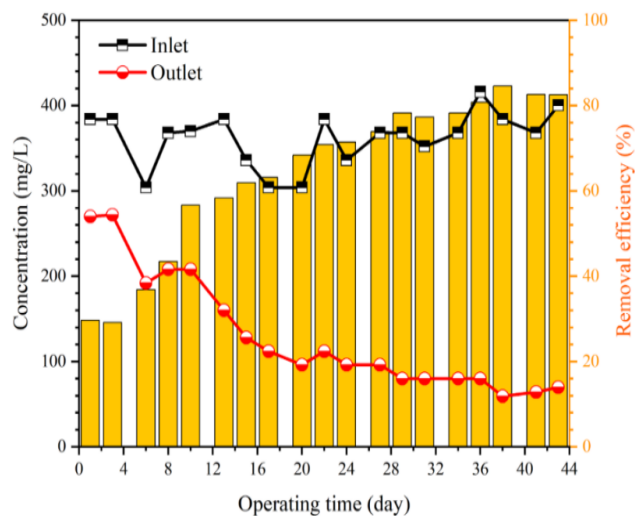


Figure 6 Trends of COD influent, effluent concentrations and removal efficiency in the RAB system

3.4 Biomass Productivity

Figure 7a shows the biomass growth in the RAB system. Biomass attached to the membrane increased from 1.85 g/m².day on day 7 to 3.85 g/m².day on day 35, before decreasing slightly to 3.45 g/m².day on day 42. Similarly, suspended biomass increased from 1.37 to 2.06 g/m².day.

The initial phase corresponds to the adaptation phase, in which the microalgae have not yet adapted to the nutrient load and C/N ratio of the wastewater, resulting in low growth rates. From day 7 to day 35, a stable growth phase was observed, marked by rapid biomass accumulation and biofilm maturation. During this phase, the microalgae efficiently assimilated inorganic nitrogen (NH₄⁺-N and NO₃⁻-N) and utilized carbon for cell synthesis, contributing significantly to wastewater treatment. The biomass accumulation followed a trend

of nitrogen removal, with the removal of NH₄⁺-N and TKN (Figure 7b). These results are consistent with previous studies [12] (Table 1).

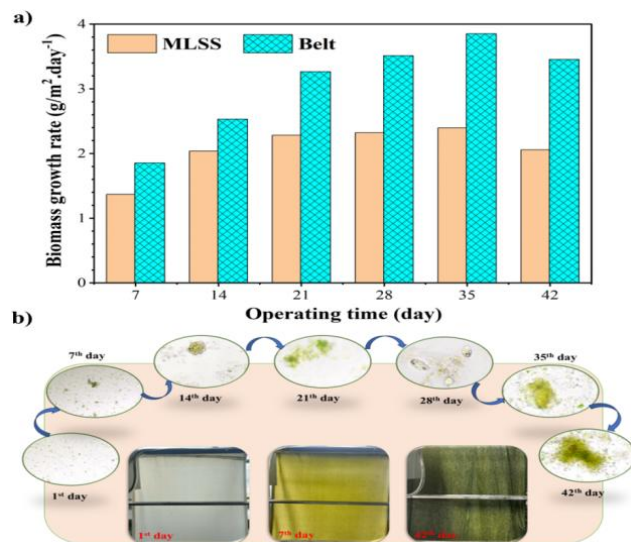


Figure 7 Biomass growth in RAB system:
a) Biomass growth rate, b) Biomass morphology development on the belt

Table 1 Comparison with previous studies

Reactor	Algae strain	Type of wastewater	Carrier material	Belt surface area (m ²)	Removal efficiency	Biomass growth rate (g/m ² .day)	Biomass retention time (days)	Ref.
RAB	<i>C. Vulgaris</i>	Shrimp farming	Cotton belt	0.6	COD: 84.8 NH ₄ ⁺ -N: 86.2 TN: 86.30 TKN: 88.0 TP: 86.80	3.85	7	This study
RABR	<i>Cyanobacteria LLC2</i>	Oilfield	Cotton belt	0.0175 - 0.51	-	~ 2.7-4.8	14 - 45	[12]
RAB - MFC	<i>Chlorella sp.</i>	Synthetic wastewater	Carbon cloth	0.09	COD: 93.50 -95.80 NH ₄ ⁺ -N: 91.10 -98.00 TN: ~88.00	> 30	180	[13]
RAB	<i>C. Vulgaris</i>	Municipal wastewater	Cotton duct	43.5	TP: 60 - 90 TKN: 80 NH ₄ ⁺ -N: 100	0.90	7	[7]
RAB	<i>Chlorella, Scenedesmus</i> ,	Aquaculture wastewater	Canvas belt	0.216	COD: 76.4 NH ₄ ⁺ -N: 91.30 TN: 85.10 TP: 87.50	6.30	7	[11]
Biofilm reactor	Natural algae in wastewater		Polyester	0.018	-	1.57-1.91	32	[14]

4 Conclusions

The RAB system demonstrated good and stable performance in treating shrimp farming wastewater. The removal efficiency of TKN and $\text{NH}_4^+\text{-N}$ consistently exceeded 80%, with peak removal rates of 4.50 and 2.71 mg/L·day, respectively. TP removal efficiency reached 86.8%, with a maximum rate of 1.40 mg/L·day. COD removal also improved over time, peaking at 84.6% with a rate of 67.67 mg/L·day. Attached and suspended biomass production peaked at

3.85 and 2.40 g/m² · d, respectively. A 6-day retention time ensured sufficient contact time for nutrient assimilation and stable biofilm development.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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Đánh giá hiệu quả xử lý nước thải nuôi tôm bằng hệ thống màng sinh học tảo quay: nghiên cứu trường hợp tại Cần Giờ, Thành phố Hồ Chí Minh

Huỳnh Đỗ Đạt¹, Lê Thị Ngọc Châu¹, Lê Vũ Anh¹, Nguyễn Văn Trục², Võ Thị Diệu Hiền^{1,*}

¹Khoa Khoa học Ứng dụng và Công nghệ, Trường Đại học Nguyễn Tất Thành, Thành phố Hồ Chí Minh, Việt Nam

²Khoa Kỹ thuật và Công nghệ, Trường Đại học Sài Gòn, Thành phố Hồ Chí Minh, Việt Nam

*vtdhien@ntt.edu.vn

Tóm tắt Nước thải nuôi trồng thủy sản chưa qua xử lý được xả trực tiếp ra môi trường mang lại rủi ro tiềm năng lớn cho hệ sinh thái. Nghiên cứu này ứng dụng hệ thống màng sinh học tảo quay (RAB) để khảo sát khả năng tăng trưởng sinh khối *Chlorella Vulgaris* và loại bỏ chất ô nhiễm trong loại nước thải này. Kết quả cho thấy hiệu quả loại bỏ TKN và $\text{NH}_4^+\text{-N}$ đạt khoảng 90 % ở giai đoạn ổn định, với tốc độ loại bỏ cao nhất lần lượt là 4,5 mg/L.ngày và 2,7 mg/L.ngày. Tốc độ loại bỏ TP lên đến 1,4 mg/L.ngày, đạt hiệu quả tối đa 86,8%, ngay cả khi nồng độ đầu vào biến động. Hiệu quả loại bỏ COD được cải thiện, đạt 84,6% với tốc độ cao nhất là 67,67 mg/L.ngày. Sinh khối bám dính trên màng tăng từ 2,53 g/m².ngày lên 3,85 g/m².ngày, trong khi sinh khối lơ lửng tăng lên 2,40 g/m².ngày. Điều này chứng tỏ hệ thống RAB tiềm năng lớn trong việc thu hồi chất dinh dưỡng và quản lý chất lượng nước trong các hoạt động nuôi trồng thủy sản bền vững.

Từ khóa Nước thải nuôi trồng thủy sản; màng sinh học tảo quay; *C. Vulgaris*; thu hồi chất dinh dưỡng.