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Design and development of pilot photobioreactor for simultaneous microalgae cultivation and aquaculture wastewater treatment

X. Christodoulou [a,*], C. M'ahmed [a], F. Zili [b], B. Bessadok [b], S. Sadok [b], I. Monney [a], R. Rothlisberger [a], M. Bagnoud [c]

[a] Institute of Energies, School of Engineering and Management Vaud, HES-SO University of Applied Sciences and Arts Western Switzerland, Switzerland
[b] Blue Biotechnology & Aquatic Bio-products-B3Aqua Laboratory, Institut National des Sciences et Technologies de la Mer—INSTM-Annexe La Goulette, 60 Port de Pêche La Goulette, La Goulette 2060, Tunisia
[c] Agroscope, Bern CH-3003, Switzerland

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ABSTRACT

This study presents an innovative and modular phototrophic biofilm photobioreactor (PBR) designed for the simultaneous cultivation of algae and the treatment of aquaculture wastewater (AWW). The vertical flat-plate BPR allows for stable microalgae growth while efficiently removing nutrients from wastewater under controlled conditions, including light, CO_2, supplementation, water recirculation and continuous monitoring of parameters such as pH, nitrate (NO_3^-N) and phosphate (PO_4^{3-}P). The PBR was operated at an aquaculture facility using AWW, with nutrient removal and microalgal growth being monitored. The microalgae consortium composed of *Chlorella sp.*, *Scenedesmus sp.* and *Phormidium sp.* were evaluated for their growth potential and wastewater remediation capabilities. Results showed high nutrient removal efficiencies with 92 % reduction of PO_4^{3-}P (removal rate: 0.07 mg/L d) and a 62 % reduction of NO_3-N (removal rate: 1.1 mg/L d), bringing nutrient concentrations below the limits set by the Waters Protection Ordinance. Maximum biomass production reached a growth rate on land surface of 25 g/m^2/d, with a favorable biochemical composition of 51 % proteins, 25 % carbohydrates and up to 8 % lipids, indicating the potential for use animal feed. This study demonstrates the feasibility of using AWW as a growth medium for microalgae while simultaneously achieving wastewater remediation, offering a sustainable solution for nutrient recycling in aquaculture operations.

1. Introduction

Water pollution is a global issue, with farms, factories and urban areas discharging untreated wastewater into natural water bodies. Conventional wastewater treatment systems have struggled to efficiently remove nutrients, prompting extensive research into economic, sustainable and efficient alternatives [1–4]. One rapidly growing contributor to wastewater generation is aquaculture, which has experienced a 60 % growth in production since the 1990s, making it one of the fastest-growing food industries globally [5]. Aquaculture wastewater rich in nutrients, such as nitrogen, phosphorus and organic matter, poses significant environmental and economic challenges [6–11].

The removal of nitrogen compounds in recirculating aquaculture systems (RAS) is crucial for maintaining optimal water quality and ensuring healthy fish production. Traditional RAS treatment involves separating solid matter and metabolic by-products to prevent decomposition, which increases the concentrations of total ammonia nitrogen (TAN), all of which are toxic to fish even at low concentrations. However, despite initial treatment, significant amounts of TAN remain in the water, necessitating further treatment through nitrification. During this process, ammonium is oxidized first to nitrite, which is also toxic, and then to nitrate. Although nitrate is more stable, it does not benefit fish nutrition and can inhibit growth at high concentrations [12, 13]. To avoid eutrophication of lakes and rivers, nitrate-rich water is regularly renewed and discharged into the environment.

To improve the sustainability and economics of the aquaculture, efficient waste management and nutrient recycling are essential. Denitrification is commonly employed for aquaculture wastewater treatment, effectively preventing the formation of ammonia (NH_3-N) and volatile ammonia. However, this process also leads to acidification, which shifts the carbonate equilibrium and increases dissolved CO_2 levels, negatively influencing water quality. The removal of CO_2 before

* Corresponding author.
E-mail address: xenia.christodoulou@heig-vd.ch (X. Christodoulou).

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recirculation requires energy-intensive processes, including oxygen supplementation.

In this context, microalgae-based systems offer promising solutions. Microalgae directly assimilate ammonia nitrogen and CO_2, converting them into biomass while producing oxygen [8,14–16]. Traditional suspended microalgae cultures; however, face limitations when used for bioremediation in aquaculture. These systems require large land areas for optimal light exposure in shallow basins and energy-intensive processes for separating algae from treated water to avoid increased turbidity in fishponds. Alternatively, microalgae biofilm-based systems, which immobilize cells, offer a more efficient and scalable solution.

Recent studies have explored biofilm-based photobioreactors (PBRs) for wastewater treatment, demonstrating their potential for nutrient removal and biomass recovery [17–19]. However, many existing biofilm PBRs are limited by low light penetration, biofilm detachment issues, and inefficient gas exchange, which can reduce overall treatment efficiency. Additionally, most designs focus solely on wastewater remediation rather than integrating biomass valorization.

To address these challenges, this study presents a newly developed biofilm PBR with an improved design that enhances light penetration, biofilm stability, and gas exchange efficiency. Unlike conventional biofilm systems, our reactor incorporates (1) an optimized surface structure for enhanced biofilm adhesion, (2) a controlled hydrodynamic environment to minimize detachment, and (3) a gas-exchange mechanism that improves CO_2 assimilation and oxygenation. These modifications improve both wastewater treatment performance and biomass yield, making the system more viable for large-scale aquaculture applications.

Furthermore, while previous biofilm PBRs have primarily been studied for secondary wastewater treatment, our study takes an integrated approach by evaluating the nutritional quality of the produced biomass and its potential as fish feed. This aligns with circular economy principles by closing the nutrient loop in aquaculture, reducing both waste discharge and dependency on external feed sources.

The use of microalgae in RAS wastewater treatment provides further advantages for fish farming. Microalgae, positioned at the base of aquatic food chains, are a key food source for fish larvae and other marine organisms. As aquaculture seeks to reduce reliance of fish oils in feed, microalgae emerge as an excellent alternative, maintaining the nutritional quality of fish through their high content of polyunsaturated fatty acids (PUFA) such as Omega 3 [20]. Many fish species, particularly larvae, have limited capacity to synthesize PUFAs, relying on zooplankton (which feed on microalgae) for these essential nutrients. Studies have shown that microalgae can significantly increase docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels in rotifers and small crustaceans, even after short-term enrichment [21]. As a result, microalgae offer a sustainable feed supplement, helping to maintain fish health and nutritional value in aquaculture [22] (Fig. 1).

Aquaculture currently utilizes over 40 species of microalgae, playing a vital role in the diets of marine animals at specific life stages. For example, studies on carp have shown that microalgae supplementation can enhance the immune system of fish depending on concentration [23,24]. The increasing demand for algal biomass in aquaculture reflects its potential as both a wastewater treatment solution and a high-value feed ingredient.

Before large-scale implementation of phototrophic-biofilm reactors in RAS, pilot-scale testing is essential to assess system efficiency, design accuracy and economic viability. This study presents a newly developed biofilm photobioreactor (PBR) designed for enhanced nitrogen and phosphorus removal from aquaculture wastewater while simultaneously generating oxygen and producing microalgal biomass. The study also evaluates the nutritional quality of the produced biomass and its potential use as fish feed, supporting circular economy approaches in aquaculture.

2. Materials and methods

2.1. Design of pilot photobioreactor

The PBR was designed and assembled in the laboratory of the Institut des Energies (HEIG-VD, Switzerland). The PBR consists of two compartments: 1) an internal compartment for distributing low-pressure CO_2-rich gas and 2) an external compartment for the gravity-laminar counter current flow of nutrient-rich water. These compartments are separated by a microporous membrane that supports the growth of the phototrophic biofilm (Fig. 1).

To enhance light concentration, the PBR is housed within, a 2 m × 2 m box covered with a silver reflective panel, which features a matte finish to promote uniform light diffusion. The system is equipped with three light sources: two high-intensity HQI (Metal Hydride) lamps and

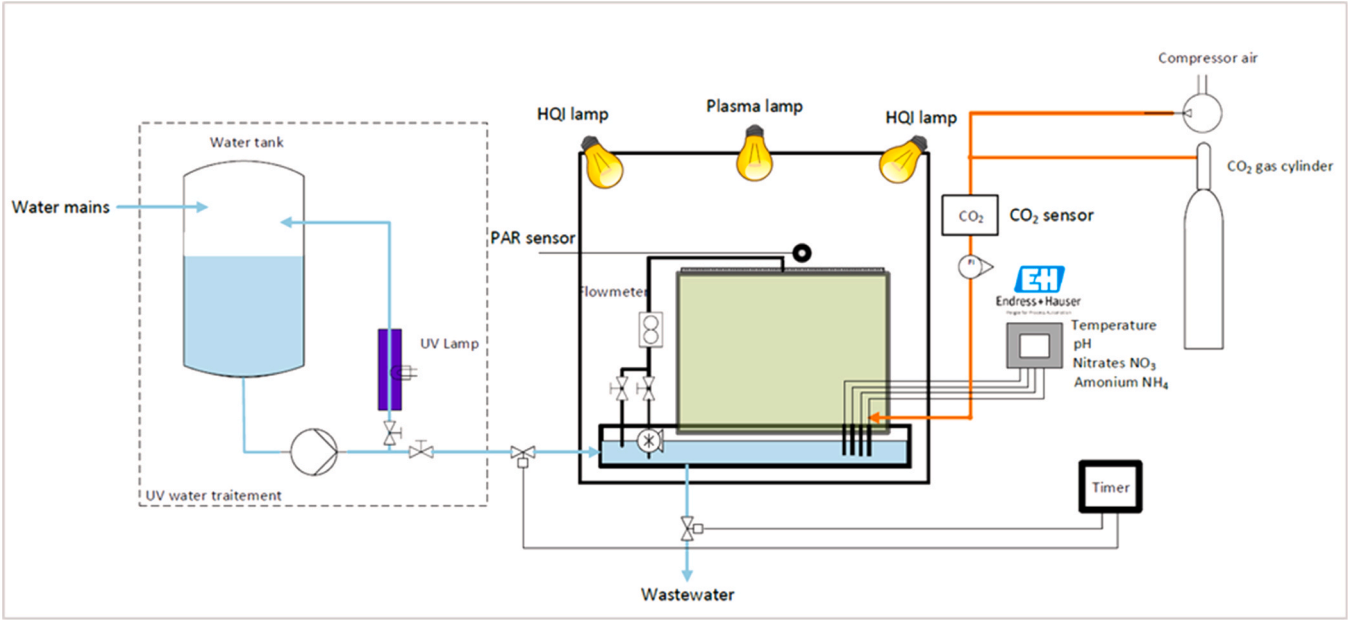

Fig. 1. PBR installation scheme (HQI lamp: hydrargyrum quartz iodide lamp, PAR sensor: Photosynthetically active radiation sensor).

one plasma lamp emitting spectra closely resembling natural sunlight. The photon flux is monitored using a photosynthetically active radiation (PAR) sensor (PQS 1), calibrated to deliver a photon flux of 200 μmol photons/m^2s, simulating conditions of a sunny day. The lights are positioned on the side of the PBR to ensure maximum coverage and operate on a 12- h photoperiod.

Water, used as the growth medium, was provided by an immersion pump (Okay 250 W) with a fixed flow rate of 20 L/min ± 1 L/min, regulated by a bypass and monitored with a magnetic inductive flow meter (Monitor MIK, Kobold NK). Continuous measurements of pH, temperature, nitrate, and ammonium concentrations are recorded using a measuring plate (*Endress Hauser* type *Liquiline*) A weekly timer manages the filling and emptying valves, while a float mechanism sets the water level in the tank by cutting off power to the filling valve. The temperature of the microalgal medium in the PBR is maintained between 20 and 30 °C.

Microalgal growth occurs on a porous substrate immobilization matrix, with a CO_2 enrichment level of 10 % supplied by Carbagas, Switzerland. This CO_2 is mixed with air to achieve the desired concentration. The gas is injected into the PBR through a diffuser located at the bottom of the porous substrate, facilitating stable gas exchange between the CO_2 and the algae. The CO_2 injection system is regulated by a solenoid valve and a pH sensor, with the set point fixed at a pH of 7.6.

The central diffuser ensures uniform irrigation throughout the system. Each component of the PBR is designed for easy disassembly, allowing for efficient cleaning and transport. The frame and tank are constructed from stainless steel (316L) to prevent corrosion, while PVC-U pipes are employed for the water circulation. The dimensions of the PBR are 30 cm in width, 135 cm in length, and 146 cm in total height (see Fig. 2). The design minimizes the footprint to just 0.4 m^2, with a total water volume of 140 L and an algal surface area of 2 m^2.

2.1.1. Evaluation of support materials

Ten different support materials were tested to evaluate their effectiveness as immobilization films in the photobioreactor (PBR). The materials included cotton fabric, three variations of polypropylene (PP) geotextiles, Tyvek® fabric, PVC crystal, PVC with polyester textile, rubber, silicone, and standard PVC. The specific properties of these materials are detailed in Supplementary Material C.

The evaluation was performed in duplicate using 100 mL glass bottles over a three-week period. Each bottle was filled with 49 mL of Bold's Basal (BB) medium, prepared as described in Supplementary Material B, and inoculated with 1 mL of a concentrated algal suspension. The bottles were maintained under controlled conditions to ensure uniform light exposure and temperature.

To assess algal growth, the immobilized biofilm was scraped from the support material once per week, and the collected algal biomass was transferred to tubes containing 2 mL of sterile water. The samples were then homogenized and measured for optical density at 750 nm using a DR3900 spectrophotometer (Hach Lange).

At the end of the experiment, the material that exhibited the highest algal growth and stability was selected for incorporation into the PBR assembly.

2.2. Collection and pre-culture of microalgae

Three pure microalgae cultures were utilized in this study: *Chlorella sp.*, *Scenedesmus sp.*, and *Phormidium sp*. All species were isolated from the water treatment basins at a facility located in Yverdon-les-Bains, Switzerland. The microalgal cultures were cultivated and maintained

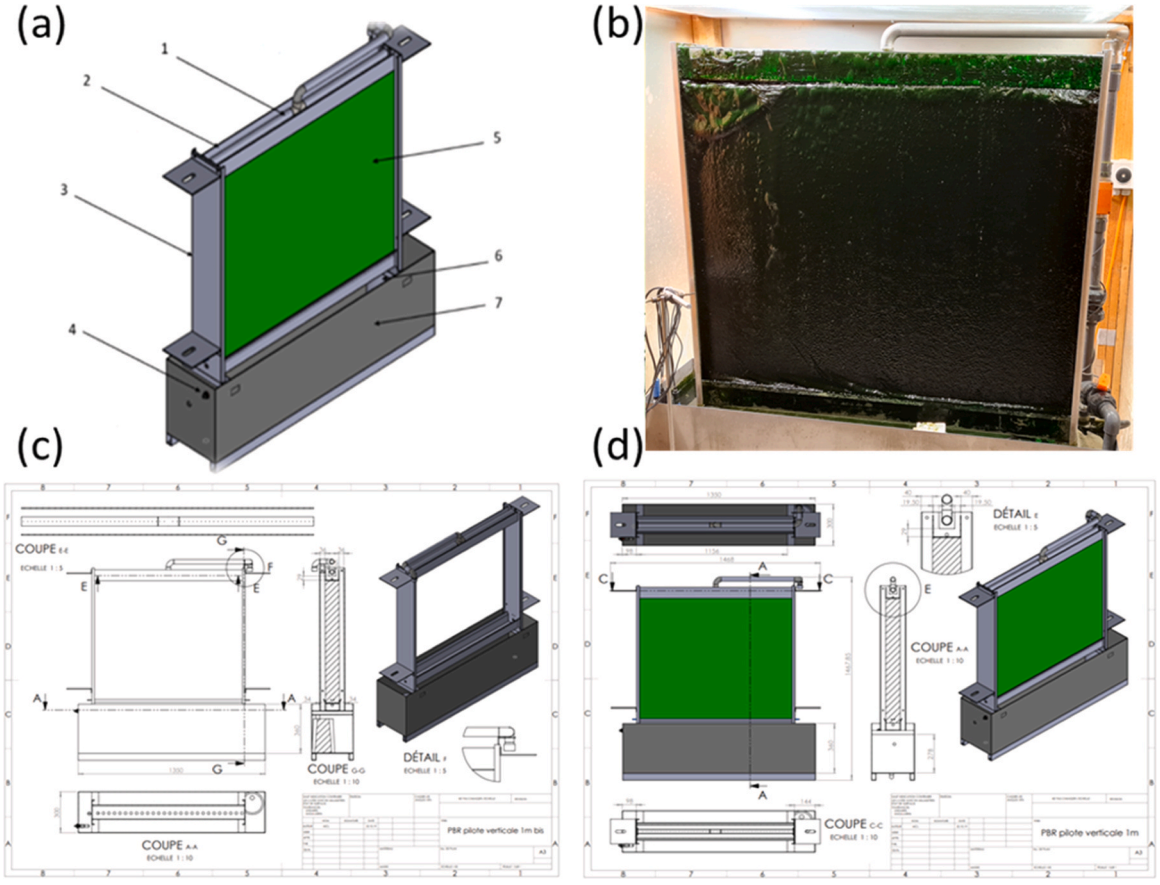

Fig. 2. Photobioreactor (PBR) - (a) Design: 1-Diffuser, 2-Overflow gutter, 3-Chassis structure, 4-Safety float, 5-Algae biofilm, 6-Recirculation pump, 7-water recipient, (b) algae biofilm on PBR, (c) and (d) PBR schematics.

in sterile Bold's Basal (BB) media and incubated at room temperature (25 ± 2 °C) under a light intensity of 200 μmol photons/m^2·s using white light. The relationship between OD and biomass was established experimentally using cell counts and absorbance (OD_{750}).

All solutions and equipment were sterilized and/or autoclaved prior to use to ensure the prevention of contamination.

2.3. Wastewater collection and characterization

Wastewater samples were collected from the effluent of the aquaculture facility Percitech in Chavornay, Switzerland. After collection, various analytical parameters of the wastewater, including orthophosphate (PO_4^{3-}-P), nitrate (NO_3^-), pH, temperature, and conductivity, were measured using standard sample kits and/or protocols. For the monitoring of wastewater quality during the study, only PO_4^{3-}-P and NO_3^- were used as pollution indicators, as ammonium (NH_4^+) was not detectable in the initial tests.

During PBR operation, pH, temperature, and NO_3^-, concentrations were continuously monitored using the Endress + Hauser EH system. Conductivity was measured with a portable conductivity meter (HACH HQ30d Flexi).

Before analysis, all samples were filtered through 0.45 μm syringe filters or centrifuged at 4000 rpm for 10 min to eliminate particulate matter and suspended solids, depending on the particle density. Total nitrogen concentration was determined using the peroxodisulphate digestion method (HACH sample kit LCK338; detection limit 20–100 mg/L). For the determination of orthophosphate (PO_4^{3-}-P), the molybdovanadate method was employed (HACH sample kit LCK350; detection limit 2–20 mg/L).

All samples were stored at 4 °C (unless otherwise stated) and analyzed within 24 h of collection to ensure the accuracy and integrity of the results.

2.4. PBR operation

2.4.1. Phase 1: Algae inoculation and conditioning

The PBR was initially operated in the laboratory for a period of three months using Bld's Basal Medium (BBM) nutrient media (see supplementary material B), prepared for a total water volume of 140 L. To enhance algae growth during the start-up phase of the PBR, a concentrated nutrient solution containing 8.6 mM of NO_3^--N and 0.7 mM of PO_4-P was administered for 3 cycles, with each cycle lasting 2 weeks).

2.5. Aquaculture wastewater treatment

The PBR was initially tested under laboratory conditions using artificial algal medium to assess its functionality. Following successful preliminary trials, the system was installed on-site at the *Percitech* aquaculture facility in *Chavornay, Switzerland*, where it operated for a total of five months using aquaculture wastewater (AWW) as the nutrient source.

Before use in the PBR, AWW was collected and stored in a 300 L tank, where it underwent sterilization with ultraviolet (UV) lamps for approximately 92 h to prevent contamination of the algal culture. Once sterilization was complete, the PBR was inoculated with a co-culture biofilm containing *Phormidium sp*., *Scenedesmus sp*., and *Chlorella sp*. in a 70:15:15 vol ratio. This ratio was selected to mimic the natural composition observed during strain isolation, where *Phormidium*—a filamentous cyanobacterium—rapidly colonizes surfaces, facilitating the attachment of *Scenedesmus* and *Chlorella*. The total inoculation volume was approximately 2 L, and the biofilm was established by spreading the culture onto an immobilization film using a roller apparatus, similar to a painting process.

Following inoculation, the PBR was operated continuously under a 12-h light/12-h dark cycle. The system was initially started at an OD_{750} of 0.2 (equivalent to 3.5×10^4 cells/mL). Algal biofilm growth was monitored, and biomass was harvested every two weeks by scraping the biofilm.

2.6. Nutrient removal performance

The nutrient removal rate was calculated using following equation (Eq. (1)) based on NO_3^-, NH_4^+, and PO_4^3-P concentrations before and after microalgae treatment.

$$Nutrient\ removal\ rate\left(mgL^{-1}d^{-1}\right) = \left(\frac{N_i - N_f}{No.of\ days}\right) \quad (1)$$

Where N_i corresponds to the concentration of nutrients in wastewater in the beginning of a cycle and N_f corresponds to the concentration of nutrients in wastewater (after treatment) at the end of a cycle.

2.7. Biochemical composition of biomass: proteins, lipids, and carbohydrates

At the end of each cycle, the algae produced in the PBR using enriched water and aquaculture wastewater (AWW) were harvested and subsequently lyophilized using a freeze dryer (VaCo2, Zirbus). The freeze-drying process consisted of three stages: 3 h at 0.5 mbar, 6 h at 0.3 mbar, and 72 h at 0.1 mbar, all at −80 °C. The dried algal biomass was analyzed for protein, carbohydrate, lipid, ash, moisture content, amino acids, and fatty acids at the Blue Biotechnology & Aquatic Bioproducts Laboratory (B3Aqua) at INSTM in Tunisia, which operates under the ISO/ILAC/IAF standard. All analytical parameters were accredited according to the international standard ISO/IEC 17025.

For the analyses, 5 g samples of the dried algae were finely powdered using a blender. Moisture and ash content were determined following international standards [25,26]. Crude protein content was measured using the method described by Hartree et al. [27]), while lipid content was determined using the Folch method [28].

The trans-esterification and esterification of the total lipid fraction obtained from the different samples were conducted as outlined in ISO 12966-2 [29] and ISO 12966-4 [30]. Fatty acid methyl esters (FAME) were analyzed using gas chromatography (GC). The identification and quantification of FAMEs (g of fatty acid/100 g of sample) were performed by comparing the retention times of the samples with those of standard methyl esters from PUFA3-SUPLECO (Sigma, Germany).

Amino acid extraction was performed following hydrolysis of the sample with a concentrated 6 N HCl solution, as described by [31]. The hydrolyzed samples were analyzed using a High-Performance Liquid Chromatography (HPLC, Agilent 1260 Infinity) system equipped with a diode-array detector (DAD).

Biochemical productivity was determined using Eq. (2).

$$Productivity\left(gL^{-1}d^{-1}\right) = Biomass\ productivity\left(gL^{-1}d^{-1}\right) \times \left(\frac{Content(\%)}{100\ \ \%}\right) \quad (2)$$

3. Results and discussion

3.1. Photobioreactor design

This study focused on the design of a flat-plate phototrophic biofilm PBR system, encased in reflective panels to concentrate photons from artificial light sources for the nutrient removal of aquaculture wastewater. The cultivation system ensures stable microalgae growth while minimizing uncontrolled variability in environmental conditions such as salinity, pH variations, metal toxicity, external microorganism contamination and product inhibition. Consequently, these controlled conditions facilitate the growth of high-density microalgae biomass in a non-destructive manner [32–34].

The vertical flat-plate PBR design is advantageous due to its large

specific surface area for the photo-biofilm development providing 2 m^2 of light exposure while requiring only a small land area, 0.4 m^2. Its modular design allows for easy scale up in both indoors and outdoors spaces [35,36]. Additionally, studies have shown that the flat-plate PBRs achieve higher photosynthetic efficiency compared to horizontal tubular photobioreactors, alongside lower dissolved oxygen accumulation and easier sterilization when necessary [37,38].

The system enhances gas-liquid mass transfer and optimizes the photosynthetic efficiency of the biofilm. The positioning of the biofilm as a permeable barrier between the liquid phase and gaseous phases is particularly effective for promoting phototropic biofilm growth, aided by the maintenance of the liquid-gas interface as described by biofouling theories. Furthermore, the design allows CO_2 molecules to diffuse more efficiently into the cells due to low resistance in gas-solid mass transfer (Fig. 2).

To mitigate the risk of biotic contamination while using wastewater, a UV lamp sterilization system was integrated into the PBR design. This method effectively reduces costs and complexities associated with wastewater sterilization for large-scale applications. While the system addresses biotic contamination risks, air contamination remains outside the scope of this study; however, it is recognized as an important factor warranting evaluation and control measures in future work.

Fig. 3 illustrates the algae growth on ten different materials tested as immobilization support. The results show that PVC with polyester textile (PVC+tex-PET) outperformed standard PVC by 46 % and surpassed the other tested materials by approximately 81–96 %, making it the preferred support for PBR assembly. The polymeric material in the flat-plate PBR provides mechanical stability during algae immobilization and promotes strong surface colonization through natural immobilization methods. Microalgae adhere to the surface by secreting extracellular substances, enhancing their attachment to the polymeric material [39,40]. These immobilization mechanisms not only ensure mechanical and chemical stability but also facilitate nutrient capture from the wastewater necessary for cell growth.

The choice of a flat-plate PBR design also enhances microalgae biomass recovery. Effective and smart microalgae harvesting methods are crucial for downstream processing after wastewater treatment. Various methods are available, such as centrifugation (the most commonly used), sedimentation, filtration and flocculation [38,40], but these remain costly and energy-intensive [41–44]. In this study, harvesting was performed by manual scrapping; however, the automation of the process is highly favorable and could optimize biofilm thickness, and consequently, algal production.

3.2. Effluent characterization

The pilot PBR was tested for microalgae cultivation and nutrient removal from AWW. The AWW was characterized for different parameters such as NO_3^--N and PO_4^{3-}P, COD content, pH and colour of wastewater, as presented in Table 1. The AWW contained 31 mg/L NO3–N and 1.3 mg/L PO_4^3—P, with nutrient concentrations exceeding the discharge limits specified in the Waters Protection Ordinance (WPO), which are set at 25 mg/L for NO3–N, 0.8 mg/L for PO_4^3-P, 20 mg/L for COD, and a pH range of 6.5–9.0 [45].

The wastewater exhibited an alkaline pH of 8, which was within the acceptable discharge limits. The pH level is significant as it influences nutrient availability; high pH values (pH $\geq$ 9) can lead to the formation of calcium phosphate, rendering it unavailable for microalgae uptake and potentially disrupting the functional groups on the microalgae surface, thereby impairing their ability to bind to different ions [46]. The COD concentration was recorded at 12.00 mg/L, below the discharge limits; thus, the COD was not monitored further in this study.

The color of the wastewater was transparent, allowing for low light absorbance, which is advantageous for stimulating photosynthetic activity in microalgae. This characteristic promotes enhanced growth and nutrient uptake compared to darker, opaque wastewaters [47,48].

Several studies have demonstrated the feasibility of utilizing aquaculture wastewater sources for microalgae cultivation due to their high nutrient content, reducing the need for expensive chemical inputs. This approach not only lowers costs associated with microalgae cultivation but also promotes the resource utilization of wastewater [49–52].

3.3. Removal of nutrients from the effluents by microalgae

Fig. 4 shows the nutrient removal performance of the AWW. The presence of nitrogen in the growth medium is crucial, even though certain algal specie can utilize gaseous CO_2 or carbonate ions as carbon

Table 1
Effluent characteristics of AWW (aquaculture wastewater) (n = 5).

Wastewater parameters	WPO discharge limits [45]	AWW
NO_3^--N (mg/L)	25	30.9 ± 3.0
PO_4^{3-}-P (mg/L)	0.8	1.3 ± 0.3
COD (mg/L)	20	12 ± 3
pH	6.5–9.0	8.0 ± 0.2
Color of wastewater	NA	Transparent

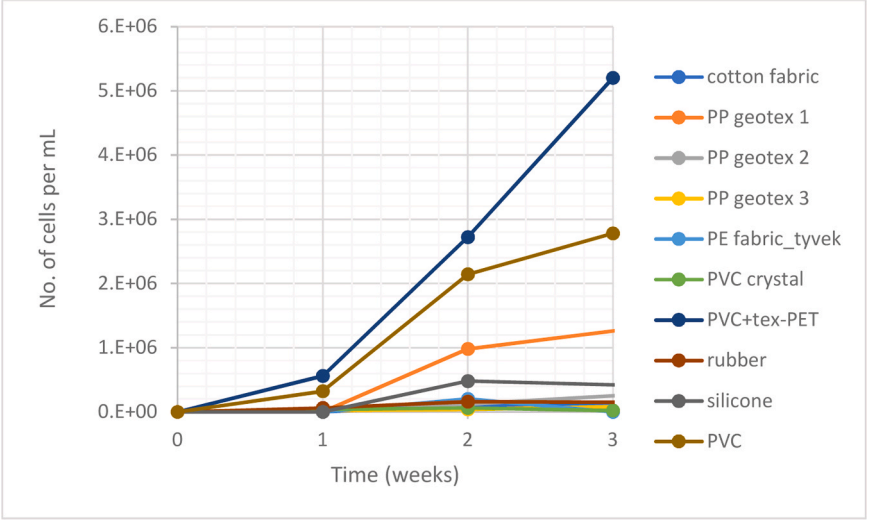

Fig. 3. Algae growth on different supports during 3 weeks; cotton fabric, PP geotex 1, PP geotex 2, PP geotex 3, PE fabric Tyvek, PVC crystal, PVC with polyester textile, rubber, silicone and PVC (PP: polypropylene, PE: polyethylene, PVC: polyvinyl chloride).

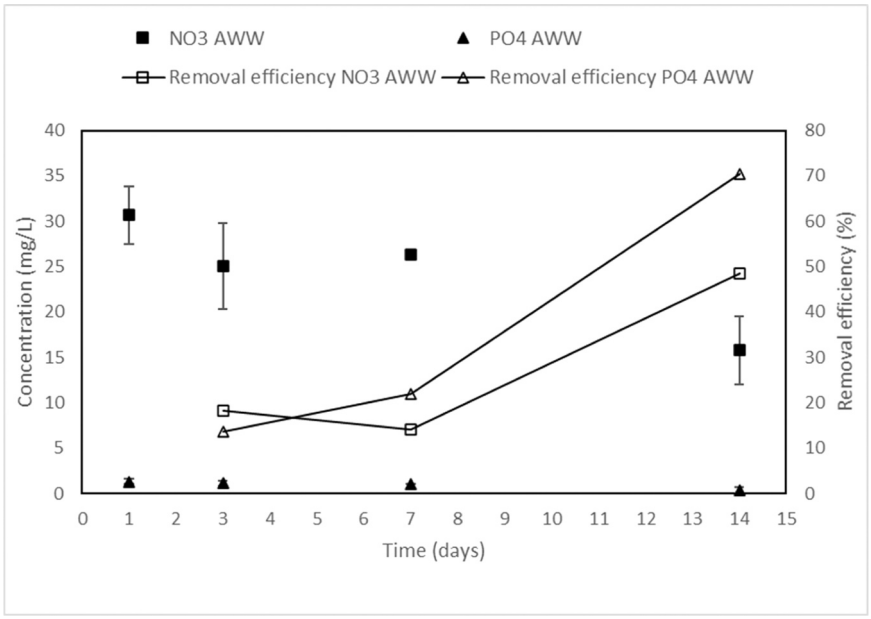

Fig. 4. Characteristics of AWW (aquaculture wastewater) during the 2 weeks cycle in the photobioreactor (PBR) (n = 5).

sources. In the context of wastewater treatment, the carbon-to-nitrogen and carbon-to-phosphorus ratios play a vital role in microalgal cultivation. Previous studies have demonstrated that microalgae cultures can effectively remove high concentrations of nitrogen and phosphorus when adequately supplied with carbon sources [53,54]. On day 14, the pollutant indicators were reduced to below the limit set by the WPO (Table 1) [45]. The NO_3^-N was reduced to *approx.* 15 mg/L (48 % removal efficiency) and the PO_4^{3-}-P to approx. 0.4 mg/L (70 % removal efficiency).

Existing technologies for treating aquaculture wastewater through mechanical filtration have reported nutrient removal efficiencies of up to 89 % [55]. This variation is influenced by effluent characteristics and the mesh size used for filtration. Low efficiencies are typically observed when larger mesh sizes are employed with dilute effluents, whereas higher efficiencies are noted when treating more concentrated waste. The nutrient removal efficiency reported in this study falls within the established range, suggesting the suitability of this approach for wastewater treatment.

3.4. Microalgae biomass production

Fig. 5 demonstrates the algae biomass production using the AWW over a 2-week cultivation cycle. The growth of *Chlorella sp.*, *Scenedesmus sp.* and *Phormidium sp.* on a 2 m^2 algal surface (with a 0.4 m^2 land surface used to support the PBR) resulted in a daily algae production of 3.6 g/m^2 d. In comparison, a similar PBR setup, fed with secondary effluent from municipal wastewater treatment, produced approx. 6 g/m^2 d [56]. However, these yields are still consistent with those reported in the literature for various PBR configurations and wastewater types. For example, [57] noted that algae production values typically range between 2 and 6 g/m^2 d in different systems. Additionally, a review by Gross et al. [58] highlighted that algal biofilm reactors typically exhibit surface productivities in similar range.

The high biomass yields in AWW can be attributed to its high nutrient contents, particularly nitrogen and phosphorus. The availability of these nutrients is known to significantly influence microalgal metabolism and growth. Specifically, the high rates of nitrogen NO_3^-N and PO_4^{3-}P removal observed suggest an efficient nutrient uptake by the algae. Ref. [59] have demonstrated that nutrients concentrations can directly affect algae growth rates. Furthermore, the COD of the AWW, which averaged at 12 ± 3 mg/L, even at relatively low concentration (< 20 mg/L), was linked to the enhanced algae growth [54]. This suggests that the available organic carbon in the AWW was fully utilized by the microalgae contributing to 51 % higher biomass production.

These findings indicate a mixotrophic behavior, where the studied algae species are not only using the supplied inorganic CO_2 but also

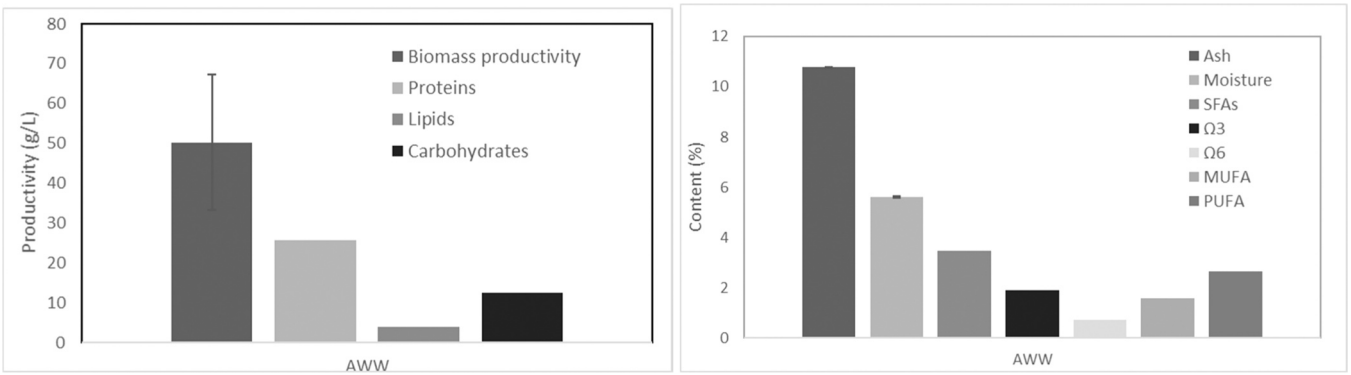

Fig. 5. Characteristics of algae produced using AWW (aquaculture wastewater). Left: Biomass and biochemical productivity. Right: Ash, moisture, SFAs (Saturated fatty acids), Ω3, Ω6 and MUFA (Monounsaturated fatty acids) yield.

metabolizing organic carbon from the wastewater. This dual carbon use suggests that either the inorganic CO_2 supply was insufficient to support optimal growth, or the algae inherently shifted to using organic carbon for growth. Some algae species, such as Chlorella, are known for their ability to use both organic and inorganic carbon sources for growth and maintenance. Studies have shown that low to moderate COD levels promote faster algal growth and accelerate the depletion of organic carbon in the medium [60]. It is also possible that the biofilm communities may have been colonized by heterotrophic organisms, further contributing to the organic carbon utilization.

The selected microalgae species in this study are known for their rapid growth rate and their high tolerance of stressful environmental conditions such as temperature fluctuations, pH changes, and varying light intensities. This makes them promising candidates for bioremediation and wastewater treatment, improving water quality for safe discharge. *Chlorella* is particularly well-researched due to its photosynthetic efficiency and high nutritional value. Studies have demonstrated *Chlorella*'s significant biosorption capacity, allowing it to effectively remove pollutants from aqueous media. In addition, it was demonstrated that *Chlorella* can adapt to different wastewater types and is extremely efficient at removing a variety of pollutants [61–64].

Likewise, *Scenedesmus* and *Phormidium* species exhibit strong biosorption capacities, especially for nutrient removal and heavy metals uptake, making them suitable for wastewater treatment [65–70].

This study also observed some variability in biomass productivity, likely due to fluctuations in nutrient levels throughout the growth cycles. Further research is necessary to better understand the causes of this variation and optimize the system for more consistent productivity.

3.5. Biochemical composition and algae valorisation

The harvested biomass was analysed for its biochemical composition. Fig. 5 shows the productivity of proteins, lipids and carbohydrates in the cultivated algae species using AWW. Lipid and carbohydrate contents were measured at 4 g/L d and 12.5 g/L d, respectively. The presence of organic carbon in AWW, along with high biomass productivity suggests mixotrophic behaviour in the microalgae, which likely contributed to the increased content in lipids and carbohydrates as observed in similar studies [71]. Additionally, stress conditions, such as low nitrogen and phosphorus levels in AWW, may have promoted the accumulation of lipids and carbohydrates. Microalgae tend to accumulate more lipids and carbohydrates when exposed to nutrient limitations or mixotrophic conditions [72].

Protein content was found at 26 g/L d., with proteins being the most dominant biochemical component, representing 51 % of the total biomass. This proportion is comparable to that found in *Spirulina* samples (68 %) and higher than levels reported for *Phormidium sp.* (35–45 %) [73,74]. The carbohydrate content in the algae cultivated with AWW was 25 %, while lipid yield was 8 % - lower than the 28–72 % range reported in the literature [72,75–77].

The composition of lipids, carbohydrates and proteins in microalgae is highly dependent on growth conditions and the nutrient content of the cultivation medium. A comparison by Ansari et al., (2017) shows that microalgae grown in synthetic media tend to have higher protein content and lower lipid levels, while real wastewater tends to result in lower protein and higher lipid concentrations due to stress conditions.

Fig. 5 illustrates the analysis of the algae composition in terms of ash, moisture, and fatty acid profiles, including saturated fatty acids (SFAs), omega-3 (Ω3), omega-6 (Ω6) and monosaturated fatty acids (MUFA). The ash content of the algae was 11 %, which falls within the range reported in literature (1.9–37 %) [78,79], suggesting a relatively low mineral content. Moisture content was measured at 5 % and SFAs constituted 35 % of the total fatty acids. The MUFA content was approx. 15 %, while PUFA made up 26 % of total fatty acids,

The cultivated algae from AWW exhibited high nutritional quality, with elevated levels of Ω3 fatty acids (18 %) and an Ω3:Ω6 ratio of 1:2.6. This favorable balance of essential fatty acids is associated with numerous health benefits and suggests that the algae biomass could be utilized as a nutritional supplement in animal feed [80].

The amino acid (AA) profile of the AWW-derived biomass was also evaluated (Data shown in Supplementary Material F). The total amino acid content was 41.36 g/100 g, with essential amino acids accounting for 44 % of the total. Arginine and leucine were the most abundant, making up 20 % of the total amino acid content.

The presence of essential amino acids in the algae biomass further supports its potential use as a feed source in animal diets. However, additional research is required to assess the digestibility of the biomass and the bioavailability of these amino acids within animal intestines, to ensure efficient nutrient absorption.

4. Conclusion

This study demonstrates the potential of a vertical flat-plate PBR for aquaculture wastewater treatment using a mixed consortium of microalgae; *Chlorella sp.*, *Scenedesmus sp.* and *Phormidium sp.* The system, which uses a polymeric material microalgae immobilization, proved effective for stable algae growth and nutrient removal, achieving over 70 % phosphate and 48 % nitrate reduction. With a biomass production rate of 3.6 g/m^2 d, as well as the modularity of the PBR system highlights the feasibility of scaling up for efficient wastewater treatment. The high protein and lipid content in the biomass also indicates its potential use as a fish feed supplement, adding value beyond environmental remediation.

The integration of algae-based treatment provides a sustainable solution to prevent nutrient pollution while enabling the reuse of both treated water and algal biomass. This approach promotes a circular economy, turning waste into valuable resources. Further research should focus on optimizing system performance and exploring the economic potential of large-scale applications.

CRediT authorship contribution statement

X. Christodouloua: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. C. M'ahmed: Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. F. Zili: Writing – review & editing,Formal analysis. B. Bessadok: Methodology, Formal analysis. S. Sadok: Writing – review & editing, Visualization, Resources, Conceptualization. I. Monney: Methodology. R. Rothlisberger: Writing – review & editing, Funding acquisition. M. Bagnoud: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.procbio.2025.03.016.

Data availability

Data will be made available on request.

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