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Aquaculture wastewater treatment using microalgae cultivated in open raceway ponds

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Dedication

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LIST OF ABBREVIATIONS

AWW: Aquaculture Wastewater

WW: Wastewater

TWW: Tilapia Wastewater

RAS: Recirculating Aquaculture System

BT: Biofloc Technology

ABFT: Autotrophic Biofloc Technology

IMTA: Integrated Multi-Trophic Aquaculture

RWP: Raceway Pond

HRAP: High Rate Algal Pond

OD: Optical Density

μ: Specific Growth Rate

Td: Doubling Time

BG11: Standard culture medium for microalgae

DO: Dissolved Oxygen

COD: Chemical Oxygen Demand

TSS: Total Suspended Solids

TN: Total Nitrogen

TP: Total Phosphorus

NH_4^+: Ammonium ion

NO_2^-: Nitrite ion

NO_3^-: Nitrate ion

PO_4^{3-}: Phosphate ion

CFU/mL: Colony Forming Units per milliliter

VAB: Vertical Algal Biofilm

Rpm: Revolutions per minute

g/L: Grams per liter

μmol/L: Micromoles per liter

pH: Hydrogen potential

nm: Nanometer

m^2: Square meter

°C: Degrees Celsius

R^2: Coefficient of determination

r: Pearson correlation coefficient

Abstract

This study evaluates the efficiency of local microalgae strains for treating raw, unfiltered Tilapia aquaculture wastewater (TWW) using laboratory-scale raceway ponds. Initially, the halotolerance of two strains (4CNR and 5CNR) was assessed under 10 g/L NaCl, confirming their tolerance but revealing optimal growth in freshwater conditions. The 5CNR strain was then selected, along with a freshwater strain (2ST), to evaluate growth and nutrient removal in BG11 medium and non-sterile TWW. Both strains showed strong growth in BG11, with 5CNR achieving higher biomass (1007.14 mg/L) and 2ST slightly higher cell density. In TWW, both strains exhibited reduced growth and fluctuating kinetics. Correlation analysis indicated that optical density (OD) is a reliable proxy for biomass in defined media like BG11 but not in complex, non-sterile media such as TWW. In raceway pond trials, the inoculated strains outcompeted the native algal population. Under batch conditions, 2ST achieved superior removal of phosphate, nitrate, and nitrite compared to the control. However, in semi-continuous mode, low nutrient input and limited biomass recovery prevented steady-state establishment. This led to modest performance, with biomass productivity at 0.087 g/L·day and nutrient removal rates of 0.006 mg/L·day for NH_4^+, 1.89 for NO_2^-, 9.7 for NO_3^-, and 0.07 for PO_4^{3-}.

Keywords: Local microalgae, growth indicators, Tilapia wastewater, raceway ponds, nutrient removal.

Résumé

Cette étude évalue l'efficacité de souches locales de microalgues pour le traitement des eaux usées brutes non filtrées issues de l'aquaculture du tilapia (TWW), en utilisant des bassins à chenal à l’échelle du laboratoire. Dans un premier temps, la tolérance au sel de deux souches (4CNR et 5CNR) a été testée à 10 g/L de NaCl, confirmant leur tolérance tout en révélant une croissance optimale en eau douce. La souche 5CNR a ensuite été sélectionnée, ainsi qu’une souche d’eau douce (2ST), pour évaluer leur croissance et la capacité d’élimination des nutriments dans le milieu BG11 et dans la TWW non stérile. Les deux souches ont montré une forte croissance dans le BG11, avec une biomasse plus élevée pour 5CNR (1007,14 mg/L) et une densité cellulaire légèrement supérieure pour 2ST. Dans la TWW, les deux souches ont présenté une croissance réduite et des cinétiques fluctuantes. L’analyse de corrélation a indiqué que la densité optique (DO) est un indicateur fiable de la biomasse dans un milieu défini comme le BG11, mais pas dans un milieu complexe et non stérile comme la TWW. Lors des essais en bassin à chenal, les souches inoculées ont surpassé la population algale native. En mode discontinu, la souche 2ST a présenté une meilleure élimination du phosphate, du nitrate et du nitrite par rapport au témoin. Cependant, en mode semi-continu, la faible concentration en nutriments et la récupération limitée de biomasse ont empêché l’établissement d’un état stationnaire. Cela a conduit à une performance modérée, avec une productivité en biomasse de 0,087 g/L·jour et des taux d’élimination de 0,006 mg/L·jour pour NH_4^+, 1,89 pour NO_2^-, 9,7 pour NO_3^- et 0,07 pour PO_4^{3-}.

Mots-clés : Microalgues locales, indicateurs de croissance, eaux usées de tilapia, bassins à chenal, élimination des nutriments.

الملخص

تهدف هذه الدراسة إلى تقييم كفاءة سلالات محلية من الطحالب الدقيقة في معالجة مياه الصرف غير المصفاة الناتجة عن تربية أسماك التيلابيا (TWW)، باستخدام برك مائية من نوع "رايسواي". على نطاق مخبري، في البداية، تم تقييم تحمل الملوحة لسلالتين (5CNR و 4CNR) بتركيز 10 غ/ ل من NaCl، وقد أظهرتا قدرة على التحمل مع نمو أفضل في ظروف المياه العذبة. تم اختيار السلالة 5CNR إلى جانب سلالة مياه عذبة (2ST)، لتقييم النمو وإزالة المغذيات في وسط BG11، وفي مياه TWW غير المعقمة. أظهرت السلالتان نموًا قويًا في BG11، حيث حققت 5CNR أعلى إنتاجية للكتلة الحيوية (1007.14 ملغم/ل)، بينما سجلت 2ST كثافة خلوية أعلى قليلاً. في مياه TWW، انخفض نمو السلالتين وظهرت تقلبات في ديناميكيات النمو. أظهرت نتائج التحليل الارتباطي أن الكثافة الضوئية (OD) تعد مؤشرًا موثوقًا للكتلة الحيوية في الأوساط المُعرفة مثل BG11، لكنها ليست كذلك في الأوساط المعقدة وغير المعقمة مثل TWW. خلال التجارب في برك "رايسواي"، تفوقت السلالات المزروعة على الطحالب المحلية. في النظام المتقطع، أظهرت السلالة 2ST كفاءة عالية في إزالة الفوسفات والنترات والنتريت مقارنةً بالشاهد. أما في النظام شبه المستمر، فقد أدت قلة توفر المغذيات وضعف استرجاع الكتلة الحيوية إلى عدم تحقيق حالة توازن. وأسفرت التجربة عن أداء متوسط، بقدرة إنتاجية للكتلة الحيوية بلغت 0.087 غ/ل· يوم، ومعدلات إزالة: 0.006 ملغم/ل· يوم للأمونيوم NH_4^+، و 1.89 للنتريت NO_2^-، و 9.7 للنترات NO_3^-، و 0.07 للفوسفات PO_4^{3-}.

الكلمات المفتاحية : طحالب دقيقة محلية، مؤشرات النمو، مياه صرف التيلابيا، برك رايسواي، إزالة المغذيات

INTRODUCTION

INTRODUCTION

The rapid expansion of aquaculture has led to increased environmental concerns, particularly due to the discharge of nutrient-rich wastewater. Aquaculture wastewater (AWW), primarily composed of uneaten feed, fecal matter, and metabolic by-products, contains high concentrations of nitrogen (NH_4^+, NO_3^-), phosphorus (PO_4^{3-}), and organic matter. If released untreated into natural ecosystems, this effluent can cause eutrophication, harmful algal blooms, oxygen depletion, and overall degradation of aquatic habitats (Bhuyar et al., 2019; Lugo et al., 2022).

Conventional wastewater treatment methods—such as activated sludge, constructed wetlands, and chemical precipitation—are often associated with high operational costs, complex maintenance, and limited nutrient recovery (Guo et al., 2022). In contrast, microalgae-based treatment systems offer a promising and sustainable alternative. Microalgae can assimilate nitrogen and phosphorus during their growth, transforming pollutants into valuable biomass. When coupled with bacteria in a symbiotic system, they enhance wastewater remediation efficiency: bacteria degrade organic compounds and produce CO_2, which is then used by microalgae for photosynthesis (Sánchez-Zurano et al., 2021).

Several studies have demonstrated the dual benefits of using microalgae for wastewater treatment and biomass production. For example, native Tunisian microalgae strains such as Scenedesmus sp. showed nutrient removal efficiencies exceeding 90% and are considered potential candidates for biofuel feedstock due to their high lipid and protein content (Jebali et al., 2018). Likewise, species like Chlorella vulgaris and Nannochloropsis oculata have been effectively cultured in aquaculture effluents for simultaneous water remediation and aquafeed production (Filali et al., 2019).

Among the different cultivation systems, open raceway ponds have gained considerable attention for large-scale wastewater treatment due to their low cost, simple construction, and ease of operation (Ansari et al., 2017). In particular, Scenedesmus almeriensis cultivated in open raceways under optimized conditions achieved over 90% removal of nitrogen and phosphorus and showed a balanced microalgae-bacteria composition that enhanced treatment efficiency (Sánchez-Zurano et al., 2021). Innovations like vertical algal biofilm integration and modified pond geometry have further improved productivity and reduced operational costs.

Given the increasing demand for sustainable aquaculture practices, integrating microalgae-based treatment in raceway pond systems presents an efficient and eco-friendly solution for nutrient remediation. Moreover, the biomass generated through this process can be valorized into biofuels, aquafeed, or fertilizers, contributing to circular bioeconomy principles (Hawrot-Paw, 2019).

This thesis aims to evaluate the performance of an open raceway pond system using Chlorella.sp microalgae strains for the treatment of tilapia aquaculture wastewater. By analyzing nutrient removal efficiencies, biomass productivity, and operational parameters, this study contributes to developing scalable, low-cost, and sustainable solutions for aquaculture wastewater management.

This will be structured in three chapters : General background, Methodology, Results and disscusion and we finish by conclusion.

CHAPTRE I. GENERAL BACKGROUND

I. CHAPTRE I. GENERAL BACKGROUND

I.1. Aquaculture industry speciation

The global aquaculture industry has experienced significant growth over recent decades, with projections indicating that by 2028 approximately 58% of the fish consumed by humans will originate from aquaculture, up from 52% in earlier years (Tom et al., 2021). This expansion underscores the industry's vital role in ensuring food security and alleviating pressures on wild fish populations. In 2018, global aquaculture production reached 114.5 million tonnes, comprising 82.1 million tonnes of aquatic animals and 32.4 million tonnes of aquatic algae (FAO, 2020). Inland aquaculture alone contributed 51.3 million tonnes, representing 62.5% of farmed food fish, predominantly freshwater finfish. By 2022, inland aquaculture continued to grow, reaching 59.1 million tonnes, with finfish accounting for approximately 89.7% of production and crustaceans making up around 8.7% (FAO, 2024)

Concurrently, the industry has undergone a process of diversification, termed aquaculture industry speciation, which involves expanding farmed species beyond traditional finfish to include mollusks, crustaceans, and photosynthetic microorganisms such as microalgae (Filali, 2019). Although thousands of aquatic species are technically cultivated, about 20 finfish species make up approximately 84% of global finfish aquaculture output, Commonly farmed species in pond aquaculture include grass carp (6.07 Mt), tilapia (5.38 Mt), silver carp (5.30 Mt), and common carp (4.56 Mt) (Verdegem & Bosma, 2009).

Among these, farmed tilapia alone reached 6.03 million tonnes in 2018, with Nile tilapia representing about 75% of that volume (FAO, 2018).

Among finfish, tilapia (*Oreochromis spp.*) has become one of the most widely cultivated species globally, owing to its resilience, fast growth rate, and adaptability to diverse environmental conditions. Despite these advantages, intensive tilapia farming generates nutrient-rich effluents that pose significant environmental challenges, including eutrophication and water quality deterioration if not properly treated. To address these issues, recent advancements in aquaculture practices have integrated microalgae into wastewater management systems, leveraging their natural capacity for nutrient removal and biomass production (Kashem et al., 2023).

Research demonstrates that microalgae such as *Chlorella vulgaris*, *Scenedesmus bijuga*, *Spirulina platensis*, and *Neochloris sp*. are highly effective in bioremediation applications. For instance, *Chlorella vulgaris* and *Scenedesmus bijuga* have achieved near-complete removal of ammonium and phosphate from tilapia effluents, while their biomass can be recycled as a supplementary feed for tilapia, establishing a circular nutrient economy, Similarly, *Spirulina platensis* has shown high efficiency in removing ammonia, nitrate, and nitrite from juvenile tilapia wastewater, simultaneously producing biomass with commercial value, Additionally, *Neochloris sp.,* cultivated in brackish tilapia wastewater, has demonstrated over 70% removal of total nitrogen, alongside high biomass productivity and moderate energy input for harvesting (Kashem et al., 2023) .

This ongoing diversification of the aquaculture sector not only addresses global demand but also opens the door to more sustainable production systems. As environmental concerns intensify, particularly regarding nutrient pollution and resource use, integrating species like microalgae into aquaculture aligns with broader goals of circular economy and ecological stewardship. Microalgae contribute significantly to bioremediation, nutrient cycling, and biomass valorization, helping transform aquaculture from a resource-intensive industry into a more regenerative one

(FAO, 202). As the industry continues to evolve, adopting integrated multi-trophic and wastewater recovery systems will be essential in maintaining ecological balance while supporting food security (FAO, 2018; Kashem et al., 2023). Thus, the speciation of aquaculture is not merely a reflection of market trends but a necessary adaptation toward resilient and environmentally responsible food systems.

1.1 Properties of Aquaculture Wastewater

Aquaculture wastewater (AWW) is characterized by a high concentration of organic and inorganic pollutants resulting from uneaten feed, fish excreta, and metabolic byproducts in intensive aquaculture systems. These effluents are typically nutrient-rich, containing elevated levels of nitrogen and phosphorus, which pose serious risks to natural aquatic ecosystems if discharged untreated (Ansari, 2017). A typical AWW sample was collected from aquaculture research facility Durban, South Africa. In this aquaculture research facility, Nile tilapia were reared in 5000 L black painted tanks in controlled temperature (27–32 °C) with continuous aeration, The sample shows a slightly alkaline pH of 7.28 ± 0.32, an average temperature of 27.3 ± 1.5 °C, and a moderate chemical oxygen demand (COD) of approximately 96 mg/L, reflecting the presence of biodegradable organic substances (Table 1). Dissolved oxygen (DO) levels are generally sufficient for aerobic microbial activity, averaging around 4.17 ± 0.31 mg/L (Ansari, 2017).

Moreover, aquaculture effluent includes essential and trace minerals such as iron, molybdenum zinc, nickel, magnesium, and sodium, which may affect both ecosystem health and the suitability of the wastewater for reuse in phycoremediation (Table 1). Salinity levels (~0.26 mg/L) and total suspended solids (0.42 mg/L) vary depending on system design and filtration practices. Microbial analyses have shown bacterial densities of approximately 1.8×10^3 CFU/mL, indicating an active biological community involved in nutrient cycling (Ansari, 2017).

Table 1: Characterization of a Typical Aquaculture Wastewater from a Nile Tilapia Farming System, Collected at an Aquaculture Research Facility in Durban, South Africa (Ansari, 2017).

Parameter	Unit	Value
pH	*	7.28 ± 0.32
Temperature	°C	27.3 ± 1.5
Total solid	mg L^{-1}	0.42 ± 0.02
Total dissolve solid	mg L^{-1}	0.352 ± 0.04
Salinity	mg L^{-1}	0.26 ± 0.02
DO	mg L^{-1}	4.17 ± 0.31
COD	mg L^{-1}	96 ± 0.00 (80–150*)
NH_4^+–N	mg L^{-1}	5.32 ± 0.45 (4–8*)
NO_2^-–N	mg L^{-1}	5.52 ± 0.18
NO_3^-–N	mg L^{-1}	46.07 ± 0.84 (25–100*)
PO_4^{3-}–P	mg L^{-1}	8.82 ± 0.02 (4–25*)
Fe	mg L^{-1}	4.05 ± 0.01
Mo	mg L^{-1}	3.00 ± 0.08
Zn	mg L^{-1}	0.85 ± 0.01
Na	mg L^{-1}	66.25 ± 1.49
Ni	mg L^{-1}	0.3 ± 0.000
Mg	mg L^{-1}	8.45 ± 0.04
K	mg L^{-1}	5.9 ± 0.02
Bacterial density	cfu/mL	$1.795 \times 10^3 \pm 0.049 \times 10^3$

* Annual range.

Another example of AWW originated from the fish tanks of a recirculating aquaculture system (RAS) where *Oreochromis niloticus* Nile Tilapia was the species farmed, provides similar nutrients concentrations and organic matter, but have a low pH (5.22) that may hinder microbial remediation processes (e.g. phycoremediation). This acidity is primarily due to CO_2 accumulation from fish respiration and hydrogen ion release during nitrification, The bacterial density also showed comparable contamination levels, with protozoa present in the samples (Lugo, 2020).

Table 2: Chemical Composition of Aquaculture Wastewater Extracted from a Recirculating Aquaculture System (RAS) Rearing *Oreochromis niloticus* Nile Tilapia (Lugo, 2020).

Parameter	**Value** (Mean ± SD)
pH	5.22 ± 0.08
EC (S/m)	0.4
Temperature °C	21.2 ± 0.4
TSS (mg/L)	41.3 ± 7.6
DO (mg/L)	4.6 ± 10
COD (mg/L)	64.3 ± 12.3
Nitrate (NO_3^-) (mg/L)	52.0 ± 4.0
Ammonium (NH_4^+) (mg/L)	12.8 ± 2.1
Nitrite (NO_2^-) (mg/L)	<0.01
Bacterial Density (CFU/mL)	2050 ± 50
N (mg/L)	41.5 ± 5.9
P (mg/L)	11.2 ± 3.6
K (mg/L)	8.3 ± 1.3
Mg (mg/L)	3.3 ± 0.8
Cl (mg/L)	24.5 ± 1.0
Na (mg/L)	28.5 ± 1.0
Zn (mg/L)	0.28 ± 0.05
Ni (mg/L)	0.018 ± 0.005
Fe (mg/L)	0.016 ± 0.005
Mo (mg/L)	<0.02
QAC (mg/L)	1.0 ± 0.1

Marine aquaculture wastewater (AWW), often originating from intensive mariculture systems, contains elevated levels of nutrients and suspended solids, which if discharged untreated, can lead to serious environmental issues such as eutrophication and biodiversity loss in coastal ecosystems. The composition of marine AWW is influenced by factors such as species cultured, feed composition, system design, and water exchange rates. Key parameters typically include total ammonia nitrogen (TAN), total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), total suspended solids (TSS), pH, salinity, and dissolved oxygen (DO). Table X summarizes typical ranges of these parameters reported in marine fish farming effluents. Understanding these baseline characteristics is critical for designing effective treatment strategies using biological approaches such as microalgae-based systems, which can efficiently assimilate these nutrients and contribute to sustainable effluent management (Cai et al., 2013).

Table 3: Typical Parameter Ranges of Marine AWW(Wang et al., 2020).

Parameter	Typical Range
TAN	0.08 – 90 mg/L
Total N (TN)	6.8 – 263 mg/L
Total P (TP)	0.21 – 17.5 mg/L
COD	0.56 – 11.12 mg/L
TSS	~90 – 214 mg/L
DO	7.3 – 10.4 mg/L
PH	7.9 – 8.4
Salinity	28.5 – 32.1 ppt

I. 2. Technological options for aquaculture wastewater treatment

Aquaculture wastewater management has become a critical focus in the pursuit of sustainable aquaculture, particularly as production intensifies to meet rising global seafood demand. This intensification results in the discharge of nutrient-rich effluents containing high concentrations of nitrogen, phosphorus, suspended solids, organic matter, and chemical residues, all of which pose serious environmental threats if left untreated. Traditional treatment methods such as nitrification-denitrification, chemical precipitation, and filtration though effective, are often energy-intensive, costly, and generate secondary waste. In response, a range of treatment technologies has been developed, tested, and scaled to mitigate the ecological impact of aquaculture wastewater. Increasingly, sustainable, biologically based solutions are gaining prominence due to their dual benefits of environmental protection and resource recovery. This section highlights the most common and proven remediation technologies used in aquaculture wastewater treatment, with emphasis on their efficiency, economic feasibility, and suitability for large-scale implementation (Martins et al., 2010; Troell et al., 2009; Zhang et al., 2018; Tom et al., 2021).

I. 2.1. Recirculating Aquaculture Systems (RAS)

The Recirculating Aquaculture System (RAS) is one of the most widely adopted and industrially scaled technologies for sustainable aquaculture. As a closed-loop system, RAS continuously treats and reuses water within fish culture units, significantly reducing freshwater consumption and minimizing effluent discharge. These systems integrate mechanical and biological filtration processes, including solids removal, nitrification through biofilters, degassing, and occasionally denitrification and disinfection. RAS has been effectively applied in the farming of species such as salmon and tilapia and forms the technological foundation for large-scale land-based operations like Atlantic Sapphire in the United States (Martins et al., 2010; Badiola et al., 2012). Recent innovations have expanded RAS capabilities through integration with microalgal cultivation, which enhances water quality via nutrient uptake and oxygen production while also enabling the recovery of valuable compounds such as lipids, proteins, and pigments from algal biomass.

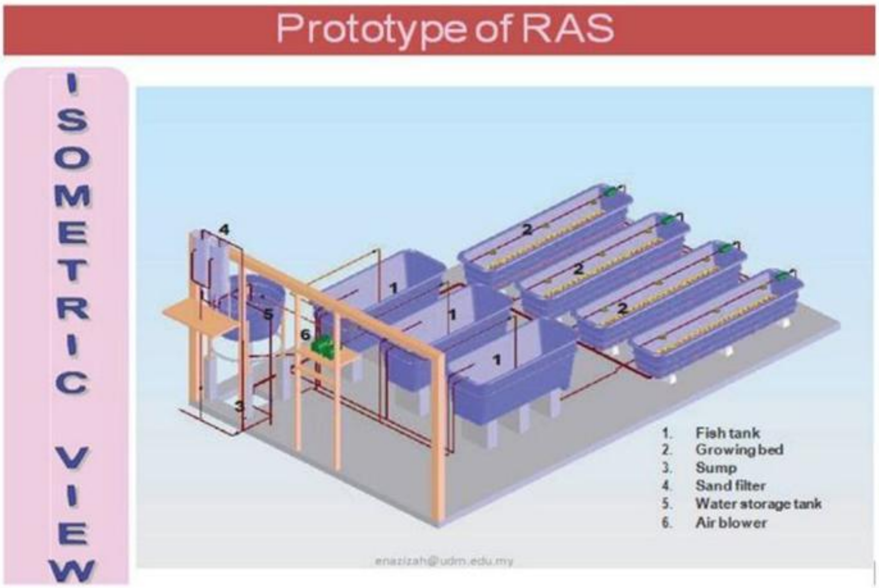

Figure 01: The isometric view of University Malaysia Terengganu (UTM's) aquaponic recirculating system 8 (Tom et al, 2021).

I. 2.2. Biofloc Technologies (BT)

Biofloc Technology (BFT) is a sustainable aquaculture approach that has been successfully implemented at large scales, particularly in the farming of shrimp and tilapia. It operates by manipulating the carbon-to-nitrogen (C:N) ratio in culture water to promote the growth of heterotrophic bacteria, which assimilate inorganic nitrogen and form bioflocs. These flocs serve as an additional protein-rich feed source for the cultured species, thereby reducing feed costs and enhancing nutrient recycling. A notable advancement of this method is Autotrophic Biofloc Technology (ABFT), which integrates microalgae alongside bacterial communities. In ABFT systems, the co-cultivation of microalgae and bacteria further enhances water purification and nutrient cycling, eliminating the need for water replacement. Experimental studies have demonstrated that such integrated systems can achieve nearly 100% fish survival, minimize water use, and even support high-yield crop production using recycled effluents (Emerenciano et al., 2013; Hargreaves, 2013; Kim et al., 2019).

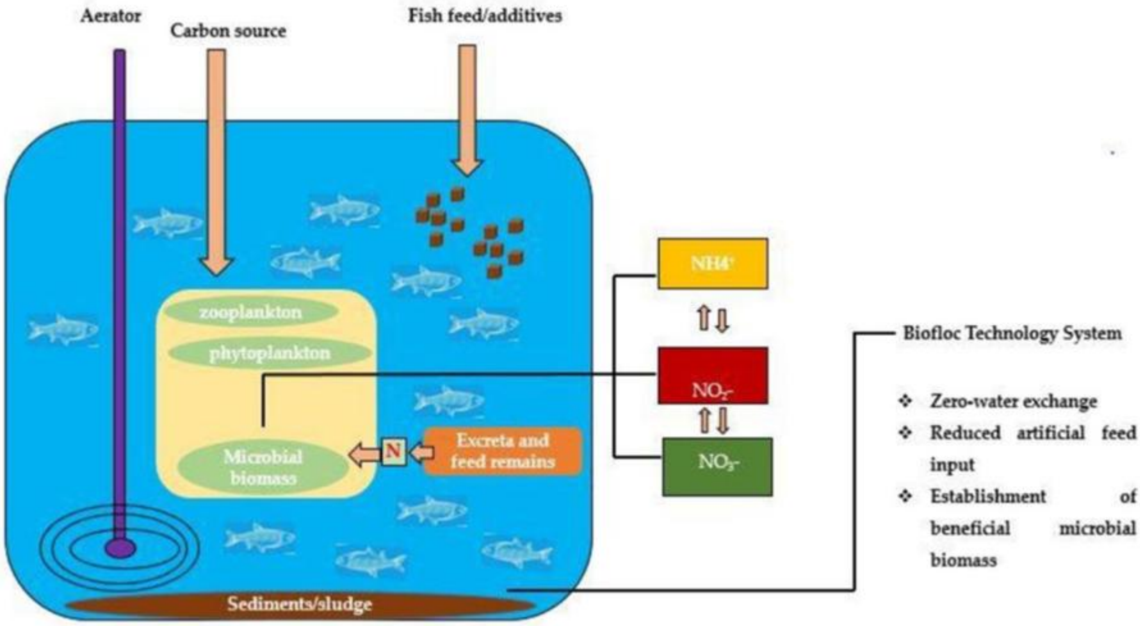

Figure 02: Shematic diagram of a biofloc technology system (Mugwanya et al.,2021).

I. 2.3. Integrated Multi-Trophic Aquaculture (IMTA)

IMTA utilizes a multi-species approach in which the waste from one species serves as a nutrient source for another. For instance, finfish effluents rich in nitrogen compounds are used to nourish algae or filter feeders, thus reducing waste and creating an internal recycling loop. This strategy not only minimizes pollution but also diversifies farmed products, increasing resilience to market fluctuations and environmental changes (Milhazes-Cunha & Otero, 2016).

IMTA systems allow for the production of valuable algal biomass while simultaneously improving water quality. High-Rate Algal Ponds (HRAPs), when applied within IMTA, can achieve BOD removal rates far exceeding those of conventional stabilization ponds, thus enabling faster detoxification and resource reuse (Milhazes-Cunha & Otero, 2016).

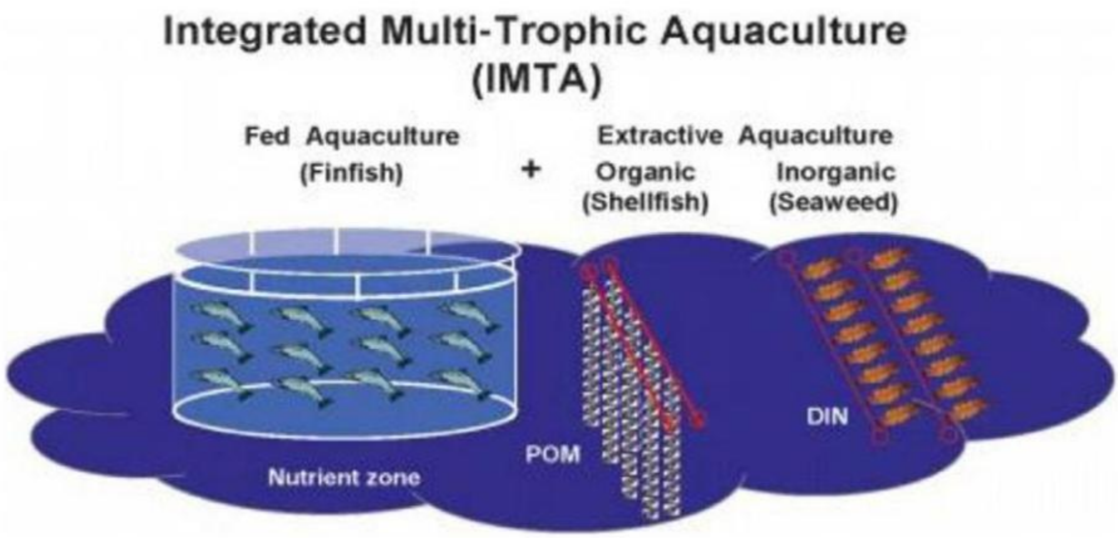

Figure 03: Integrated Multi-Trophic aquaculture (Chopin, 2008).

I. 3. Microalgae-Based Wastewater Remediation

Microalgae play a central role in biologically driven wastewater treatment due to their ability to assimilate nitrogen, phosphorus, and heavy metals from effluents. Through photosynthesis, they contribute to oxygen production, reduce biological oxygen demand (BOD), and serve as a low-cost option for nutrient removal. Algae can grow in saline, brackish, or industrial wastewater, and their cultivation allows for phosphorus and nitrogen recovery, which are non-renewable and critical agricultural inputs (Lee & Lee, 2002; Aslan et al., 2006; Sengupta et al., 2015).

Phycoremediation, a process employing microalgae to treat wastewater, has been shown to be both cost-effective and sustainable (Hawrot-Paw et al., 2020; Lugo et al., 2020). A study on the treatment of AWW with Chlorella minutissima demonstrated effective nutrient removal. Initial concentrations of total nitrogen (TN) and total phosphorus (TP) were 31.83 mg/L and 1.1 mg/L, respectively, with removal efficiencies reaching 87.9% for TN and 99.1% for TP after 10 days of treatment. Nitrate and orthophosphate were almost completely eliminated, confirming the viability of microalgae in wastewater remediation (Table 3) (HawrotPaw, 2015).

Table 4: The chemical composition of wastewater from RAS used for salmon farming in Jurassic Salmon company (Hawrot-Paw, 2015).

Time (Days)	**TN** (mg/L)	**TP** (mg/L)
0 (initial stage)	31.83 ± 0.3	1.1 ± 0.0
10 (final stage)	3.83 ± 0.2	0.01 ± 0.0

These systems benefit further from CO_2 enrichment and pH control, which enhance nutrient uptake and biomass productivity (Lugo et al., 2020; Han et al., 2019).

I. 3.1. Mechanisms of Waste Assimilation

Microalgae play a pivotal role in wastewater remediation due to their ability to assimilate inorganic nutrients—particularly nitrogen and phosphorus—as well as organic compounds and heavy metals. These organisms absorb nitrogen in various forms (ammonium, nitrate, nitrite) and phosphorus as phosphate, incorporating them into cellular structures and metabolic pathways (Skifa et al., 2025; Filali et al., 2019; Han et al., 2019). Certain strains, such as Chlorella, Scenedesmus, and Spirulina, have demonstrated over 90% removal efficiency for total nitrogen and phosphorus in both municipal and aquaculture effluents (Viegas et al., 2021; Guo et al., 2013).

The assimilation of nutrients is influenced by environmental factors such as pH, light, and carbon availability. Ammonium is often the preferred nitrogen source due to its lower energy requirement, though it acidifies the medium, necessitating pH regulation (Lugo et al., 2020). In contrast, nitrate raises pH levels and requires enzymatic reduction before uptake. This dual pH impact requires careful monitoring to maintain optimal growth (Lugo et al., 2020).

Microalgae also interact symbiotically with bacteria, which support growth through nutrient recycling and vitamin production (e.g., B_{12}). However, uncontrolled bacterial proliferation can inhibit algal productivity by outcompeting them for nutrients or releasing inhibitory compounds (Kumar et al., 2015). Algal-bacterial consortia can enhance overall nutrient removal and biomass yield by facilitating degradation of complex organics into bioavailable forms (Han et al., 2019; Zhang et al., 2018).

Additionally, microalgae are capable of removing heavy metals and pharmaceutical residues through bioaccumulation and biodegradation. Although this extends their application to more complex pollution scenarios, it can limit the downstream use of biomass for food or feed (Abdel-Raouf et al., 2012; Viegas et al., 2021).

The oxygen produced during photosynthesis by microalgae enhances aerobic microbial degradation of organic matter, reducing reliance on mechanical aeration. This is particularly advantageous in aquaculture wastewater, where oxygen demand is high (Filali et al., 2019; Milhazes-Cunha & Otero, 2016).

I. 3.2. Microalgae Cultivation Systems

Microalgae can be cultivated in various systems categorized into open (e.g., raceway ponds, high-rate algal ponds) and closed (e.g., photobioreactors). Among these, open raceway pond systems (RWPs) are the most commonly used due to their low construction and operation costs as well as their scalability for wastewater treatment. Their widespread adoption is also attributed to their ease of construction and suitability for large-scale operations, particularly in aquaculture wastewater remediation (Skifa et al., 2025; Filali et al., 2019). However, RWPs are not without limitations; they are vulnerable to contamination and environmental fluctuations such as temperature and light intensity, making them better suited for robust microalgal strains like Spirulina and Dunaliella (Filali et al., 2019). Additionally, challenges such as suboptimal mixing and light distribution can affect treatment efficiency and biomass yield.

To mitigate these limitations, various strategies have been developed. Key operational parameters for any microalgae cultivation system include light intensity, pH, nutrient concentration, temperature, and mixing. In RWPs, uniform distribution of light and nutrients is typically achieved through paddlewheel-driven mixing, although care must be taken to avoid excessive shear stress on algal cells (Acien et al., 2013). Efficient vertical mixing is particularly important as it enhances photosynthetic productivity by cycling cells between light-exposed and nutrient-rich zones (Kumar et al., 2015). Accordingly, design innovations such as paddlewheel optimization, baffle implementation, and airlift-driven flow have been introduced to improve hydrodynamics and reduce energy costs (Kumar et al., 2015; Filali et al., 2019).

Further improvements have emerged through the development of hybrid systems that combine the simplicity of RWPs with the control advantages of photobioreactors (PBRs). For instance, the integration of PBRs with RWPs enables high-density cultivation under controlled conditions before transferring algal cultures to open systems for biomass accumulation (Kumar et al., 2015). Likewise, the introduction of vertical-algal-biofilm (VAB) designs within RWPs has enhanced light utilization and surface area, enabling greater productivity and simplified harvesting. These systems have demonstrated pollutant removal rates including nitrogen, phosphorus, and COD comparable to or exceeding conventional techniques (Zhang et al., 2018). Algal biofilm reactors such as the Rotating Algal Biofilm (RAB) system further improve efficiency by allowing biomass to grow on rotating substrates, thereby eliminating the need for biomass separation before discharge (Han et al., 2019).

In more advanced applications, autotrophic biofloc systems (ABFTs) represent a closed-loop approach where microalgae are co-cultivated with fish. These systems have shown considerable promise by maintaining fish health, achieving high water savings, and allowing nutrient-rich effluents to be reused in agriculture (Kim et al., 2019). Additional techniques, including the use of microalgal-bacterial flocs, periphyton-based reactors, and immobilized cell systems, have further improved nutrient removal efficiency while also simplifying biomass recovery (Milhazes-Cunha & Otero, 2016).

Real-world implementations underscore the practicality of these systems. For example, semi-continuous cultivation using untreated aquaculture wastewater has achieved high biomass productivity and pollutant removal without the need for pre-filtration or sterilization (Viegas et al., 2021; Hawrot-Paw et al., 2020). In these contexts, the efficiency of biomass harvesting becomes crucial. Techniques such as bioflocculation, centrifugation, and membrane filtration not only help prevent the eutrophication of effluent discharge streams due to uncollected biomass but also allow recovery of valuable algal components including lipids, proteins, and pigments for use in biofuels, animal feed, and pharmaceuticals (Jusoh et al., 2020; Tom et al., 2021).

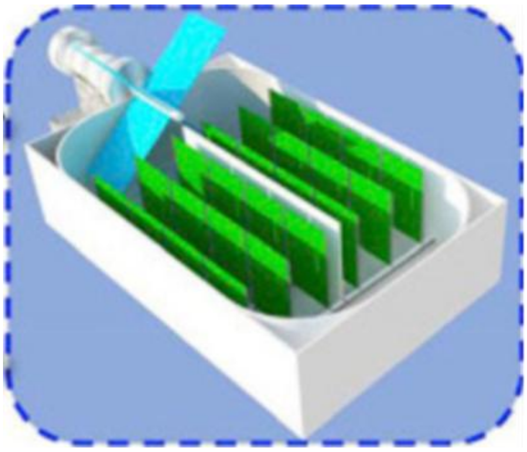

Figure 4: Vertical-algal-biofilm (VAB) enhanced RWP (Zhang et al., 2018).

I. 3.3. Challenges and Future Perspectives

Despite the clear advantages of microalgae-based systems—such as low energy use, high nutrient recovery, and added economic value—several challenges remain. Open pond systems are particularly susceptible to contamination by bacteria, fungi, or competing algal species, which can compromise the stability and effectiveness of the treatment process (Skifa et al., 2025). Environmental fluctuations, such as changes in temperature, pH, and light availability, further complicate process control and biomass consistency (Filali et al., 2019; Kumar et al., 2015). Maintaining monocultures and optimizing nutrient ratios and CO_2 delivery are essential for achieving consistent treatment performance and high-quality biomass (Viegas et al., 2021; Zhang et al., 2018).

Moreover, while many pilot-scale systems have demonstrated high nutrient removal and biomass productivity, large-scale economic viability remains a challenge. Operational costs, harvesting methods, and biomass valorization pathways must be optimized to justify commercial investment (Guo et al., 2013; Lugo et al., 2020). Future efforts should focus on hybrid designs combining open raceway ponds with vertical biofilm systems, enhanced CO_2 transfer techniques such as carbonation columns, and automated system monitoring to stabilize productivity. Ultimately, integrating these innovations into a circular bioeconomy framework that couples waste remediation with biomass production appears to be the most promising strategy for sustainable aquaculture (Milhazes-Cunha & Otero, 2016; Skifa et al., 2025).

I. 4. Raceway ponds for aquaculture wastewater treatment

I. 4.1. General factors affecting raceway pond

I. 4.1.1. Hydrodynamics and Flow Management .

Hydrodynamic conditions within raceway ponds are critical for maintaining efficient algal productivity. It is essential to sustain flow velocities above 0.1 m/s to prevent sedimentation and dead zones, which can hinder nutrient distribution and biomass growth (Filali et al., 2019).

Proper flow ensures uniform mixing and prevents oxygen accumulation that could damage algal cells. Utilizing Computational Fluid Dynamics (CFD) simulations allows for detailed analysis of flow patterns, facilitating the optimization of pond design parameters such as channel length towidth ratio, ideally between 6 and 7, to promote effective circulation and minimize shear stress (Skifa et al., 2025).

I. 4.1.2. Mixing and Aeration

Adequate mixing is paramount for maximizing algal biomass productivity, as it ensures homogenous distribution of nutrients, gases, and light exposure. Paddlewheels are commonly employed to induce vertical and horizontal mixing, which enhances photosynthesis and prevents localized oxygen buildup, thereby reducing oxidative stress on cells (Filali et al., 2019; Kumar., 2015).Proper mixing can increase productivity by nearly tenfold, but it also accounts for a significant portion of operational costs—approximately 69%, emphasizing the need for cost-effective design and operation strategies (Filali et al., 2019).

I. 4.1.3. Light Penetration and Photobiology

Light availability directly influences the rate of photosynthesis and biomass accumulation. The depth of the pond affects how much light reaches the microalgae; shallower ponds (0.1–0.3 m) typically allow better light penetration but may increase evaporation and land use costs (Filali et al., 2019; Skifa et al., 2025). Biomass concentration also affects light attenuation. Thus, maintaining optimal cell densities is crucial to prevent self-shading and ensure efficient photosynthesis. Turbulence and mixing further aid in distributing light uniformly, mitigating photoinhibition (Kanhaiya Kumar., 2015).

I. 4.1.4. Temperature, pH, and Salinity Control

Maintaining optimal environmental conditions is essential for microalgal growth. The ideal temperature range is between 20°C and 25°C, which supports metabolic activity and biomass productivity (Filali et al., 2019; Kumar., 2015). pH levels should be kept between 7 and 9; deviations can cause osmotic stress or alter cellular ionic balances, inhibiting growth. Salinity fluctuations can induce osmotic and ionic stress, adversely affecting cell health and composition. Therefore, regular monitoring and control are necessary to sustain stable culture conditions (Kanhaiya Kumar., 2015).

I. 4.1.5. CO_2 Delivery and Gas Transfer

Efficient carbon dioxide transfer is critical for photosynthesis and biomass production. The cost of CO_2 can account for 8–27% of daily production expenses; thus, optimizing gas-liquid contact through spargers and other transfer mechanisms is vital (Skifa et al., 2025; Kumar., 2015). An optimal CO_2 concentration of at least 65 μmol/L at a pH of 8.5 enhances algal growth. Factors such as mixing intensity, liquid velocity, and the volumetric mass transfer coefficient influence the efficiency of CO_2 delivery, directly affecting productivity and operational costs (Filali et al., 2019).

I. 4.1.6. Site Selection and Environmental Factors

Selecting an appropriate location is fundamental to the success of raceway ponds. Sites should receive high solar radiation—preferably exceeding 4.65 kWh/m²/day—to maximize photosynthetic activity (Kumar, 2015). Additionally, the area should experience minimal rainfall—preferably less than 1 meter annually—to reduce water management challenges. Land slope should be gentle, not exceeding 5%, to minimize earth-moving costs and facilitate construction. These environmental considerations help optimize operational efficiency and reduce expenses (Kumar., 2015).

I. 4.1.7. Biological Considerations and Predation Control

The biological stability of algal cultures depends on the resilience of selected strains and the management of predators. Open ponds are vulnerable to various predators, including insects, fungi, bacteria, viruses, and larger organisms such as birds and frogs (Skifa et al., 2025; Kumar, 2015). Implementing biological controls and selecting robust algal strains are necessary to maintain healthy cultures, prevent contamination, and ensure consistent biomass production (Filali et al., 2019).

I. 4.2. Raceway pond design

I. 4.2.1. Construction Materials and Structural Design

Raceway ponds (RWPs) are constructed using a variety of materials and techniques tailored to the scale of operation, environmental conditions, and financial constraints. Common construction materials include compacted earth, concrete, and plastic liners. While compacted earth and plastic-lined ponds are cost-effective and suitable for small- to medium-scale applications, they often demand more maintenance and offer a shorter operational lifespan (Skifa et al., 2025). Concrete-lined ponds, although more expensive, provide superior durability and structural integrity over time. The design typically consists of excavated trenches with gently sloping earth embankments. To prevent seepage, especially in permeable soils, compacted clay or synthetic liners are recommended. In some cases, crushed rock surfaces are incorporated to prevent erosion. However, these may limit light penetration critical for algal photosynthesis (Skifa et al., 2025).

An alternative, more durable construction method includes building vertical, free-standing walls using corrugated asbestos-cement panels installed into shallow concrete-filled trenches. Silicone sealants are applied at the joints to ensure water tightness. Fiberglass materials are preferred for paddlewheel walls due to their light weight and mechanical robustness (Filali et al., 2019). Fully concrete raceways may be coated with epoxy, particularly in saline environments, to prevent corrosion. Covered systems using transparent plastic sheets can retain heat and enable effective CO2 delivery but may lead to overheating in high solar radiation areas, thereby restricting algal species selection (Skifa et al., 2025).

I. 4.2.2 Geometrical Configuration and Dimensions

Raceway pond geometry significantly influences operational efficiency and energy demand. RWPs generally consist of two parallel channels separated by a central wall, forming a closedloop, racetrack configuration. Recommended raceway areas range between 100 to 5000 m², with larger installations achieved by increasing the number of modular ponds (Skifa et al., 2025). The length-to-width (L/W) ratio is a critical parameter; optimal values range between 10 and 20 to minimize head losses. Designs minimizing bends further enhance hydraulic efficiency.

Channel depths typically range from 0.1 to 0.3 m, which provides a favorable balance between light penetration, temperature stability, and biomass productivity. Shallower depths, around 5–8 cm, help reduce sedimentation and improve microalgal exposure to light (Filali et al., 2019). The surface-to-volume (S/V) ratio, usually between 1 and 10 m^{-1}, is another key factor, influencing both light availability and overall culture volume (Skifa et al., 2025).

I. 4.2.3 Mixing Systems and Hydraulic Considerations

Mixing within RWPs is vital for maintaining homogeneity in nutrient and gas distribution, as well as for preventing biomass sedimentation. Paddlewheels are the most widely employed mixing devices due to their compatibility with high-volume, low-head applications. Positioned either above flat pond bottoms or within floor recesses to counteract reverse flow, paddlewheels gently circulate the culture medium at recommended velocities of 0.15 to 0.3 m/s (Skifa et al., 2025). Standard eight-blade paddlewheels strike a balance between mechanical efficiency and construction cost.

Back-curved paddlewheel blades have been developed to reduce blade contact surface and energy use. Rotor speeds of approximately 20 rpm and bottom clearances of 5–8 cm are typical settings that promote effective circulation while limiting shear stress on sensitive algal cells (Filali et al., 2019). To model energy demands and liquid motion, Manning's formula and Bernoulli's equation are often applied. The hydraulic parameters of Shape Ratio (SR): defined as L/B, where L is the channel length (m) and B is the channel width (m). It influences power requirements per unit surface area, with larger SRs typically resulting in lower power demands, Aspect Ratio (AR): defined as B/d, where B is the channel width (m) and d is the fluid depth (m). It impacts hydraulic power requirements, and Head loss (HL) in a channel between two locations can be computed using experimental data and by utilizing Bernoulli's Equation $HL = (\alpha V1^2/2g + d1) - (\alpha V2^2/2g + d2)$, where HL is the head loss (m), V is the average velocity of the section (ms^{-1}), d is the depth of the fluid (m), g is the gravitational acceleration (Nsm^{-2}), and α is the kinetic energy correction coefficient these parameters directly affect power requirements. (Skifa et al., 2025).

I. 4.2.4. Design Innovations and Performance Enhancements

Recent developments in RWP design focus on enhancing mixing efficiency and energy savings. These include sump-assisted CO_2 injection systems that prolong gas-liquid contact time, as well as airlift-driven and jet-type circulation systems offering alternatives to paddlewheels. However, the latter may be less compatible with certain algal strains and increase operational complexity (Skifa et al., 2025). Box-like bend geometries and flow deflectors are also utilized to minimize energy losses and improve mixing uniformity.

Tracers such as Rhodamine WT and sodium chloride (NaCl) are commonly used to assess flow behavior and residence time. The Peclet number, derived from such tracer studies, is instrumental in evaluating axial dispersion and optimizing channel design (Filali et al., 2019).

I. 4.2.5. Environmental and Operational Factors

Raceway pond performance is heavily influenced by environmental conditions such as light availability, ambient temperature, and wind exposure. Shallow depths support stable light penetration and mitigate thermal stratification, both essential for maximizing photosynthetic efficiency and biomass yield. However, open systems are susceptible to high evaporation rates and CO_2 losses, necessitating careful management of depth and pond covering materials (Filali et al., 2019).

Construction material selection must account for potential toxicity, resistance to corrosion, and ease of maintenance. PVC liners, epoxy-coated concrete, and fiberglass are commonly employed materials. Operational factors such as routine maintenance, shear stress regulation, and efficient

CO2 delivery systems are crucial to ensuring sustained microalgal productivity and water treatment performance.

I. 4.2.6. System Configuration for Aquaculture Wastewater Treatment

In aquaculture applications, RWPs are particularly well-suited for the treatment of nutrient-rich effluents. The high nutrient loads provide a growth medium for microalgae, which, in turn, assimilate nitrogen and phosphorus while producing valuable biomass. The typical High Rate Algae Pond (HRAP) design—a shallow, elongated system with a central wall—ensures consistent culture mixing and oxygenation. Paddlewheels, air-lifts, and low-shear pumps are all used to sustain circulation. Given the variability in climate, pond design must accommodate fluctuations in light intensity, temperature, and evaporation to maintain effective treatment outcomes (Filali et al., 2019).

Collectively, raceway pond design for microalgae-based aquaculture wastewater treatment demands a multidisciplinary approach. Structural configuration, hydraulic optimization, environmental adaptation, and biological compatibility must be simultaneously addressed to create an efficient, sustainable, and scalable system

Table 4 suggest a general optimal design of a RWP dedicated to wastewater treatment and Figure 2 present a Schematic view of a RWP equipped with a paddlewheel

Table 5: Optimized RWP design information's for wastewater treatment (process with flow rate of 1 m3/h) (Filali et al, 2019).

Parameters	**Technical solutions**
Type of construction	Wall without membrane
Wall material construction	Corrugated asbestos-cement panels
RWP geometry	Asymmetric island in each bend of middle wall
Additional device RWP	Three semi-circular deflectors at each bend
Paddlewheel material	Fiberglass
Blades form	Back- and forward-curved blades
Paddlewheel flow velocity	0.2 m/s
Length	50 m
Width	2 m
Culture depth	0.2 m
Gas supply device	Airlift system (sparger for CO_2 injection)
Optimization device	Control system of pH culture (by CO_2 injection)

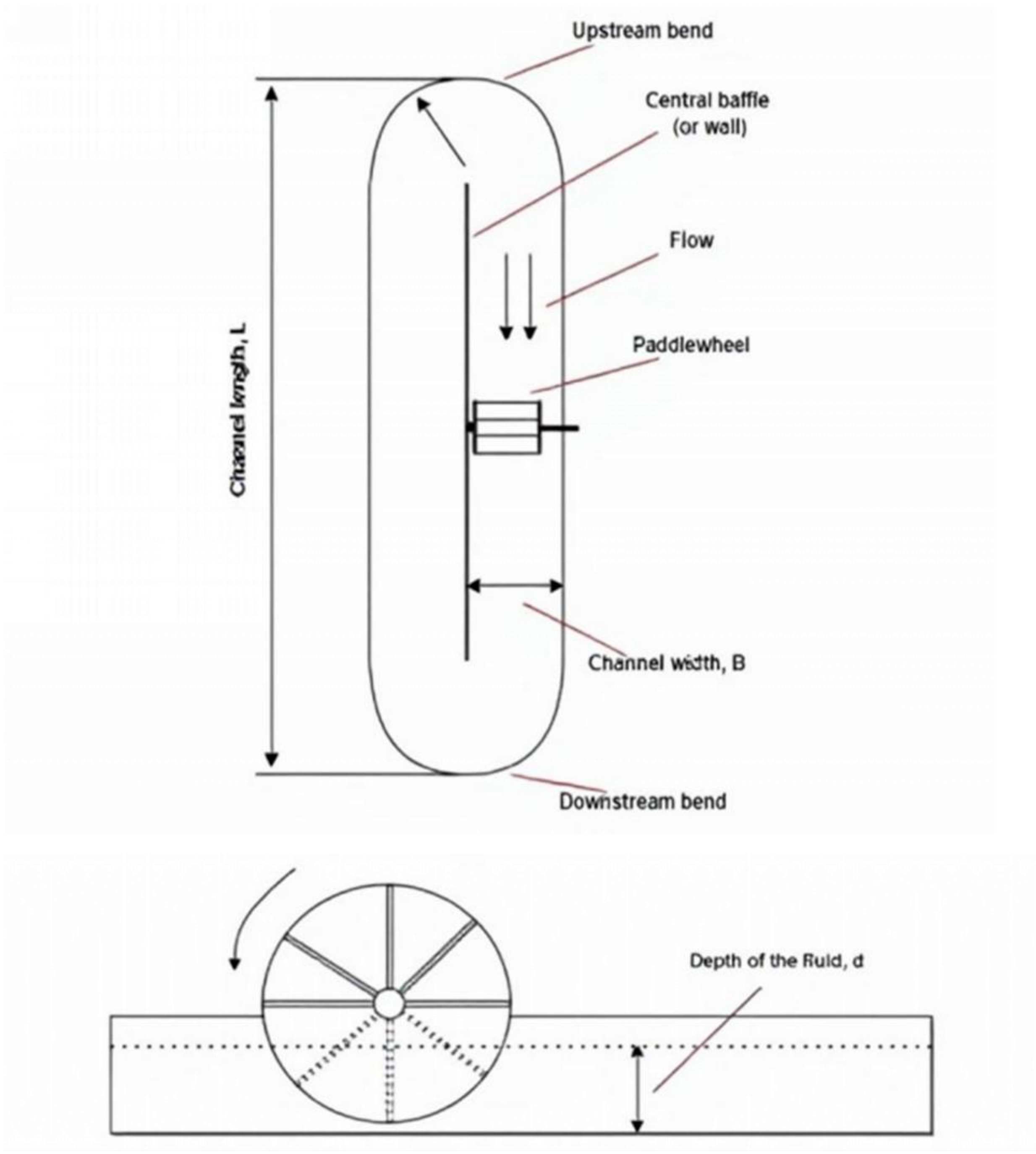

Figure 5: Schematic view of a RWP, (L) is length, (B) is width and (d) is depth (Skifa et al., 2025).

CHAPTRE II. METHODOLOGY

CHAPTRE II. METHODOLOGY

II. 1. Preparation of liquid and agar BG11 media for subcultring microalgal strains

Liquid and agar BG11 media were prepared according to Mouheb (2024). Strains of microalgae, previously isolated (Meberbeche and Hamadouche, 2023) from wastewater samples, were used in this work.

For liquid subcultring, each strain was reactivated by inoculating a loopful of colonies into 5 mL of sterile liquid BG11 medium prepared in test tubes. The test tubes were incubated at 25 °C under continuous illumination of 2000 lux and were handling shaking every day until obtaining a green color of the culture.

For solid cultivation, two methods were used:

Streak plate technique: Sterile BG-11 agar medium was prepared and poured into Petri dishes. A loopful of each microalgal strain was inoculated by streaking it across the agar surface. Petri dishes were inverted and incubated under the same light and temperature conditions.

Stab inoculation: A loopful of microalgae was aseptically taken from a well-isolated colony grown on solid BG-11 agar and carefully inoculated into sterile test tubes completely filled with solid BG-11 medium using the stab technique. The loop was inserted vertically into the center of the medium to ensure deep deposition of cells, using a sterile needle or Pasteur pipette. Tubes were incubated upright under continuous light at 25 ± 2°C to monitor growth along the stab line for preservation and viability assessment (Figure 6).

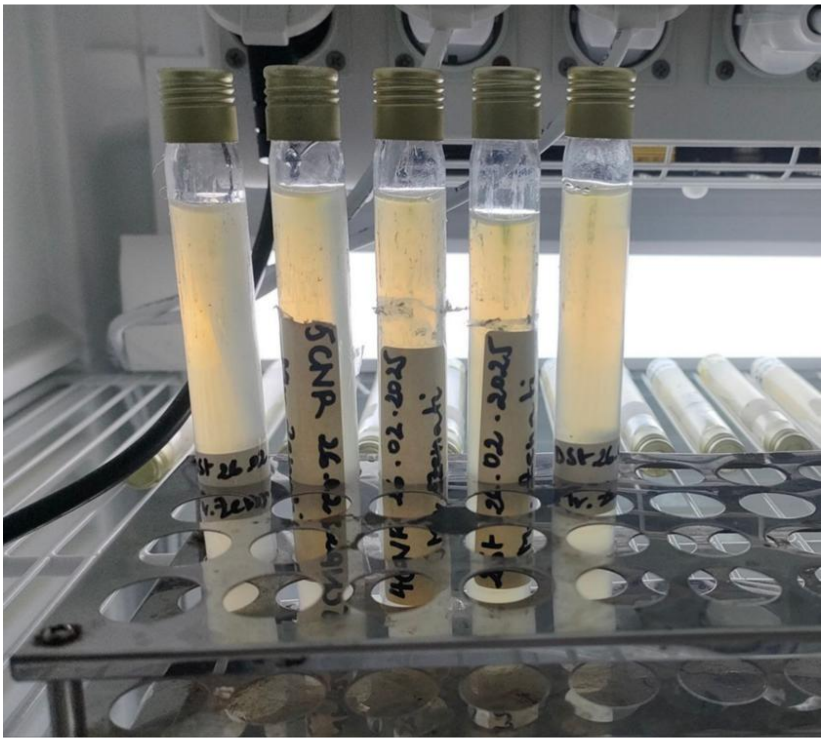

Figure 6: The stab inoculation technique used for microalgae subculturing

II. 2. Effect of salinity on microalgae strains

The study of the salinity effect focused on two microalgae strains, 4 CNR and 5 CNR, previously isolated from brackish wastewater.

Precultures of both strains were first prepared by inoculating 5 mL of each strain into 250 mL Erlenmeyer flasks containing 100 mL of BG-11 medium, with and without salinity (10 g/L NaCl).

After one week of incubation, duplicate cultures for each condition were established by transferring a volume of the preculture into fresh sterile BG-11 medium (final volume of 100 mL), prepared in 500 mL Erlenmeyer flasks, and adjusting the initial optical density (OD) at 680 nm to 0.2.

The flasks were incubated on an orbital shaker at 25 °C, under continuous illumination at 2000 lux and agitation at 150 rpm.

The growth of the two microalgae strains was monitored over a 6-day period by measuring the optical density (OD) at 680 nm and 750 nm of the culture.

II. 3. Comparative study of microalgae growth performance cultivated in BG11 medium and Tilapia wastewater

The growth performance of two microalgae strains, 2 ST and 5 CNR, was evaluated in non-sterile Tilapia wastewater compared to a favorable culture medium (i.e., BG11 medium). These strains were selected due to their demonstrated rapid and maximal growth in BG11 medium.

III. 3.1. Preculture of microalgae strains

Precultures of the 2 ST and 5 CNR strains were initiated by inoculating 20 mL of each liquid culture into 500 mL Erlenmeyer flasks containing 250 mL of fresh sterile BG11 medium. The cultures were incubated at 25 °C under 2000 lux continuous illumination and aerated using a bubbling system.

II. 3.2. Cultivation of microalgae strains in BG11 medium

After one week of incubation—corresponding to the exponential growth phase—an appropriate volume of each culture was transferred into 1 L Erlenmeyer flasks and adjusted to a final volume of 900 mL with fresh sterile BG11 medium, in order to obtain an initial optical density (OD) of approximately 0.2 at 680 nm. Cultures were maintained under the same incubation conditions (25 °C, 2000 lux, continuous aeration via bubbling) for a period of 10 days.

II. 3.3. Cultivation of microalgae strains in Tilapia WW

For the Tilapia wastewater experiments, 200 mL of microalgal culture (previously grown in BG11 medium) was added to 800 mL of Tilapia wastewater in 1 L Erlenmeyer flasks, and the cultures were maintained under the same incubation conditions.

II. 3.4. Growth measurements

Daily sampling was performed to monitor quantitative growth using the following methods:

- Optical density measurements: Approximately 2 mL of culture were analyzed at 680 nm and 750 nm using a spectrophotometer.
- Cell counting: A 100 µL aliquot of culture was examined using a Malassez counting chamber under a microscope at 400× magnification (Mouheb, 2024).

- Dry biomass estimation: 15 mL of culture were filtered through pre-weighed GF/C glass fiber filters and subsequently dried at 105 °C for 2 hours until a constant weight was achieved, in order to determine the dry biomass (mg/L).
-

II. 3.5. Determination of key growth parameters

To evaluate and compare the growth performance of microalgal strains under different culture conditions (BG11 medium and Tilapia wastewater), the following key growth parameters were calculated:

- **Specific Growth Rate (μ, day⁻¹):**

Calculated during the exponential growth phase using the formula:

$$\frac{\ln(OD_2) - \ln(OD_1)}{t_2 - t_1}$$

Where OD1 and OD2 are the optical density values at 680 nm at times t1 and t2, respectively.

- **Doubling Time (Td, days):**

Estimated from the specific growth rate using the formula:

$$Td = \frac{\ln(2)}{\mu}$$

II. 3.6. Correlation between growth measurements

To assess the consistency and reliability of the different growth indicators, correlation analyses were carried out between the following measurement methods:

- Optical Density (OD_{680} and OD_{750})
- Cell Counts
- Dry Biomass

Linear regression analyses were performed using **Microsoft Excel** to determine the nature of the relationships between the measured parameters and to derive regression equations. The coefficient of determination (R^2) was used to assess the strength of the linear association between variables.

In addition, **Pearson correlation coefficients (r)** were calculated using **XLSTAT** software to quantify the degree of linear relationship between each pair of variables. The strength and direction of the correlations were interpreted as follows:

- Strong correlation: $|r| > 0.9$
- Moderate correlation: $0.7 < |r| \leq 0.9$
- Weak correlation: $0.5 < |r| \leq 0.7$
- Negligible: $|r| \leq 0.5$

II. 4. Tilapia wastewater treatment using microalgae strains cultivated in open raceways ponds

II. 4.1. Conception and dimension of the laboratory raceways ponds

Three open raceway ponds (Figure 1, a) were custom-fabricated using grey polyvinyl chloride (PVC) sheets, selected for their mechanical strength and resistance to corrosion and chemical attack. Each tank measured 700 mm in length, 200 mm in width, and 75 mm in height, with a curved U-shaped design at both ends to facilitate continuous water circulation (Figure 1, b). The tanks were firmly fixed onto a flat base to ensure stability during operation. Such a scale-down geometric configuration was previously described by Radmann et al. (2007) in their study on Spirulina cultivation in raceway ponds.

A central paddle wheel system was installed above the three tanks to provide uniform mixing. The paddle wheels were connected to a single iron shaft, which in turn was driven by an electric motor coupled to a gear reducer, ensuring a stable rotation speed of 18 revolutions per minute (rpm). The paddles were precisely aligned within each tank to allow smooth, continuous movement of the culture medium along the raceway loop. This configuration guaranteed homogeneous distribution of microalgal cells, nutrients, and light exposure, preventing sedimentation and ensuring optimal hydrodynamic condition.

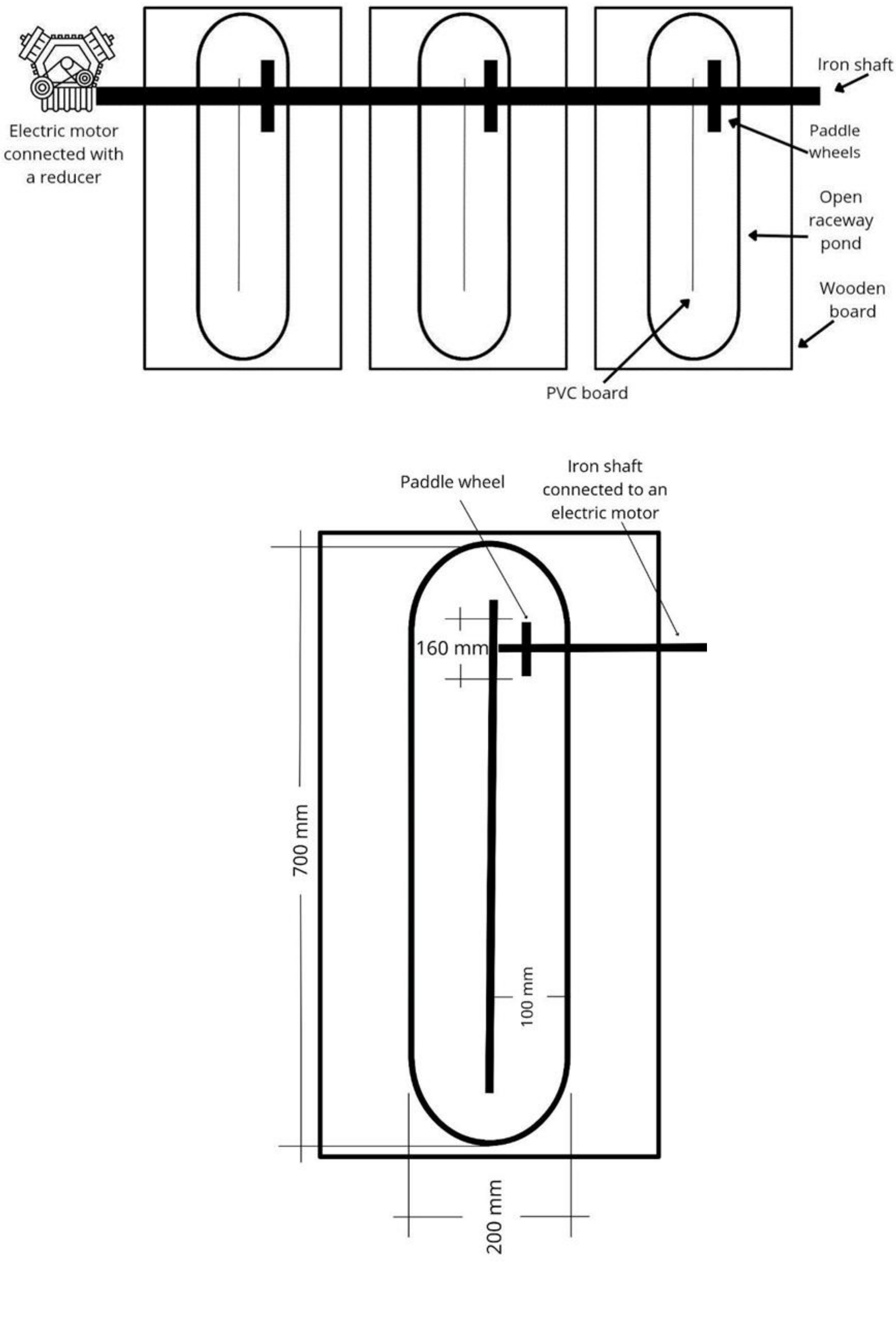

Figure 7: Schematic representation of the open raceway pond used for Tilapia wastewater treatment. (a) General configuration; (b) Dimensions of the raceways.

Prior to use, all raceway units were thoroughly cleaned, rinsed, and disinfected to avoid contamination. The proper functioning of the paddle wheel and flow circulation was verified before inoculation to ensure consistent and reproducible experimental conditions.

II. 4.2. Cultivation Conditions of Microalgae in Tilapia Effluent

Tilapia aquaculture effluent was collected from an active rearing tank at the Garden Nursery aquaculture farm in Chéraga, Algiers. The effluent had not undergone water renewal for over one month prior to sampling, thus representing a realistic, nutrient-rich wastewater condition.

Cultivation was carried out in raceway ponds located in a non-sterile room, maintained at an ambient temperature of 25 ± 2 °C. Agitation was provided by paddle wheels, and continuous illumination at 2000 lux was supplied using fluorescent lamps. Each raceway pond was filled to a total volume of 6 liters, composed of tilapia wastewater (TWW) and a pre-inoculated microalgae culture.

II. 4.3. Batch Cultivation Experiment

The batch cultivation experiment was conducted using three open raceway tanks, each with a total working volume of 5.8 liters, configured as follows:

- Tank 1 (Control): Contained 5.8 L of raw tilapia effluent without any microalgal inoculation.
- Tank 2 (2 ST Treatment): Contained 5.0 L of tilapia effluent supplemented with 800 mL of 2 ST microalgae pre-culture.
- Tank 3 (5 CNR Treatment): Contained 5.0 L of tilapia effluent supplemented with 800 mL of 5 CNR microalgae pre-culture.

All tanks were incubated for two weeks under identical environmental conditions. The pH was maintained at 8.0 ± 0.2 through periodic adjustments using 6 N hydrochloric acid (HCl). Daily evaporation losses were compensated with tap water, administered via serum valves to maintain a consistent volume and system balance (Sánchez Zurano et al., 2021).

Daily measurements of pH, temperature, and dissolved oxygen were performed using a Hanna HI 98194 multiparameter probe, which was directly immersed into the tanks containing the effluent. Additionally, optical density (OD) at 680 nm and 750 nm and dry biomass concentration were measured for the 2 ST, 5 CNR, and control treatments to evaluate the growth performance of the inoculated microalgae strains compared to the indigenous microalgae present in the tilapia wastewater (TWW).

Microscopic examination of the cultures in each tank was also carried out to confirm the presence of the inoculated strains and to identify the native microalgal population in the TWW. Photomicrographs were captured using the microscope's integrated digital camera at 1000× magnification.

At the end of the experiment, nutrient concentrations—including NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-}—were analyzed in each of the treatment raceway tanks.

II. 4.4. Semi-Continuous mode for Tilapia Wastewater (TWW) treatment using the best-performing microalgae strain

After completion of the batch trial, the 2 ST strain was selected for semi-continuous cultivation based on its performance. The three raceway tanks were thoroughly cleaned and refilled with tilapia wastewater (TWW), supplemented with the 2 ST strain as described previously in Section 2.4.3.

Following inoculation, the 2 ST culture was allowed to grow under batch conditions for eight days, during which it reached a stable biomass concentration. Thereafter, a semi-continuous culture regime was initiated by renewing 50% of the culture volume daily with fresh TWW. This renewal process continued until at least two complete volume replacements were achieved to ensure steady-state conditions.

In-situ measurements and OD (at 680 nm and 750 nm) determination, were recorded daily. Nutrient concentrations (NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-}) were analyzed on the first day of semi-continuous operation (immediately after the initial volume renewal) and again during the last three consecutive days of the experiment, prior to daily tank renewal, to assess nutrient removal efficiency under steady-state conditions.

The removal rate of each nutrient in the semi-continuous mode of the raceway pond was calculated using the following formula (Jebali et al., 2015; Sánchez-Zurano et al., 2021) :

Nutrient removal (mg/L.day)= (Cin-Cout)×D

Cin: Intial concentration of TWW in the raceway pond (mg/L)

Cout: Final concentration of TWW in the raceway pond (mg/L)

D: the dilution rate, 0.5 in our case.

Biomass productivity of the 2 ST strain cultivated in semi-continuous mode using TWW was calculating using the following formula:

$$Biomass\ productivity\ \left(\frac{g}{m^2.day}\right) = \frac{C_b.D.V}{A}$$

Where Cb is the biomass concentration (g/L), D is the dilution rate (day−1), V is the cultures' volume (L) and A is the area of the raceway pond (m2)

II. 4.5. Nutrient Analysis Protocols

To evaluate the nutrient load in tilapia aquaculture effluent before and after treatment, the concentrations of ammonium (NH_4^+), phosphate (PO_4^{3-}), nitrite (NO_2^-), and nitrate (NO_3^-) were determined using colorimetric spectrophotometric methods, based on reagent-specific protocols described by Aminot and Chaussepied (1983). Prior to analysis, all samples were filtered using GF/F glass fiber filters to remove suspended solids. Depending on the expected nutrient levels, appropriate dilution factors were applied to ensure that the absorbance readings fell within the linear range of the calibration curve for each compound.

For ammonium (NH_4^+) analysis, 100 mL of filtered effluent was mixed with 2 mL of reagent R1 and 2 mL of reagent R2. The mixture was incubated in complete darkness for a period of 6 to 8 hours to allow full color development. Absorbance was then measured at 630 nm using a UV-Vis spectrophotometer.

Phosphate (PO_4^{3-}) determination was performed by treating 100 mL of effluent with four reagents. The volumes added were 2 mL of reagent R1, 5 mL of reagent R2, 2 mL of reagent R3, and 1 mL of reagent R4. To ensure uniformity, the required quantities of each reagent were precalculated based on the number of samples, then mixed together in a beaker before being added to each effluent sample. After a reaction time of 10 minutes, absorbance was measured at 885 nm.

For nitrite (NO_2^-) analysis, a 50 mL sample of filtered effluent was used. First, 2 mL of reagent R1 was added, followed by 2 mL of reagent R2 after an interval of 1 to 2 minutes. The sample was allowed to react for 10 minutes before measuring absorbance at 543 nm.

Nitrate (NO_3^-) concentrations were determined by reducing nitrate to nitrite using a cadmium reduction column. The procedure began with the addition of 2 mL of ammonium chloride (NH_3Cl) solution to 100 mL of filtered effluent. This solution was passed through the pre-rinsed cadmium column, and the reduced sample was collected into a 50 mL volumetric flask. Subsequently, 2 mL of reagent R1 was added, followed 1 to 2 minutes later by 2 mL of reagent R2. After a 10-minute reaction period, absorbance was measured at 543 nm.

All spectrophotometric measurements were carried out using clean cuvettes, and absorbance values were compared against standard calibration curves specific to each nutrient.

CHAPITRE III. RESULTS AND DISCUSSION

3.1. Effect of salinity on the growth of microalgae strains cultivated in BG11 medium

The effect of salinity on the growth of 4 CNR, monitored by optical density (OD) at 680 nm and 750 nm, and by cell concentration, is presented in Figures 8 and 9, respectively. Similarly, figures 10 illustrate the salinity effect on the growth of 5 CNR, monitored by OD (at 680 nm and 750 nm) and cell concentration.

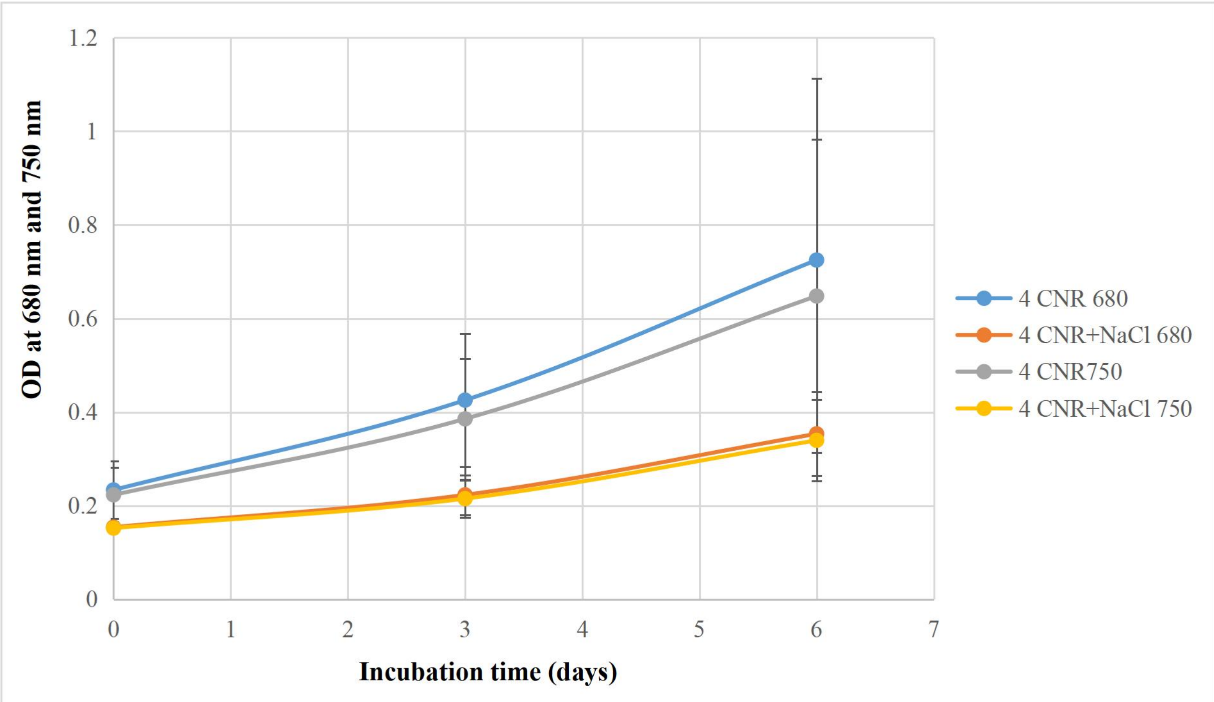

Figure 8: Salinity effect on the growth of 4 CNR monitored by OD cultivated in BG11 medium.

At both wavelengths, the *4CNR* strain exhibited higher growth in the control BG11 medium compared to the salinity-stressed condition. Specifically, the OD at 680 nm—which reflects chlorophyll *a* content and is directly related to photosynthetic activity—increased significantly from 0.23 on Day 0 to 0.72 on Day 6 in BG11, indicating active growth and chlorophyll production. In contrast, the OD680 in the NaCl-supplemented medium (4CNR N) only increased from 0.15 to 0.35 during the same period, showing reduced photosynthetic performance under salinity stress.

Similarly, OD750 (indicative of total biomass or turbidity) followed the same trend: growth was higher in BG11, increasing from 0.22 to 0.64, while in the presence of NaCl, it remained lower, rising only from 0.15 to 0.34. These results indicate that the *4CNR* strain is moderately tolerante to salinity stress, as shown by the slowed growth rate and lower pigment content in the saline medium.

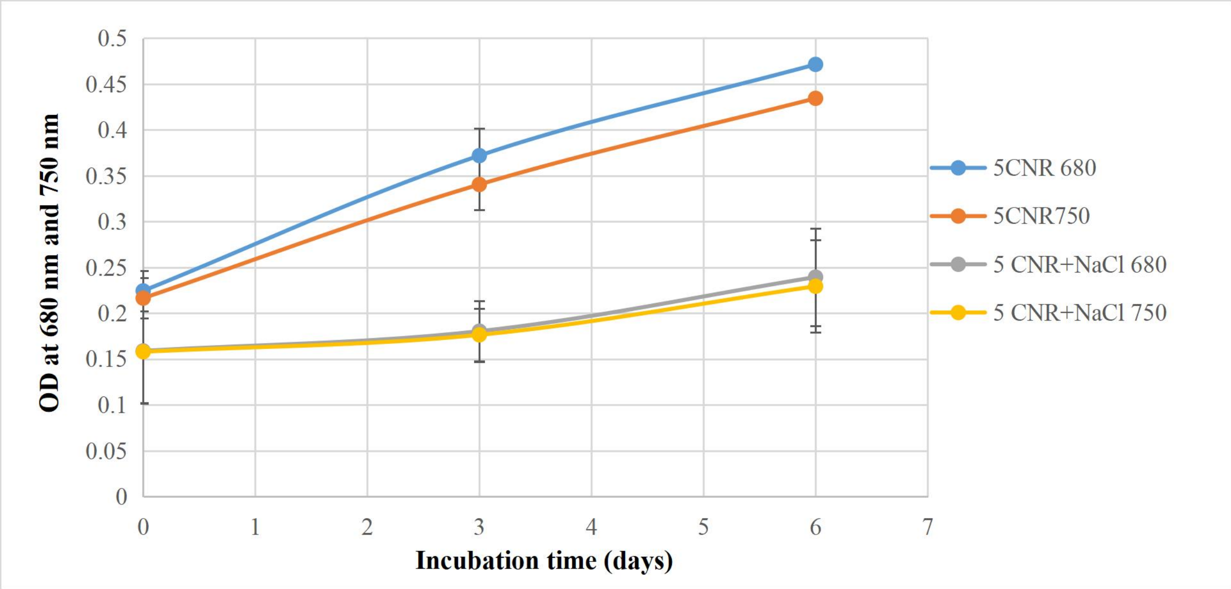

Figure 9: Salinity effect on the growth of 5 CNR monitored by OD cultivated in BG11 medium.

In the control medium (BG11), *5CNR* exhibited a steady increase in OD at both 680 nm and 750 nm. OD680 (linked to chlorophyll a and photosynthetic activity) rose from ~0.22 on Day 0 to ~0.47 by Day 6, while OD750 (indicative of cell density/biomass) increased from ~0.21 to ~0.44. This indicates healthy growth, good pigment production, and biomass accumulation under non-stress conditions.

In contrast, under salinity stress (5CNR N), growth was considerably reduced. OD680 increased only modestly from ~0.18 to ~0.26, and OD750 from ~0.16 to ~0.24. These values indicate that salinity stress negatively affected both photosynthetic pigment content and overall cell proliferation.

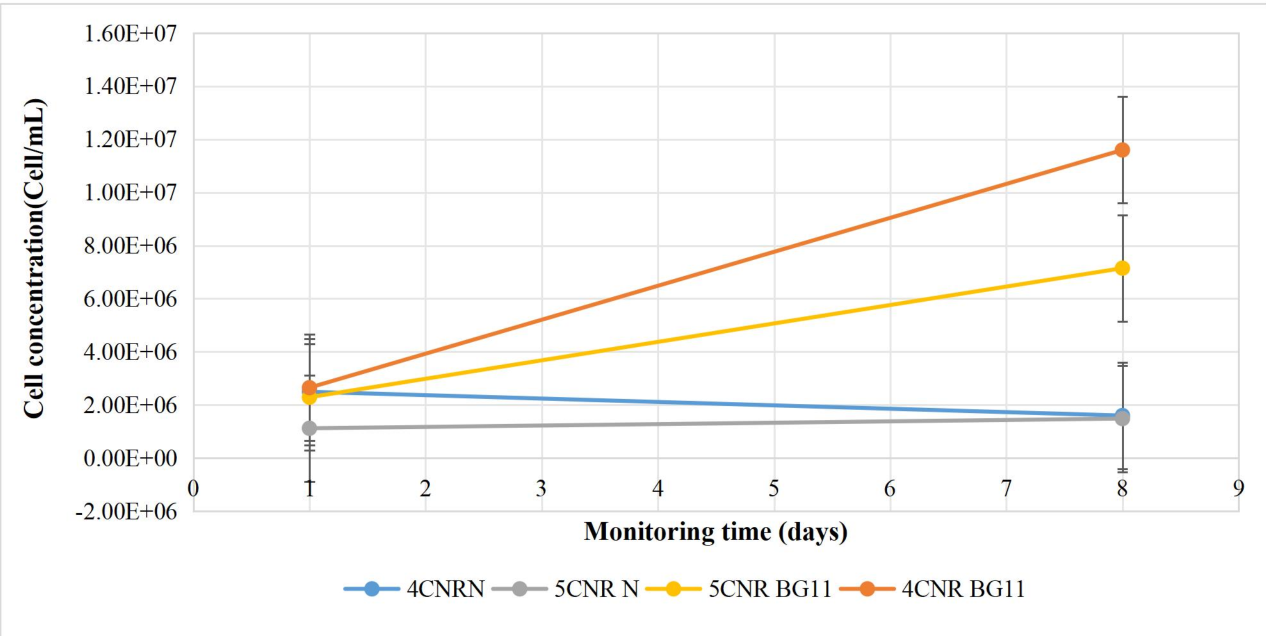

Figure 10: Salinity effect on the growth of 5 CNR monitored by Cell concentration cultivated in BG11 medium

Under non-saline conditions (BG11), both strains demonstrated an increase in cell concentration over time. Notably, strain 4CNR exhibited the highest growth, In contrast, the addition of NaCl had a significant inhibitory effect on the growth of both strains.

To summarize, both strains exhibited moderate growth under salinity stress, but *4CNR* clearly outperformed *5CNR* in both OD680, OD750 and cell concentration throughout the monitoring period. At Day 6, *4CNR* reached an OD680 of 0.35 and OD750 of 0.34, indicating greater pigment production or denser cell structure while *5CNR* reached only 0.23 (OD680) and 0.22 (OD750). *5CNR* showed a more stable increase in cell concentration, while *4CNR* experienced a decline, suggesting that *5CNR* has better tolerance to salt stress in terms of maintaining cell proliferation. *Chlorella* sp. still showed the ability to survive and adapt to increased salt concentrations, which aligns with previous findings on its salinity tolerance. According to Figler et al., (2021), *Chlorella* species are among the most salt-tolerant green microalgae, especially compared to freshwater-restricted species such as *Scenedesmus obliquus*. Also, another study showed that regarding salt tolerance, the results showed that halophytic microalgae such as *Chlorella vulgaris* were able to survive and grow in a sea salt medium with salinity levels up to 30 g/L and achieved 40 - 60 million cells/mL after 15 days of cultivation (Hau and Nguyen., 2022).

3.2. Comparative study of microalgae strains grown in BG11 and Tilapia WW

This study aimed to compare the growth performance of microalgae strains cultivated in two different media: BG11, a nutrient-rich synthetic medium commonly used to support optimal microalgal growth, and Tilapia aquaculture wastewater (TWW), a more variable and nutrient-limited medium. The objective of this comparative analysis is to evaluate how each strain responds to these contrasting nutrient environments in terms of growth behaviour and physiological adaptation. In addition to assessing growth differences, the study also investigated whether optical density (OD) measurements—specifically at 680 nm and 750 nm—serve as reliable proxies for estimating actual biomass accumulation (as dry weight in mg/L) and cell concentration. By establishing the correlation between OD and direct growth indicators (e.g. biomass and cell concentration).

3.2.1. Growth of microalgae strains in BG11 medium

Figure 11 and 12 present the growth curves of 5 CNR and 2 ST strains monitored by cell concentration and biomass, respectively.

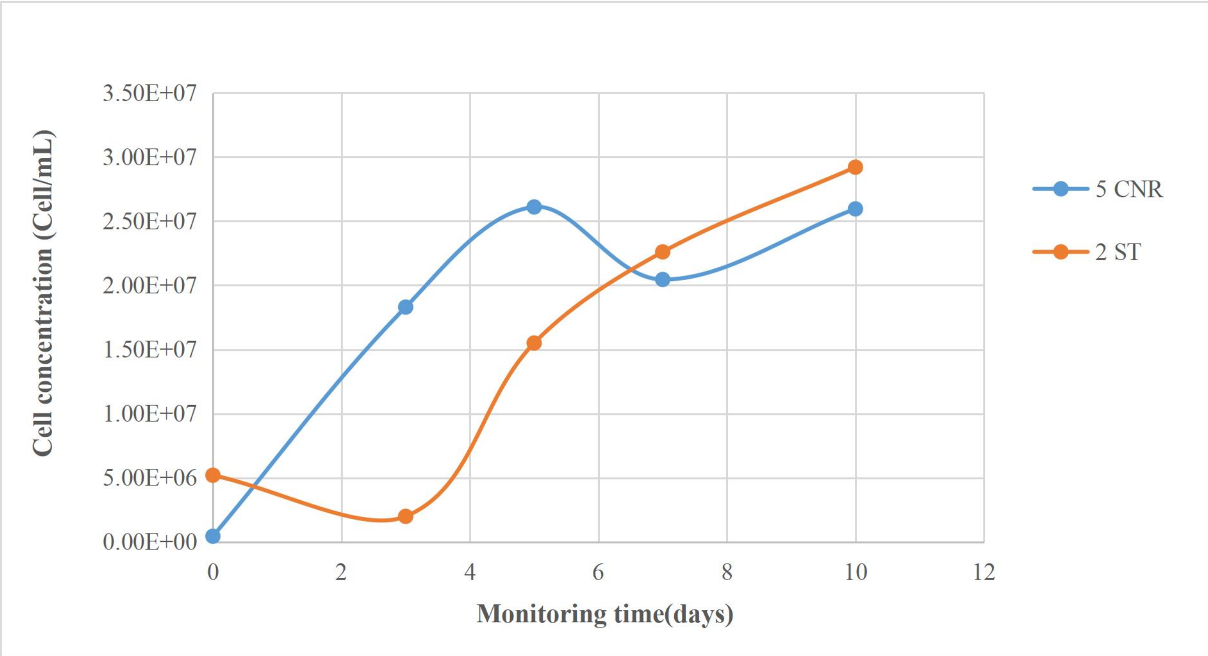

Figure 11: Growth monitoring of 2 ST and 5 CNR microalgae strains based on Cell Concentration in BG11 medium

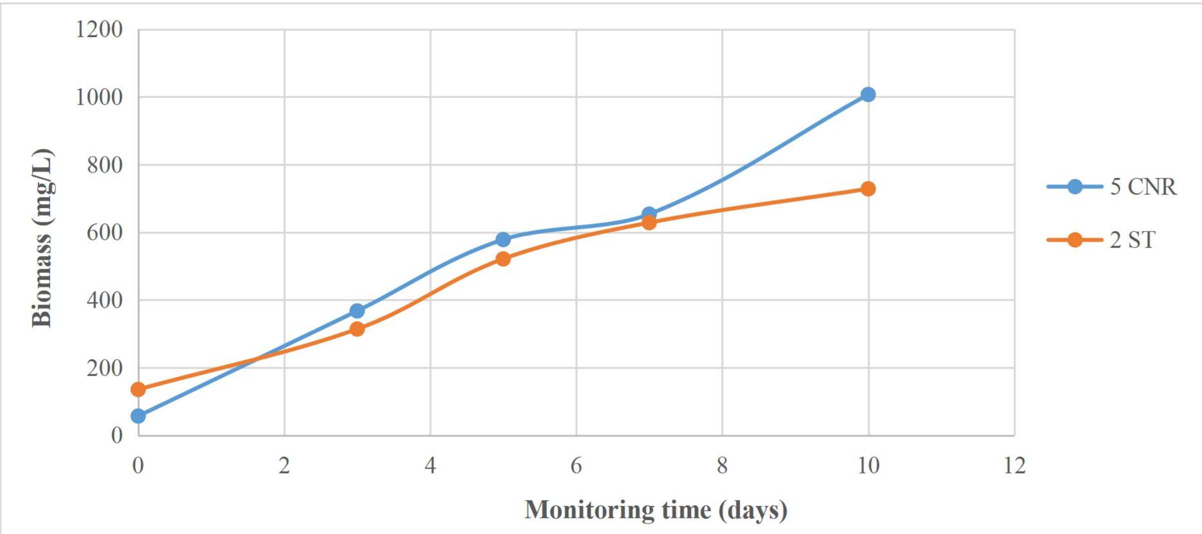

Figure 12: Growth Monitoring of 2 ST and 5 CNR microalgae strains based on biomass in BG11 medium

From the growth curves, key growth parameters—such as specific growth rate and doubling time—were determined. Table 5 and Table 6 present the growth parameter results for the 2 ST and 5 CNR strains, respectively.

Table 5: Key growth parameters of 2ST strain cultivated in BG11 medium.

	OD 680	OD 750	Cell concentration (Nbr/mL)	Biomass (mg/L)
Max value	2,53	1,674	2,92E+07	728,57
Specific growth rate μ (1/day)	0,48	0,45	1,024	0,28
Doubling time Td (day)	0,63	0,67	0,29	1,08

Table 6: Key growth parameters of 5 CNR strain cultivated in BG11 medium.

	OD 680	OD 750	Cell concentration (Nbr/mL)	Biomass (mg/L)
Max value	2,95	1,8035	2,61E+07	1007,14
Specific growth rate μ (1/day)	0,45	0,41	1,235	0,62
Doubling time Td (day)	0,67	0,73	0,24	0,48

3.2.2. Correlations between parameters for microalgae strains cultivated in BG11 medium

Table 7 and Table 8 present the linear correlations between quantitative growth measurements for the 2 ST and 5 CNR strains cultivated in BG11 medium, respectively. On the other hand, the correlations between these growth measurements for the 2 ST and 5 CNR strains were statistically analyzed using the Pearson correlation matrix. The results are shown in Table 9 and Table 10, respectively.

Table 7 : Linear correlation between quantitative growth measurements for 2 ST strain cultivated in BG11 medium.

Correlation	Equation	R^2
Biomass vs OD 680	Y= 274,82x+22,32	0.97
Biomass vs OD 750	Y=417,56x+29,849	0,92
Cell concentration vs OD 680	Y=1E+07x-4E+06	0,74
Cell concentration vs OD 750	Y=2E+07x-5E+06	0,62

Table 8 : Linear correlation between quantitative growth measurements for 5 CNR strain cultivated in BG11 medium.

Correlation	Equation	R²
Biomass vs OD 680	Y= 274,82x+22,32	0.97
Biomass vs OD 750	Y=417,56x+29,849	0,92
Cell concentration vs OD 680	Y=1E+07x-4E+06	0,74
Cell concentration vs OD 750	Y=2E+07x-5E+06	0,62

Table 9 : Pearson r correlation between quantitative growth measurements for 2 ST strain cultivated in BG11 medium.

	r	p-values	Significance
Biomass vs OD 680	0,984	0,002	Very strong
Biomass vs OD 750	0,961	0,009	Very strong
Cell concentration vs OD 680	0,861	0,06	Not significant
Cell concentration vs OD 750	0,789	0,11	Not significant

Table 10 : Pearson r correlation between quantitative growth measurements for 5 CNR strain cultivated in BG11 medium.

	r	P values	Significance
Biomass vs OD 680	0,986	0,002	Very strong
Biomass vs OD 750	0,955	0,011	weak
Cell concentration vs OD 680	0,859	0,062	Not significant
Cell concentration vs OD 750	0,926	0,024	weak

Our study shows that OD is a highly reliable indicator for biomass but less so for cell concentration, with cell size influencing outcomes. 5CNR outperforms 2ST in biomass production (1007.14 mg/L vs. 728.57 mg/L) and biomass growth rate (0.62 day^{-1} vs. 0.28 day^{-1}), while 2ST achieves a slightly higher cell concentration (2.92×10^7 vs. 2.61×10^7 cells/mL). Strong, significant correlations between biomass and OD ($P = 0.002–0.011$, < 0.05) for both strains confirm that OD 680 and OD 750 are effective for non-invasive biomass monitoring.

However, OD-cell concentration correlations are weaker, with 5CNR showing a significant correlation at OD 750 ($P = 0.02$) but not at OD 680 ($P = 0.06$), and 2ST showing no significant correlations ($P = 0.06–0.11$). This suggests that larger cells in 5CNR enhance OD 750's reliability for cell counts, while 2ST's smaller cells reduce it, necessitating direct methods like hemocytometry for precise cell counts.

Compared with typical *Chlorella* sp. references (500-800 mg/L biomass, growth rate 0.2-0.5 day[1]), the performance of 5CNR is exceptional, indicating strain-specific optimisation or advantages, while the 2ST results are typical but suggest further potential for improvement (Blair et al., 2014).

3.2.3. Growth of microalgae strains in Tilapia WW

Figure 13 present the growth curves of 5 CNR and 2 ST strains monitored based on biomass measurements.

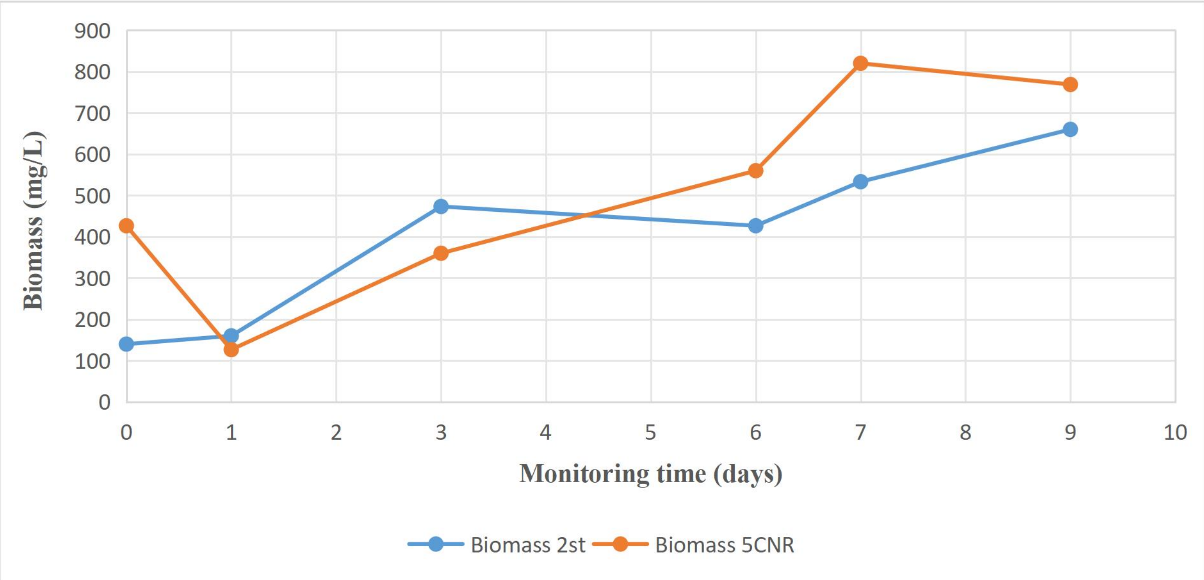

Figure 13: Growth monitoring of 2 ST and 5 CNR microalgae strains based on biomass in Tilapia WW

Table 11 and Table 12 present the key growth parameter results for the 2 ST and 5 CNR strains, respectively.

Table 11: Key growth parameters of 2ST strain

	OD 680	OD 750	Biomass (mg/L)
Max value	0.628	0.601	660
Specific growth rate μ (1/day)	0.198 (3-0)	0.193 (3-0)	0.54 (3-1)
Doubling time Td (day)	3.48	3.58	1.27

Table 12 : Key growth parameters of 5 CNR strain

	OD 680	OD 750	Biomass (mg/L)
Max value	0.755	0.706	820
Specific growth rate μ (1/day)	0.453 (7-3)	0.126 (7-3)	0.165 (6-1)
Doubling time Td (day)	1.52	5.48	4.18

The results from Figure 13 and the growth indicators in Tables 11 and 12 show that the 5CNR strain outperforms the 2ST strain in biomass production (820 mg/L vs. 660 mg/L). However, the 2ST strain demonstrates faster growth kinetics, with a higher specific growth rate (μ: 0.54 day^{-1} vs. 0.165 day^{-1}) and a shorter doubling time (Td: 1.27 days vs. 4.18 days), indicating more rapid cell division.

3.2.4. Correlations between parameters for microalgae strains cultivated in Tilapia WW

Table 13 and Table 14 present the linear correlations between quantitative growth measurements for the 2 ST and 5 CNR strains cultivated in TWW, respectively. On the other hand, the correlations between these growth measurements for the 2 ST and 5 CNR strains were statistically analysed using the Pearson correlation matrix. The results are shown in Table 15 and Table 16, respectively.

Table 13 : Linear correlation between quantitative growth measurements for 2 ST strain cultivated in Tilapia WW.

	Equation	R^2
Biomass vs OD 680	**DW**= 1533.7**Abs680**-359.2	0.8801
Biomass vs OD 750	**DW**=1495.9**Abs750**-374.38	0.885

Table 14 : Linear correlation between quantitative growth measurements for 5 CNR strain cultivated Tilapia W

	Equation	R^2
Biomass vs OD 680	**DW**= 1412.2**Abs680**-257.5	0.8093
Biomass vs OD 750	**DW**=1506.3**Abs750**-261.3	0.8254

Table 15 : Pearson r correlation between quantitative growth measurements for 2 ST strain cultivated in Tilapia WW

	r	P values	Signifiance
Biomass(mg/L) vs OD 680	0.809	0.1	Not significant
Biomass(mg/L) vs OD 750	0.825	0.091	Not significant
OD680 vs OD750	1	0	Very significant

Table 16 : Pearson r correlation between quantitative growth measurements for 5 CNR strain cultivated in Tilapia WW

/	r	P values	Signifiance
Biomass(mg/L) vs OD 680	0.880	0.062	Not significant
Biomass(mg/L) vs OD 750	0.885	0.059	Not significant
OD680 vs OD750	1	0	Very significant

These results show that the linear correlations between biomass (as dry weight in mg/L) and optical density at 750 nm (OD_{750}) or 680 nm (OD_{680}) are weaker in Tilapia Wastewater (TWW) compared to the BG11 medium. This aligns with previous studies (Lee and Lee, 2001; Griffiths et al., 2011), which found that OD-based estimations of microalgal concentration are more reliable under sterile or controlled conditions. In contrast, this relationship does not hold consistently in TWW.

The Pearson correlation matrix reveals no significant correlation between biomass (as dry weight) and optical densities at 750 nm or 680 nm (p-values > 0.05). This suggests that using optical density (OD) to estimate biomass concentration in non-sterile Tilapia effluents yields unreliable results. The discrepancy may arise from the presence of organic particles, microbial interference, or other substances in the wastewater that affect light absorption or scattering, thereby distorting OD measurements without reflecting actual microalgal biomass (Azizan et al., 2020; Rasoul-Amini et al., 2014). This issue has been documented in previous studies using wastewater as a growth medium, where OD readings were influenced by abiotic particles and non-algal microbial consortia (Acién et al., 2017; Richmond, 2004).

The only significant correlation found is between OD680 and OD750 ($r = 1$, $p \ll 0.05$), reflecting a perfect positive relationship. This result simply indicates that both OD680 and

OD750 measure similar optical properties of the suspension and are not specific to biomass concentration.

3.2.5. Summary

Our results, comparing microalgal growth in BG11 medium to cultivation in tilapia wastewater (TWW), align with the findings of Mahmoud (2024). In her study, *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Haematococcus lacustris* all achieved substantial growth in aquaculture wastewater (AWW), though biomass yields were consistently higher in BG11—for example, *C. vulgaris* reached approximately 930 mg/L in BG11 versus 640 mg/L in AWW. Similarly, in our study, the 5CNR strain achieved 1007.14 mg/L in BG11 compared to 820 mg/L in TWW, while the 2ST strain reached 728.5 mg/L in BG11 versus 660 mg/L in TWW. Although AWW proved to be a viable medium for microalgal cultivation, the three microalgal species demonstrated faster growth and higher biomass accumulation in BG11. The comparatively lower nutrient concentrations in AWW may induce stress conditions that, as Mahmoud (2024) noted, enhance lipid and carbohydrate accumulation relative to BG11.

This experiment demonstrated notable differences between microalgae strains grown in BG11 and those cultivated in Tilapia wastewater (TWW). Across all measured parameters, strains grown in BG11 consistently outperformed those in TWW. Furthermore, relying on optical density (OD) measurements to estimate biomass concentration in non-sterile Tilapia effluents can lead to inaccurate conclusions, as OD does not reliably reflect actual biomass levels under these conditions. For more accurate assessment, direct methods such as dry weight, ash-free dry weight (AFDW), or chlorophyll-a quantification are recommended (Griffiths et al., 2011; Slade and Bauen, 2013).

To assess the relationship between OD and biomass or cell concentration, the Pearson correlation coefficient (r) is more suitable than the coefficient of determination (R^2). Unlike R^2, which is designed for predictive modeling and explains variance in a dependent variable, Pearson's r measures the strength and direction of a linear relationship between two continuous variables—without assuming dependency (i.e., it does not imply that OD causes biomass to change). This is crucial in exploratory biological studies where variables may be interrelated but not causally linked. Prior studies have shown that while OD can serve as a biomass proxy, the relationship is species- and condition-dependent (Myers et al., 2013; Lu et al., 2017). Pearson's r can detect whether OD increases or decreases with cell concentration, offering valuable insight into the reliability of OD in a given setup (Wungmool et al., 2019).

Moreover, microalgae strains vary widely in their pigment content, morphology, and light-scattering properties. These factors further complicate OD-based assessments. By focusing on correlation rather than causation, Pearson's r enables a more nuanced and informative interpretation across diverse strains and environmental conditions (Santos-Ballardo et al., 2015). For correlation studies, therefore, Pearson's r provides a more accurate and practical metric than R^2 (Mousavian et al., 2023)

3.3. Tilapia wastewater treatment using microalgae strains cultivated in open raceway ponds

3.3.1. Tilapia wastewater composition

Tilapia wastewater (TWW) was collected from the fish tanks at Garden City Farm. Three sampling events were conducted. Table 17 presents the in situ parameters, suspended solids, and nutrient concentrations for each sample.

Table 17 : Composition of the sampled Tilapia wastewater

Composition	Sample 1	Sample 2	Sample 3	Mean	SD	CV%
pH	8.32	9.80	9.06	9.06	0.74	8.16
PO_4^{3-}(mg/L)	1.346	0.2386	0,4534	0.67	0.58	86.5
NH_4^+(mg/L)	0.0035	0.0058	0.0164	0.008	0.006	75
NO_3^-(mg/L)	7.3306	0.6611	2.3725	3.45	3.46	100
NO_2^-(mg/L)	0.997	0.0753	0.0609	0.37	0.53	143
OD at 680 nm	0.171	0.118	0.417	0.23	0.15	65.2
OD at 750 nm	0.16	0.111	0.395	0.22	0.15	68.1

When comparing the nutrient content of our Tilapia wastewater (TWW) samples to values reported in the literature, it becomes clear that our samples are significantly lower in nutrients. For example, Ansari (2017) reported an average pH of 7.28 ± 0.32, whereas our TWW had a higher average pH of 9.06. In terms of phosphorus, their study recorded PO_4^{3-} concentrations of 8.82 ± 0.02 mg/L, while our samples contained only 0.67 mg/L. Similarly, ammonium (NH_4^+) was 5.32 ± 0.45 mg/L in their data, compared to just 0.008 mg/L in ours. Nitrate (NO_3^-) levels were 40.67 ± 0.84 mg/L in their study, significantly higher than the 3.45 mg/L we observed. Nitrite (NO_2^-) concentrations also differed, at 5.52 ± 0.18 mg/L versus 0.37 mg/L in our samples. In contrast, (Lugo et al., 2020) reported NO_2^- levels below 0.01 mg/L, while Hawrot-Pawlikowska (2019) reported 3.6 mg/L of phosphorus, highlighting the variability in nutrient levels across different wastewater sources.

These comparisons clearly demonstrate that our TWW samples are nutrient-poor relative to those in the literature. This is likely due to the long residence time of water in our aquaculture ponds, which allows biological processes such as microbial assimilation, nitrification, and phosphorus precipitation to reduce nutrient concentrations—particularly ammonium, nitrite, and phosphate. Nitrate, although also lower, showed the greatest variability in our samples, possibly due to fluctuations in nitrification activity and limited denitrification, a pattern consistent with findings in extended-hold pond systems (Crab et al., 2007). This is further supported by the coefficient of variation (CV%), which exceeded 40% for all measured parameters—indicating high variability—except for pH, which remained stable with a CV of 8.16%.

3.3.2. Batch experiments

This experiment aims to evaluate the growth performance of our isolated microalgae strains in comparison with indigenous resident microalgae from tilapia wastewater over a period of 16 days.

3.3.3.1. Microscopic observation of raceway microalgae strains

Two distinct microalgal taxa naturally present in the effluent were identified based on their morphological characteristics (Bourrely., 1990).

- *Chlorella* sp. is a widely distributed unicellular green microalga, frequently utilized in wastewater treatment and biofuel research due to its robustness and nutrient uptake efficiency.
- In contrast, *Golenkinia radiata* is a green algal species recognized by its spherical shape and radiating spines. It belongs to the class Chlorophyceae and is typically found in nutrient-rich aquatic environments. While it is less commonly used in wastewater treatment compared to ***Chlorella*** sp., its presence in aquaculture wastewater suggests favorable conditions for green algal proliferation. Some studies have highlighted the potential of ***Golenkinia*** species for lipid accumulation, making them promising candidates for biofuel production (Hodaifa et al., 2008; Metzger & Largeau, 2005).

Photomicrographs of both strains were captured at 400× magnification using the microscope's integrated digital camera and are presented in Figures 14 and 15.

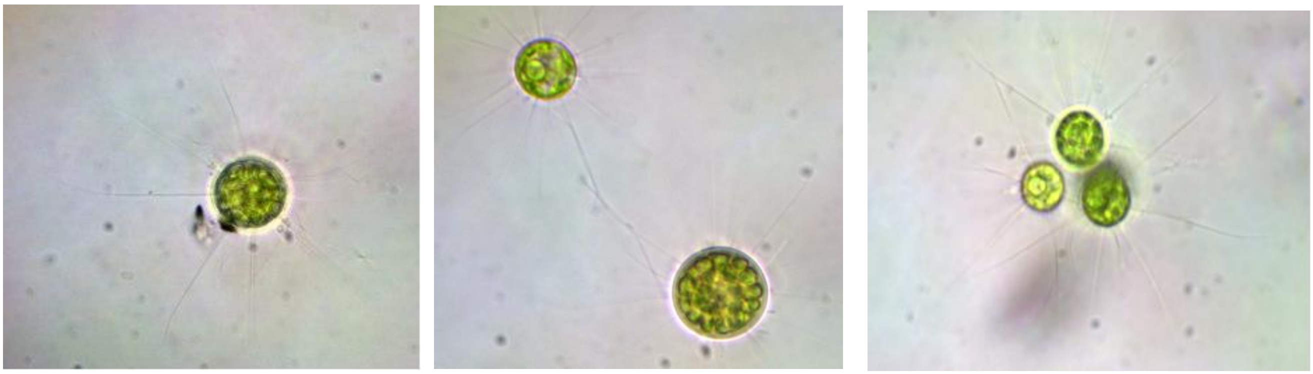

Figure 14: Photomicrograph of *Golenkinia radiata* took by the microscope's integrated digital camera 400× magnification.

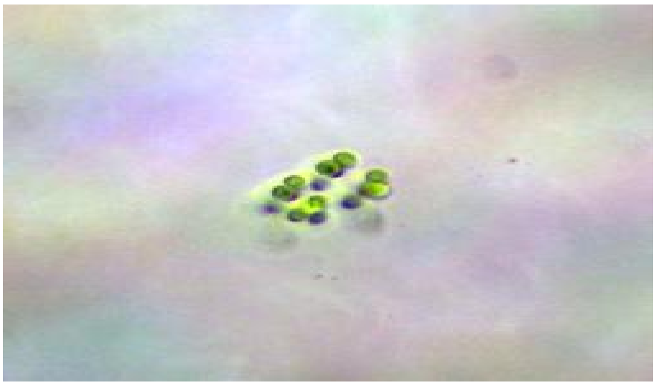

Figure 15 : Photomicrograph of *Chlorella sp* took by the microscope's integrated digital camera 400× magnification

The two microalgae strains, 5CNR and 2ST—shown in Figures 16 and 17, respectively—are likely to belong to the genus *Chlorella* sp. (Bourrely, 1990). *Chlorella* is a widely studied genus of green microalgae commonly used in phycoremediation and wastewater treatment due to its rapid growth, high adaptability, and ability to thrive in nutrient-rich environments. It is particularly effective in aquaculture wastewater treatment systems, as it can tolerate elevated concentrations of nitrogen and phosphorus (Rawat et al., 2011). Additionally, *Chlorella* is favored for its simple cellular morphology, ease of cultivation, and compatibility with various photobioreactor configurations (Cai et al., 2013).

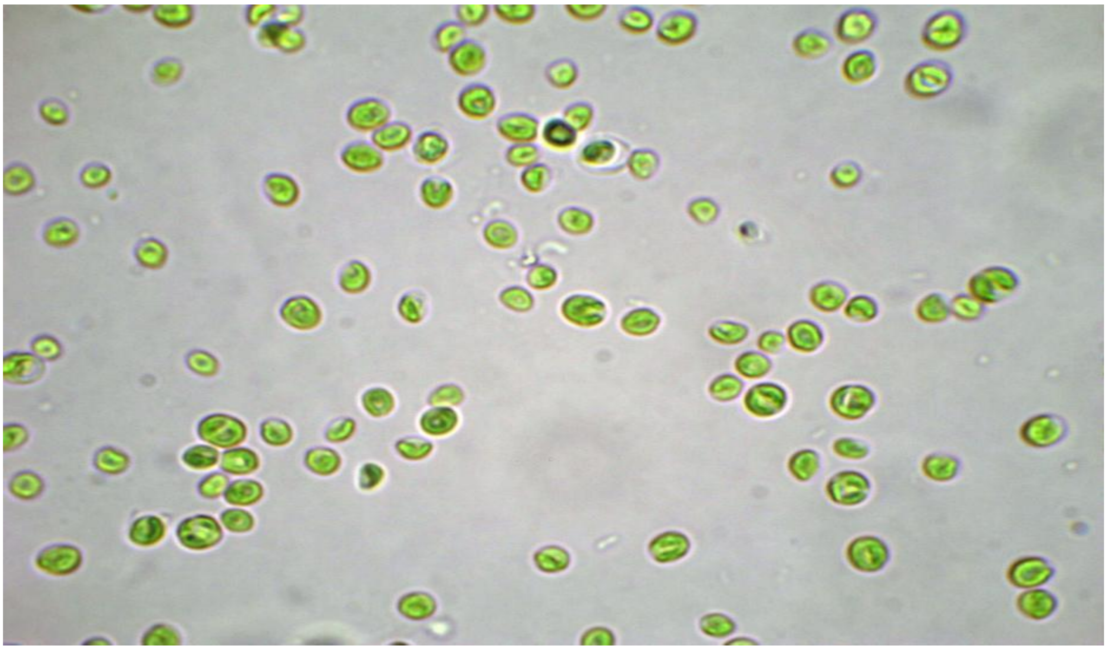

Figure 16: 5CNR microalgae strain observed under microscope's integrated digital camera at 1000× magnification

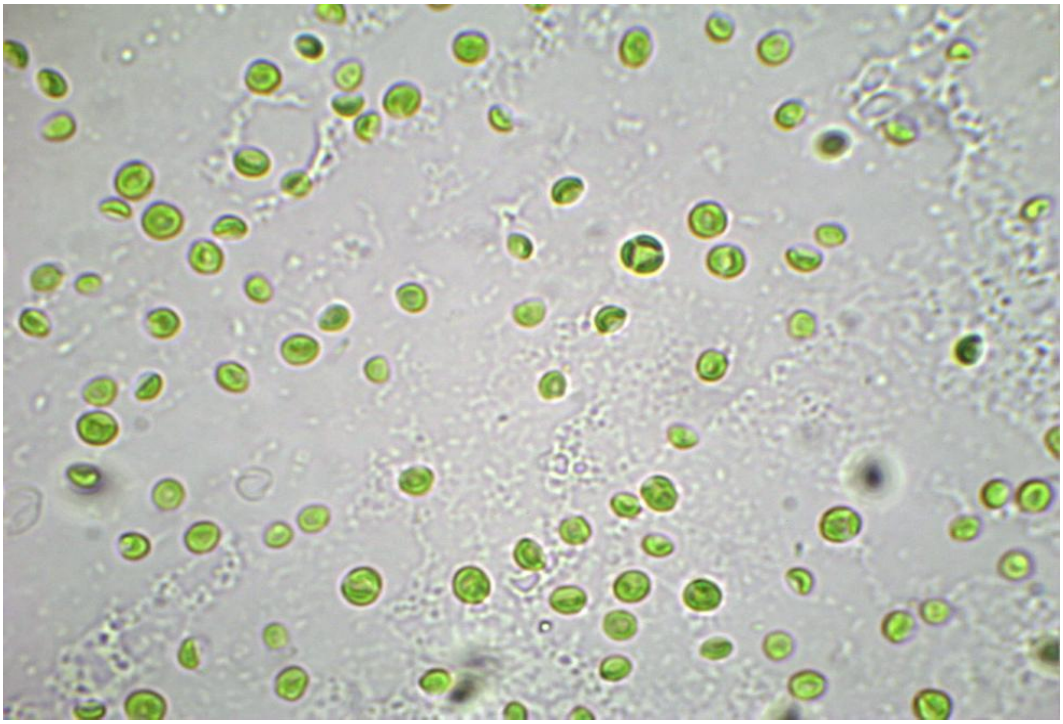

Figure 17: 2ST microalgae strain observed under microscope's integrated digital camera 1000× magnification.

3.2.2.2. Monitoring of environmental factors, growth, and nutrient removal in batch experiments

- **Monitoring of environmental factors**

Table 18 presents the changes in environmental parameters (pH, dissolved oxygen, DO%, and temperature) across the 2ST, 5CNR, and TWW treatments in raceway ponds.

Table 18: Environmental Parameters in Raceway Ponds for 2ST, 5CNR, and TWW Treatments

Parameter	Range (2ST)	Mean	SD	CV (%)	Range (5CNR)	Mean	SD	CV (%)	Range (TWW)	Mean	SD	CV (%)
pH	7.9–8.9	8.55	0.26	3.06	8.0–8.73	8.57	0.28	3.24	8.06–8.9	8.43	0.29	3.52
DO (mg/L)	11.04–12.62	11.54	0.46	3.99	10.37–12.23	11.40	0.50	4.40	11.32–12.00	11.60	0.23	2.00
DO (%)	115.3–138.6	131.0	7.17	5.47	125.2–138.1	133.0	5.19	3.90	118.1–136.4	132.3	3.73	2.30
Temp (°C)	20.08–23.50	22.70	1.08	4.84	21.00–23.07	22.46	0.82	3.67	21.07–23.30	22.38	0.77	3.45

The environmental conditions across all three treatments—2ST, 5CNR, and TWW—were relatively stable, with low coefficients of variation (CV%), suggesting consistent pond conditions during the experiments.

- pH levels ranged narrowly from approximately 8.4 to 8.6 across all treatments, with minimal variability (CV ~3%). This slightly alkaline environment is typical for microalgal growth and indicates that the presence of microalgae had limited effect on pH shifts during the batch period.

- Dissolved Oxygen (DO) concentrations (in mg/L) were high in all treatments, reflecting strong photosynthetic activity. The 2ST and 5CNR strains had DO values around 11.4–11.5 mg/L, while TWW recorded a slightly higher mean of 11.60 mg/L but with the lowest variability (CV = 2%), indicating more stable oxygen dynamics in the uninoculated pond.
- Oxygen saturation (%) was also high (115–138%), indicating supersaturation due to microalgal photosynthesis. The 2ST treatment showed slightly more variability in DO% (CV = 5.47%) compared to TWW (CV = 2.3%), which may reflect fluctuations in algal activity over time.
- Temperature remained within a narrow and optimal range for algal growth (21–23.5°C), with mean values close across all treatments (22.38–22.70°C). CV values were again low (3.45–4.84%), showing that the experimental conditions were well maintained.

In summary, environmental conditions across all treatments were suitable for microalgal cultivation. The minor variations observed (especially in DO% and pH) may relate to differences in microalgal metabolic activity, particularly between inoculated (2ST and 5CNR) and control (TWW) ponds. The low CV% values indicate that the system was stable and reliable for comparative biological assessment.

- **Growth monitoring**

Figure 18 and 19 present the temporal evolution of optical density (OD) and biomass for the two-microalgal strains and the native microalgal population cultivated in Tilapia wastewater (TWW) within the raceway ponds, respectively.

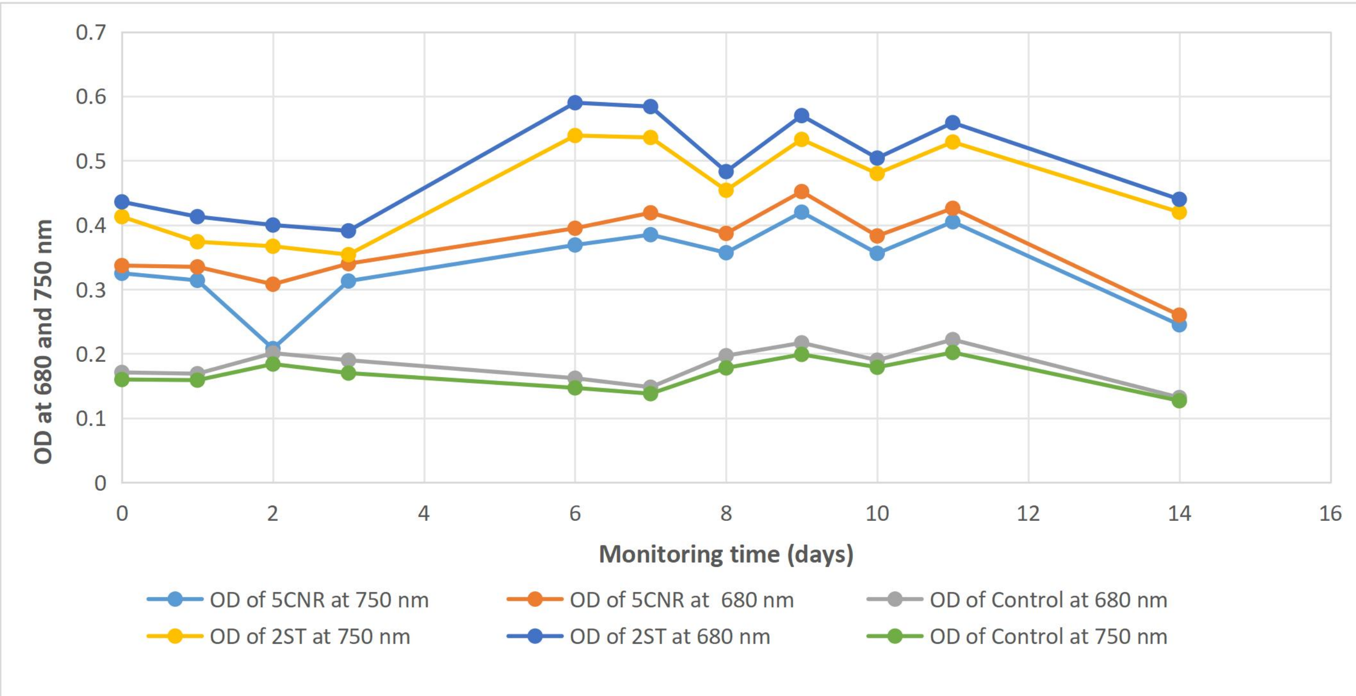

Figure 18 : Growth monitoring of 2 ST, 5 CNR, and indigenous TWW microalgae based on OD measurements

Both microalgal strains exhibited characteristic growth curves, with OD values increasing from Day 0 to Day 8, reflecting active cell proliferation and biomass accumulation. Notably, strain 2ST reached slightly higher OD values—0.56 at 680 nm and 0.53 at 750 nm—by Day 6, compared to strain 5CNR, which peaked at 0.45 (680 nm) and 0.42 (750 nm) by Day 9. In contrast, the control (Tilapia effluent without inoculation) maintained consistently low and stable OD readings throughout the experiment, confirming the minimal presence of native microalgal biomass under uninoculated conditions.

Overall, these results demonstrate successful cultivation of the microalgae strains within the raceway system, with strain 2 ST showing a marginally superior growth performance compared to 5 CNR. The low and stable OD in the control underscores the specificity of the observed growth to the inoculated strains.

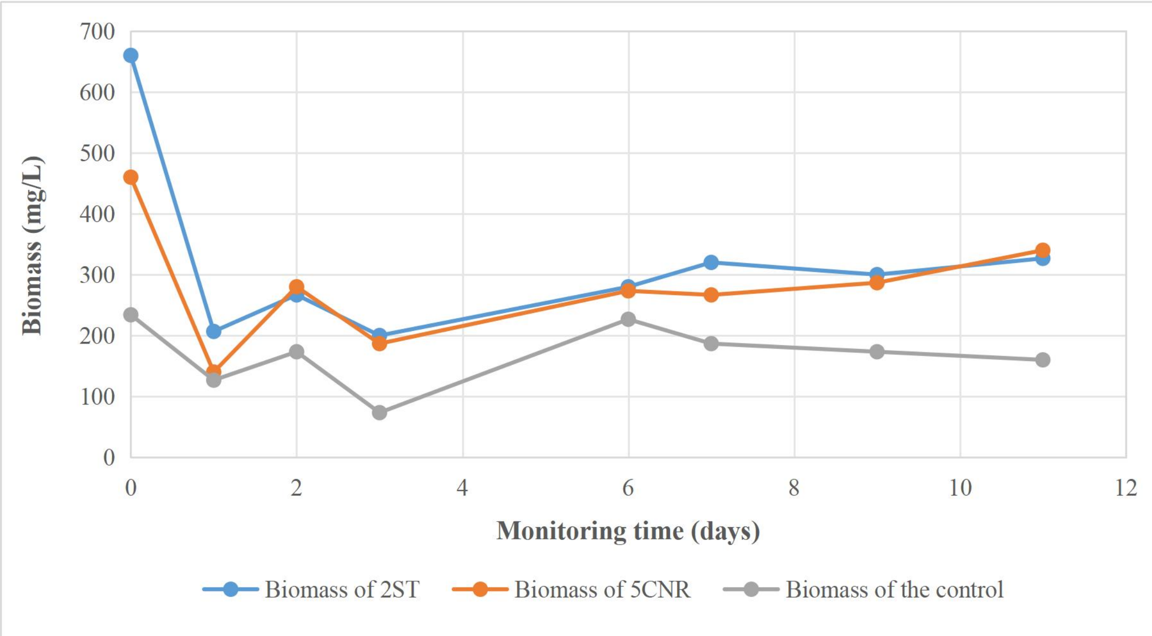

Figure 19: Growth Monitoring of 2 ST, 5 CNR, and indigenous TWW microalgae based on biomass quantification

Initially, on Day 0, biomass levels were high for all groups, with 2ST exhibiting the highest biomass concentration. A sharp decline in biomass was observed across all samples within the first two days, likely attributable to the initial adaptation phase or environmental stressors, with the control maintaining the lowest biomass levels throughout.

From Day 2 onwards, biomass values for both strains stabilized and showed gradual increases. The 5CNR strain exhibited a steady biomass increase, reaching 340 mg/L by Day 11. The 2ST followed a similar trend but consistently maintained slightly higher biomass levels compared to 5CNR marginally better growth performance under the experimental conditions.

The control maintained relatively low biomass levels throughout the experiment, with minor fluctuations, indicating minimal microalgal proliferation in the tilapia effluent without inoculation. The biomass of the control remained below 200 units after the initial decline, emphasizing the effectiveness of inoculation in promoting microalgal growth.

Comparing our results with those reported in the literature, Hawrot-Pawlikowska (2019) observed an optical density (OD) of 0.304 at 680 nm after eight days of cultivation, corresponding to a biomass concentration of 469 mg/L. Similarly, Bhuyar et al. (2021) reported a biomass yield of approximately 94.21 mg/L after 12 days of cultivation. In comparison, our microalgal strains demonstrated good growth performance, with strain 5CNR reaching a biomass concentration of 340 mg/L and an OD of 0.26 at 680 nm, while strain 2ST achieved 326 mg/L with a higher OD of 0.44. These results indicate that both strains adapted well to the cultivation conditions and exhibited competitive biomass productivity relative to previous studies.

- **Nutrient removal**

Table 19 show the concentration of nutrients at end of batch experiment for 2 ST, 5 CNR and control treatment.

Table 19: Overview of Nutrient Levels Post-Experiment in the batch experiment.

Parameter	TWW (Control)	5CNR	2ST
PO_4^{3-} (mg/L)	1.346	0.8591	1.0883
NH_4^+ (mg/L)	0.0035	0.0023	0.0035
NO_3^- (mg/L)	0.997	1.4006	0.771
NO_2^- (mg/L)	7.3306	1.1609	1.111

Table 19 presents the nutrient concentrations (PO_4^{3-}, NH_4^+, NO_3^-, and NO_2^-) measured at the end of a batch experiment using Tilapia Wastewater (TWW) treated with two microalgal strains, 2 ST and 5 CNR**,** in raceway ponds. The control (uninoculated TWW) serves as a baseline for comparison.

Phosphate (PO_4^{3-}) levels were reduced from 1.346 mg/L in the control to 0.8591 mg/L by 5 CNR and 1.0883 mg/L by 2ST. This translates to a TWW/5 CNR ratio of ~1.6 and a TWW/2 ST ratio of ~1.2, indicating that 5 CNR removed phosphate more efficiently. However, when comparing 5 CNR to 2ST directly (5 CNR/2 ST = 0.7), 2ST showed approximately 1.8 times greater removal, emphasizing its strength in phosphate uptake under certain conditions.

Ammonium (NH_4^+) was already present at very low levels in the control (0.0035 mg/L) and was slightly reduced by 5 CNR (0.0023 mg/L), with 2 ST maintaining similar levels to the control. The TWW/5 CNR and TWW/2 ST ratios were both close to 1, suggesting marginal differences. Still, 5 CNR showed modestly better performance in ammonium removal, which could be attributed to stronger assimilation or inhibition of nitrifying bacteria, as seen in similar studies using raceway-based systems (Sfez et al., 2015).

Nitrate (NO_3^-) showed contrasting behavior: 5 CNR-treated samples had a higher NO_3^- concentration (1.4006 mg/L) than the control (0.997 mg/L), possibly indicating incomplete nitrogen assimilation or active nitrification without downstream denitrification. In contrast, 2 ST reduced nitrate to 0.771 mg/L, giving TWW/2 ST and TWW/5 CNR ratios of ~1.3 and ~0.7, respectively. Comparing both strains, the 5 CNR/2 ST ratio is 1.8, confirming that 2 ST removed nearly twice as much nitrate as 5 CNR.

Nitrite (NO_2^-) was drastically reduced by both strains compared to the control (7.3306 mg/L), with final values of 1.111 mg/L for 2 ST and 1.1609 mg/L for 5 CNR**.** The TWW/2ST and TWW/5 CNR ratios were both close to 6.5, and 5 CNR/2 ST was ~1.04, indicating comparable removal efficiencies, though 2 ST had a slight advantage.

These findings are in line with previous research showing the effectiveness of microalgae for nutrient removal in batch-operated raceway systems using aquaculture wastewater. For instance, Iasimone et al. (2018) reported complete nutrient removal by microalgae in urban wastewater, while Sfez et al. (2015) observed similar trends in AWW treatment using algal-bacterial flocs in raceway ponds.

III.2.3 Semi-Continuous mode for Tilapia Wastewater (TWW) treatment using the best-performing microalgae strain

Figure 20 shows the evolution of the 2ST strain growth cultivated in semi-continuous raceway mode using TWW based on OD measurements.

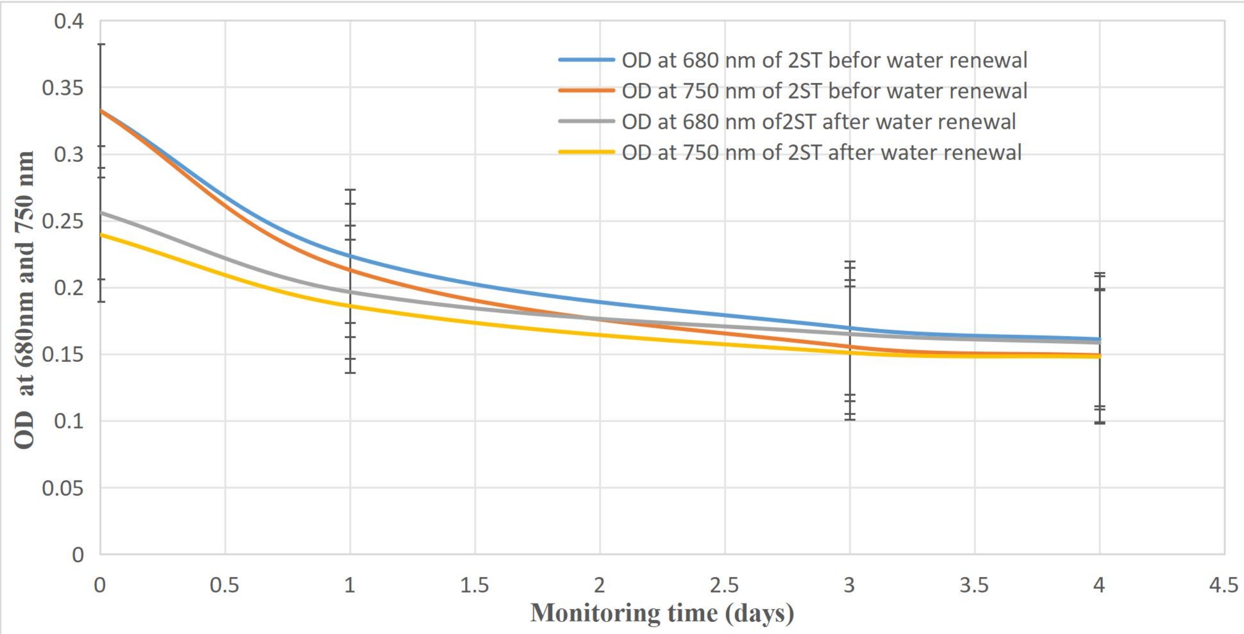

Figure 20: Growth monitoring of 2ST strain cultivated in semi-continuous raceway mode using TWW based on OD measurements

In the raceway ponds, we observed noticeable fluctuations in optical density (OD) throughout the monitoring period, particularly following each water renewal event. At 680 nm, OD values averaged between 0.16 and 0.33 before water renewal and decreased to 0.15–0.25 immediately afterward. This decline indicates that the relatively low biomass concentration at the end of the batch phase likely led to a dilution effect when fresh Tilapia wastewater (TWW) was introduced in the semi-continuous regime. The system was unable to recover biomass levels post-renewal, likely due to the combination of low initial biomass and the nutrient-poor nature of the TWW. These factors prevented the culture from reaching a steady state—defined here as a stable, biomass-rich system—which is essential for the efficient functioning of semi-continuous cultivation.

Despite these limitations, our post-renewal OD values at 750 nm (0.15–0.24) are comparable to those reported by Mayers et al. (2013), who recorded post-renewal OD values of 0.31 ± 0.01 for *Lobochlamys segnis* F12 and 0.20 ± 0.02 for *Klebsormidium flaccidum*. This indicates that, under nutrient-limited conditions, our microalgal strains maintained competitive OD performance relative to those tested in more enriched systems.

Figure 21 shows the change in the biomass productivity of the 2 ST strain cultivated in semi-continuous mode using TWW.

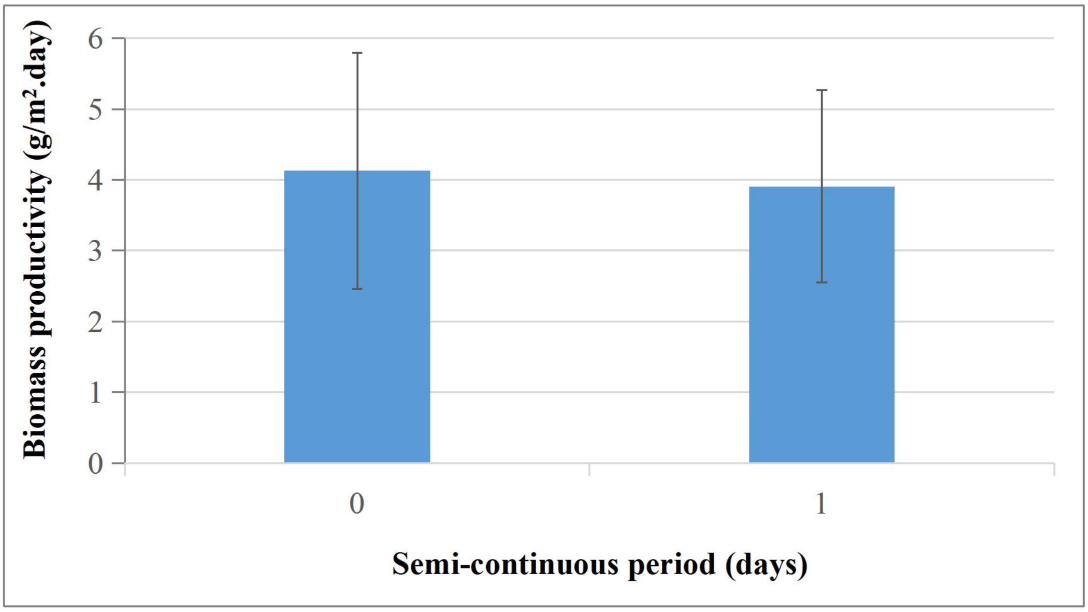

Figure 21 : Variation in the biomass productivity of the 2 ST strain cultivated in semi-continuous mode using TWW.

In this study, the semi-continuous operation of raceway ponds at a dilution rate of 0.5 day^{-1} using the 2ST strain resulted in surface biomass productivities of 4.12 and 3.90 g m^{-2}·day on Days 0 and 1, respectively. When converted to volumetric terms, this corresponds to 0.092 and 0.087 g L^{-1}·day, allowing for a direct comparison with studies reporting productivity per unit volume. For instance, Jebali et al. (2015) reported significantly higher volumetric biomass productivity of 0.9 g L^{-1}·day using a native *Scenedesmus* strain (WT6) in semi-continuous operation at a similar dilution rate (0.6 day^{-1}). The difference is primarily attributed to their use of nutrient-rich influent (~63.5 mg/L NH_4^+), controlled lab conditions, and optimized light and CO_2 supply. In contrast, Zurano et al. (2021) conducted large-scale experiments in 80 m^2 outdoor raceways using real primary wastewater and achieved surface biomass productivities between 15 and 30 g m^{-2}·day. Their superior yields were facilitated by shallower culture depths (e.g., 0.05 m), higher nutrient loading, and optimized mixing—factors that enhance light availability and growth rates. Compared to these studies, our system—operated with Tilapia wastewater of low nutrient strength and deeper culture depths—produced modest but realistic biomass yields under field conditions.

Table 20 show the Nutrient removal rates from treated wastewater (TWW) using 2 ST strain in raceway ponds operated in semi-continuous mode.

Table 20: Nutrient removal rates from treated wastewater (TWW) using 2ST strain in raceway ponds operated in semi-continuous mode

	NH_4	NO_2	NO_3	PO_4
TWW in (mg/L)	0.11±0.001	4.41±0.06	27.23±2.66	0.26±0.13
TWW out (mg/L)	0.1±0.03	0.62±0.13	7.81±0.1	0.12±0.02
Removal rate (mg/L.day)	0.006	1.89	9.7	0.07

Nutrient removal performance followed similar trends. In our system, the 2ST strain achieved daily removal rates of 0.006 mg/L·day for NH_4^+, 1.89 mg/L·day for NO_2^-, 9.70 mg/L·day for NO_3^-, and 0.07 mg/L·day for PO_4^{3-}. These values reflect effective nitrogen oxidation and moderate phosphate removal, but are lower than those reported in comparable semi-continuous systems. Jebali et al. (2015) observed much higher removal efficiencies, including 34.6 mg/L·day for NH_4^+ and 12.7 mg/L·day for PO_4^{3-}, made possible by high influent concentrations and efficient uptake by the WT6 strain. Similarly, Zurano et al. (2021) reported NH_4^+ removal rates of 60–70 mg/L·day and phosphate removal up to 0.6 g m^{-2}·day, even at comparable dilution rates (0.3–0.5 day^{-1}). Their system, fed with influent containing >60 mg/L of NH_4^+, was designed for high throughput and maintained optimal conditions for nutrient assimilation. Overall, while our system performed below the benchmarks set by these studies, the results demonstrate the ability of native strains like 2 ST to support nutrient removal and biomass generation in real aquaculture wastewater, despite challenging input conditions.

CONCLUSION

CONCLUSION

The objective of this dissertation was to evaluate the efficiency of local microalgae strains for treating raw, unfiltered, and non-sterilized aquaculture wastewater using custom-built raceway ponds.

Initially, the effect of salinity (10 g/L NaCl) on two brackish wastewater-isolated microalgae strains, 5CNR and 4CNR, was assessed. Both strains exhibited halotolerance; however, their optimal growth was observed in the absence of sodium chloride. Cell concentrations reached 1.16×10^7 and 7.15×10^6 cells/mL without NaCl, compared to 1.6×10^6 and 1.49×10^6 cells/mL with 10 g/L NaCl for 4CNR and 5CNR, respectively.

Subsequently, the growth performance of the selected strains, 2ST and 5CNR, was evaluated in both BG11 medium and non-sterile Tilapia wastewater (TWW). The study also examined the potential use of optical density (OD) at 680 nm and 750 nm as proxy indicators for biomass and cell concentration. In BG11 medium, both strains exhibited robust growth, with 5CNR outperforming 2ST in biomass production (1007.14 mg/L vs. 728.57 mg/L), while 2ST slightly exceeded 5CNR in cell concentration (2.92×10^7 vs. 2.61×10^7 cells/mL). This trend remained in TWW, though with reduced growth levels (820 mg/L for 5CNR vs. 660 mg/L for 2ST) and more variable kinetics. Correlation analysis revealed that OD measurements are unreliable proxies for biomass or cell concentration in complex, non-sterile media like TWW. However, in well-defined media such as BG11, OD may serve as a suitable indirect method when supported by statistically significant correlations (e.g., Pearson correlation coefficients).

Treatment of TWW using these local strains in laboratory-scale open raceways showed thefollowing outcomes:

- Environmental conditions (temperature, pH, and dissolved oxygen) across treatments remained suitable and stable for microalgal growth.
- The inoculated microalgae strains effectively outcompeted the native microalgae population, confirming their ecological dominance.
- Under batch conditions, both 2ST and 5CNR outperformed the uninoculated control, with 2ST achieving greater nutrient removal, particularly for phosphate, nitrate, and nitrite.
- In semi-continuous mode, however, the combination of low biomass recovery and limited nutrient availability in TWW prevented the establishment of a steady state. As a result, biomass productivity and nutrient removal rates remained low (0.087 g/L·day; 0.006 mg NH_4^+/L·day, 1.89 mg NO_2^-/L·day, 9.7 mg NO_3^-/L·day, and 0.07 mg PO_4^{3-}/L·day).

Perspectives

Future research should focus on:

- Investigating other types of aquaculture wastewater (AWW) with higher nutrient content to enhance biomass production.
- Deepening the understanding of semi-continuous AWW treatment in raceway ponds

by optimizing key operational parameters such as dilution rate, culture depth, and light availability.

- Scaling up raceway systems to larger dimensions (e.g., pilot scale) to enable the treatment of greater volumes of Tilapia wastewater (TWW) and assess performance under realistic field conditions.

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APPENDICES

Figure 22: The open raceway ponds used in the TWW treatment

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Attestation du meilleur poster

Cette attestation est délivrée à

M. ZENATI Billal

A participé à la conférence intitulée « Interactions anthropiques avec la mer et le littoral : Perspectives scientifiques et stratégiques « CIAMEL 2025 » qui a eu lieu à l'ENSSMAL le 28 mai 2025 par une communication écrite intitulée :

«Comparative grouwth analysis and paramater correlation of two local microalgae strains»,

Co-auteurs: KEDOUI Sarah, MESSAOUDI Hana, MEKNACHI Abdellah, GUENACHI Belkacem, AYAD Meriem, AIT SAIDI Adel

Alger, le 28 mai 2025

Directrice de l'ENSSMAL

Figure 23: Certificate of Best Poster Presentation in the Research Category – CIAMEL 2025 Award

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Attestation de participation

Cette attestation est dévirée à

Mme MAOUEL Djamila

A participé à la conférence intitulée « Interactions anthropiques avec la mer et le littoral : Perspectives scientifiques et stratégiques « CIAMEL 2025 » qui a eu lieu à l'ENSSMAL le 28 mai 2025 par une communication écrite intitulée :

«Analysis of consumer behavior toward aquaculture products: Tilapia»,

Co-auteurs: YAHIAOUI D., KHELIFA Oussama, CHELOUH Mourad, MESSAOUDI Hana, KEDOUI Sara

Alger, le 28 mai 2025

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Figure 24: Certificate of participation in CIAMEL 2025