OPTIMIZATION OF ENVIRONMENTAL FACTORS FOR ENHANCING GROWTH AND POLLUTANT REMOVAL EFFICIENCY OF THE CYANOBACTERIUM SYNECHOCYSTIS SALINA M8 IN DOMESTIC WASTEWATER

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Abstract

Microalgae biotechnology has attracted widespread attention due to its potential for wastewater treatment and the recovery of biomass with economic value. This study focuses on evaluating the effects of several environmental factors on the growth and domestic wastewater treatment efficiency of the photoautotrophic cyanobacterium Synechocystis salina M8, which was isolated from agricultural water sources in Vietnam. Laboratory-scale experiments were conducted using two types of culture media (BG-11 and domestic wastewater), under different pH levels and C:N:P nutrient ratios, to determine the optimal conditions for the growth of S. salina M8 and its pollutant removal performance. The study employed standard methods for sampling and wastewater analysis in the laboratory, along with techniques for assessing the growth of S. salina M8. The results showed that S. salina M8 exhibited good growth in both culture media, particularly in domestic wastewater under mixotrophic conditions with aeration. Biomass productivity reached 1.34 g/L in non-sterilized BG-11 medium and peaked at 1.64 g/L in domestic wastewater. After 8 days of cultivation under optimal conditions - including an inoculum ratio of 20–25% (v/v), pH 7, temperature of 27°C, aeration at 0.1 vvm, light intensity of 4500 Lux, and a C:N:P ratio of 100:10:1 - the treatment efficiency reached approximately 75% for COD, and over 80% for N–NH_4^+, total nitrogen (T-N), P–PO_4^{3-}, and total phosphorus (T-P). The treated water met the standards set by QCVN 14:2008/BTNMT, Column B. These findings confirm the potential of S. salina M8 for application in sustainable and environmentally friendly domestic wastewater treatment, while simultaneously generating biomass for the production of valuable products. This contributes to the advancement of green technologies and supports the development of a circular economy model.

Keywords: *Domestic wastewater, Mixotrophy, Biomass, S. salina M8.*

JEL Classifications: *Q25, Q53, Q55.*

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1. INTRODUCTION

Currently, water pollution has become a major global concern, particularly in large urban areas such as Hanoi. The rapid pace of urbanization, coupled with population growth, has led to the generation of a substantial volume of domestic wastewater each day. In Hanoi, each resident discharges approximately 100 - 150 liters of wastewater per day, bringing the total volume of domestic wastewater in the city to nearly 1 million cubic meters per day (Anh, T. T. D., 2022). However, according to the Ministry of Natural Resources and Environment (2023), only about 18% of urban wastewater is currently collected and treated at centralized treatment plants. The remaining portion is often discharged directly into the environment without treatment, resulting in serious pollution, eutrophication, and adverse impacts on aquatic ecosystems and public health. Domestic wastewater contains high levels of organic matter, nitrogen and phosphorus compounds, pathogenic microorganisms, and toxic micro-pollutants. Current treatment technologies mainly rely on aerobic, anaerobic, or anoxic biological processes. However, these conventional methods generate large amounts of sludge that require further treatment and are often not environmentally friendly.

In response to the current situation, the development of efficient, environmentally friendly, and cost-effective wastewater treatment technologies with potential for resource recovery has become an urgent necessity. Among the promising approaches, the application of photoautotrophic microorganisms - particularly microalgae and cyanobacteria has attracted significant attention. These microorganisms are capable of assimilating pollutants such as chemical oxygen demand (COD), nitrogen, and phosphorus through photosynthesis. Moreover, the biomass generated during the treatment process can

be reused for the production of fertilizers, animal feed, biofuels, or other high-value compounds. For instance, Chlorella variabilis TH03 has demonstrated the ability to remove up to 99,9% of phosphorus and 96,1% of nitrogen within just 14 - 17 days (Dang Thuan Tran et al., 2021). In another study, Synechocystis sp. cultured in wastewater enriched with NH_4^+ and PO_4^{3-} achieved nutrient removal efficiencies of 96,99% for phosphate, 80,10% for nitrate, 67,90% for nitrite, and 98,07% for ammonium (N. Krasaesueb, A. et al., 2019). Recent research has also shown that microalgae can be cultivated not only under photoautotrophic conditions but also under heterotrophic and photo-mixotrophic regimes. Under heterotrophic conditions, microalgae grow using organic carbon sources without the need for light, enabling effective removal of organic matter, nitrogen, and phosphorus from wastewater compared to conventional autotrophic cultivation (Santos, C. A bet al., 2020). On the other hand, photo-mixotrophic cultivation which has recently received increasing research interest allows microalgae to simultaneously utilize both organic and inorganic carbon sources, along with nitrogen and phosphorus nutrients in wastewater, thereby improving the efficiency of pollutant removal (Voulvoulis, N et al., 2017).

Although this has been documented in several microalgal species such as Arthrospira platensis (M. I. B. Pereira et al., 2019), the application of the photo-mixotrophic cultivation mode for the cyanobacterium Synechocystis salina M8 in domestic wastewater treatment remains limited in Vietnam. This strain exhibits the ability to grow in both freshwater and saline environments and contains phycocyanin and chlorophyll – a pigments that enhance light absorption efficiency. S. salina M8 has been shown to remove up to 96% of phosphorus and 66% of nitrogen after only a few days of cultivation (Trentin et al., 2019), while also accumulating proteins, polysaccharides, and polyhydroxybutyrate (PHB) - a key raw material for the bioplastics industry. In addition, S. salina M8 demonstrates robust growth in organic-rich environments and can accumulate a substantial amount of high-value compounds such as proteins, polysaccharides, and polyhydroxybutyrate (PHB) – a type of biodegradable biopolymer. The objective of this study is to investigate the growth performance of S. salina M8 in both the standard BG-11 medium and in sterilized and non-sterilized domestic wastewater under two mixing conditions: magnetic stirring and aeration. The experiments also explore the effects of different pH levels and C:N:P nutrient ratios. Moreover, the study evaluates the strain's wastewater treatment capability and biomass accumulation potential, aiming to develop practical applications in eco-friendly, cost-effective domestic wastewater treatment systems aligned with the principles of green technology and the circular economy.

2. MATERIALS AND METHODS

2.1. Materials and experimental equipment

2.1.1. Source of algal inoculum

The cyanobacterial strain S. salina M8 was obtained from the culture collection maintained and cultivated at the Biochemical Technology Laboratory, Institute of Chemistry, Vietnam Academy of Science and Technology, located at 18 Hoang Quoc Viet, Hanoi.

Prior to experiments, S. salina M8 was pre-cultivated in BG-11 medium (Table 1). Cultivation was carried out in 250 mL Erlenmeyer flasks with a working volume of 100 mL. The cultivation conditions were maintained at

Table 1. Composition of 1 liter of BG-11 medium

No.	Chemical Name	Formula	Concentration (g/L)
1	Sodium nitrate	$NaNO_3$	1,5
2	Dipotassium phosphate	K_2HPO_4	0,04
3	Magnesium sulfate heptahydrate	$MgSO_4{\cdot}7H_2O$	0,075
4	Calcium chloride dihydrate	$CaCl_2{\cdot}2H_2O$	0,036
5	Citric acid	$C_6H_8O_7$	0,006
6	Ferric ammonium citrate	$(NH_4)_5[Fe(C_6H_4O_7)_2]$	0,006
7	Disodium ethylene diaminete traacetate dihydrate	$Na_2EDTA{\cdot}2H_2O$	0,001
8	Sodium carbonate	Na_2CO_3	0,02
9	Trace metal mix (A5): H_3BO_3: 2,86g/L; $MnCl_2.4H_2O$: 1,81 g/L; $ZnSO_4.7H_2O$: 0,222g/L; $Na_2MO_4.2H_2O$: 0,39g/L; $CuSO_4.5H_2O$: 0,079g/L; $Co(NO_3)_2.6H_2O$: 0,049 g/L.		1 mL/L

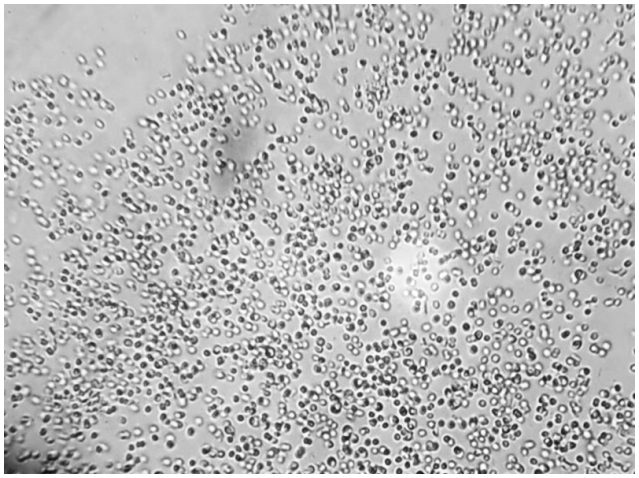

Figure 1. Morphology of cyanobacterium S. salina M8

Figure 2. Inoculum cultivation of S. salina M8 in a 250 mL Erlenmeyer flask

a temperature of approximately 25–27°C under illumination from two fluorescent lamps providing a light intensity of 4.500 Lux with a 24-hour continuous light cycle. Aeration was supplied at 0.1 vvm and magnetic stirring was set at 150 rpm using a 1.000 mL Duran flask containing 500 mL of BG-11 medium.

2.1.2. Domestic wastewater

Domestic wastewater samples were collected from two wastewater discharge drains in the residential area of Nguyen Chanh Street, Cau Giay District, Hanoi. The sampling locations were at the wastewater drainage points in alley 48 Nguyen Chanh, coordinates: X_1 = 2,325,145; Y_1 = 572,270, and alley 39 Tu Mo Street, coordinates: X_2 = 2,345,350; Y_2 = 580,543.

Table 2. Experimental instruments and equipment

No	Equipment	Model/ Specification	Manufacturer
1	Analytical balance	JF2204	Labex – UK
2	Centrifuge	Z206A	Hermle – Germany
3	Ultrasonic cleaner	Ultrasonic Cleaner	UK
4	Autoclave	LS-75LJ	Nanbei – China
5	Oven and incubator	—	Heraeus – Germany
6	Microscope	Optical microscope	OLYMPUS – Japan
7	UV-Vis spectrophotometer	U-2900/2910	Shimadzu – Japan
8	LED lighting	—	Rang Dong – Vietnam
9	Air pump	HP-400	Atman – China
10	Air flow regulator	—	Vietnam
11	Air filter head	0.22 µm	China
12	Silicone tubing	—	China
13	Glass culture flasks	1L, 2L, 3.5L, 5L	SIMAX – Germany
14	Measuring cylinders	25, 50, 100, 1000 mL	Germany
15	Volumetric flasks	1000 mL	Germany
16	Erlenmeyer flasks	50 mL, 250 mL	Germany

2.1.3. Laboratory Equipment

2.2. Experimental design

2.2.1. Investigation of the effects of artificial BG-11 medium and domestic wastewater on the growth of S. salina M8

The S. salina M8 cyanobacterial strain was maintained in 1.000 mL Duran flasks as described in Section 2,1 for 6–8 days to reach a cell density above 2,5 × 10^5 cells/mL (equivalent to OD_{750} = 2,5 Abs or 1,0 g/L). Subsequently, the S. salina M8 inoculum was transferred into 1.000 mL Duran flasks containing sterilized or non-sterilized BG-11 medium or domestic wastewater at an inoculation ratio of 20% (v/v). Cultivation was carried out under aeration conditions (0,1 vvm) and magnetic stirring (150 rpm), with the initial pH adjusted to 7, under a light intensity of 4.500 Lux for 8 days. Each experiment was conducted in triplicate. The bioreactors were equipped with three stainless steel (304 grade) ports (Φ6 mm) for aeration, sampling, and gas exhaust. The stainless steel aeration port was connected via silicone tubing to a flow meter linked to a Fujimac aeration pump.

2.2.2. Investigation of the effect of pH on the growth of S. salina M8 and the efficiency of domestic wastewater treatment.

The experiment was conducted in 1-liter Duran flasks at pH values of 5, 6, 7, 8 and 9, with pH adjusted

Figure 3. Experiment on culturing S. salina M8 in domestic wastewater

using NaOH 5M/H_2SO_4 5M. The initial inoculation ratio of S. salina M8 was set at 20% (v/v). Experimental conditions were maintained as follows: temperature of 25 - 27°C, aeration at 0,1 vvm, light intensity of 4.500 Lux under continuous illumination (24-hour light cycle), and aeration at 0,1 vvm. Each experiment was performed in triplicate. The growth rate and domestic wastewater treatment efficiency of S. salina M8 were monitored over 8 days. The water quality parameters analyzed after treatment included ammonium nitrogen ($N–NH_4^+$), total nitrogen (T-N), total phosphorus (T-P), phosphate (PO_4^{3-}) and chemical oxygen demand (COD).

2.2.3. Investigation of the effect of C:N:P ratios on the growth capability of S. salina M8 and its efficiency in treating domestic wastewater.

The experiment was conducted in 1-liter Duran flasks with C:N:P ratios of (100:10:1), (100:5:1), (100:10:0.5), and (100:15:1). Nutrient composition was adjusted by supplementing carbon sources (CO_2 or acetate), nitrogen ($NaNO_3$), and phosphorus (K_2HPO_4). The experimental conditions were as follows: temperature 25 - 27°C, aeration at 0,1 vvm, light intensity of 4500 Lux, continuous illumination (24 hours light), an initial inoculum ratio of S. salina M8 at 20% (v/v), and pH adjusted to 7. Each experiment was performed in triplicate. The growth rate and domestic wastewater treatment efficiency of S. salina M8 were monitored over 8 days. Water quality parameters analyzed in the treated wastewater included NH_4-N, total nitrogen (T-N), total phosphorus (T-P), PO_4^{3-} and COD.

2.3. Research methods

2.3.1. Sampling and laboratory analysis methods for wastewater

Wastewater samples were collected according to the Vietnamese standard TCVN 5999:1995 (ISO 5667/10:1992) on water quality – sampling – guidance on sampling of wastewater. Sample preservation was conducted following the national standard TCVN 6663-3:2016 (ISO 5667-3:2012) on water quality – sampling – Part 3: Preservation and handling of water samples. Wastewater was collected in 5–10 L plastic containers and transported to the Biochemical Technology Laboratory, Institute of Chemistry, Vietnam Academy of Science and Technology. Prior to experimentation, wastewater was pretreated by filtration through filter paper with pore sizes of 3–7 μm for 30 minutes to remove debris and suspended solids. Initial parameters including COD, NH_4^+, NO_3^-, PO_4^{3-}, total nitrogen (TN), and total phosphorus (TP) were measured immediately upon arrival at the laboratory. Final parameters were analyzed after 8 days of cultivation. Analytical methods used were as follows: NH_4 according to TCVN 6179-1:1996; NO_3 according to TCVN 6180:1996 and ISO 7890-3:1998 (E); phosphorus analysis according to TCVN 6202:2008 using the ammonium molybdate spectrophotometric method; COD according to TCVN 6491:1996. The initial quality parameters of the pretreated influent wastewater were: pH = 7,2 ± 0,2; COD = 325,6 ± 0,3 mg/L; NO_3^- = 3,2 ± 0,1 mg/L; NH_4^+ = 32,12 ± 0,42 mg/L; TN = 36,12 ± 0,52 mg/L; $P\text{-}PO_4^{3-}$ = 4,24 ± 0,15 mg/L; TP = 5,2 ± 0,2 mg/L; total suspended solids (TSS) = 1,21 ± 0,2 mg/L. The initial C:N:P ratio of the domestic wastewater was 63:7:1. The experiments were arranged using a completely randomized design with independent variables including pH and C:N:P ratio. The dependent variable was optical density (OD_{750}). Each experiment was repeated three times to ensure statistical reliability. Results are presented as mean values ± standard deviation. Raw data were processed using Microsoft Excel 2016. The removal efficiencies of COD, NH_4^+-N, total nitrogen (T-N), PO_4^{3-}P, and total phosphorus (T-P) were calculated using the formula:

$$H_i = (1 - \frac{C_i}{C_{0i}}) \times 100$$

Hi - the removal efficiency (%); C0i - the concentration of the parameter in the influent wastewater (mg/L); Ci - the concentration of the parameter in the treated wastewater (mg/L).

2.3.2. Method for evaluating the growth of S. salina M8

The growth of the cyanobacterium was monitored daily by measuring the optical density at a wavelength of 750 nm (OD_{750})

using a UV-Vis spectrophotometer (Shimadzu, Japan). Dry cell weight (DCW) was determined by collecting 10 mL of the cyanobacterial culture, filtering it through a 0,45 µm membrane, and drying it at 105°C for 24 hours to obtain the dried biomass. Biomass concentration (g/L) was calculated based on the dry weight of biomass obtained per liter of culture.The specific growth rate (µ, day^{1}) of the cyanobacterial strain was determined using the following equation (1):

$$\mu = \frac{\ln \frac{X_2}{X_1}}{t_2 - t_1} \quad (1)$$

X1 and X2 are the biomass concentrations (g/L) of Synechocystis salina M8 measured at cultivation times t1 and t2 (days), respectively.

3. RESULTS AND DISCUSSION

3.1. Analysis of domestic wastewater samples in the study area

The analysis results in Table 3 show that the filtered domestic wastewater, prior to treatment with Synechocystis salina M8, exhibited a neutral pH environment. The chemical oxygen demand (COD) concentration ranged around 325,6 ± 0,3 mg/L. The wastewater was rich in nitrogen, with a high concentration of ammonium nitrogen (NH_4^+-N) at approximately 32,12 ± 0,42 mg/L, while nitrate nitrogen (NO_3^--N) was present at a lower level of 3,2 ± 0,1 mg/L. Phosphate (PO_4^{3-}), the predominant form of phosphorus found in domestic wastewater, was recorded at a concentration of 4,24 ± 0,15 mg/L. The total nitrogen (T-N) and total phosphorus (T-P) contents were found to be 36,12 ± 0,52 mg/L and 5,2 ± 0,2 mg/L, respectively. Total suspended solids (TSS) after filtration were approximately 1,21 ± 0,2 mg/L, a level that does not hinder light penetration into the water. The pH of the domestic wastewater collected from the sewer was considered suitable for the cultivation of S. salina M8, aligning well with the optimal conditions of the standard BG-11 medium.

The research results indicate that the domestic wastewater used in this experiment contains essential nutrients such as carbon, nitrogen, and phosphorus compounds necessary for the growth of microalgae. These findings are consistent with those reported by Dang Thuan Tran and colleagues in 2021 (D.T. Tran et al., 2021). Therefore, in subsequent experiments, domestic wastewater was used as the cultivation medium for Synechocystis salina M8.

Table 3. Characteristics of domestic wastewater (after pre-treatment, before inoculation) (n=3)

No.	Parameter	Unit	Concentration	QCVN 14:2008/ BTNMT Column B
1	pH	-	7,3 ± 0,2	**5-9**
2	Temperatur	°C	25,67± 0,21	-
3	TSS	mg/L	1,4± 0,2	**100**
4	N-NO_3-	mg/L	3,2 ± 0,1	**50**
5	N-NH_4^+	mg/L	32,12 ± 0,42	**10**
6	Total Nitrogen	mg/L	36,12± 0,52	-
7	P-PO_4^{3-}	mg/L	4,24 ± 0,15	**10**
8	Total Phosphorus	mg/L	5,2 ± 0,2	-
9	COD	mg/L	325,6 ± 0,3	-

3.2. Effect of culture medium and mixing regime on the growth of S. salina M8

The culture medium is a key factor that directly affects the growth performance of S. salina M8. Figure 2 presents the specific growth rate and biomass productivity of S. salina M8 when cultured in standard BG-11 medium and in domestic wastewater, under both sterilized and non-sterilized conditions, using two mixing regimes: magnetic stirring and aeration. The results demonstrated that the type of culture medium whether standard BG-11 or domestic wastewater, sterilized or not - did not exhibit significant effects on the growth of S. salina M8. In contrast, the mixing regime had a substantial impact on both growth rate and biomass productivity. Specifically, under magnetic stirring at 150 rpm in sterilized BG-11, S. salina M8 reached a specific growth rate of (0,16 ± 0,01) day^{1} and biomass productivity of (0,72 ± 0,01) g/L (Figure 2A). In non-sterilized BG-11, the specific growth rate and biomass productivity increased slightly to (0,21 ± 0,01) day^{-1} and (0,78 ± 0,02) g/L, respectively (Figure 2B). When aeration was applied at a rate of 0.1 vvm, the specific growth rate and biomass productivity of S. salina M8 increased to (0,46 ± 0,02) day^{-1} and (1,12 ± 0,015) g/L in sterilized BG-11 medium (Figure 2A), and to (0,42 ± 0,02) day^{-1} and (1,34 ± 0,01) g/L in non-sterilized BG-11 medium (Figure 2B). Similarly, under magnetic stirring conditions, S. salina M8 cultured in sterilized and non-sterilized domestic wastewater achieved specific growth rates and biomass productivities of (0,28 ± 0,01) day^{-1} and (0,85 ± 0,02) g/L (Figure 2C), and (0,42 ± 0,03) day^{-1} and (0,92 ± 0,02) g/L (Figure 2D), respectively. Under aeration, S. salina M8 showed even higher performance in domestic wastewater: in the sterilized condition, the specific growth rate and biomass productivity reached (0,54 ±

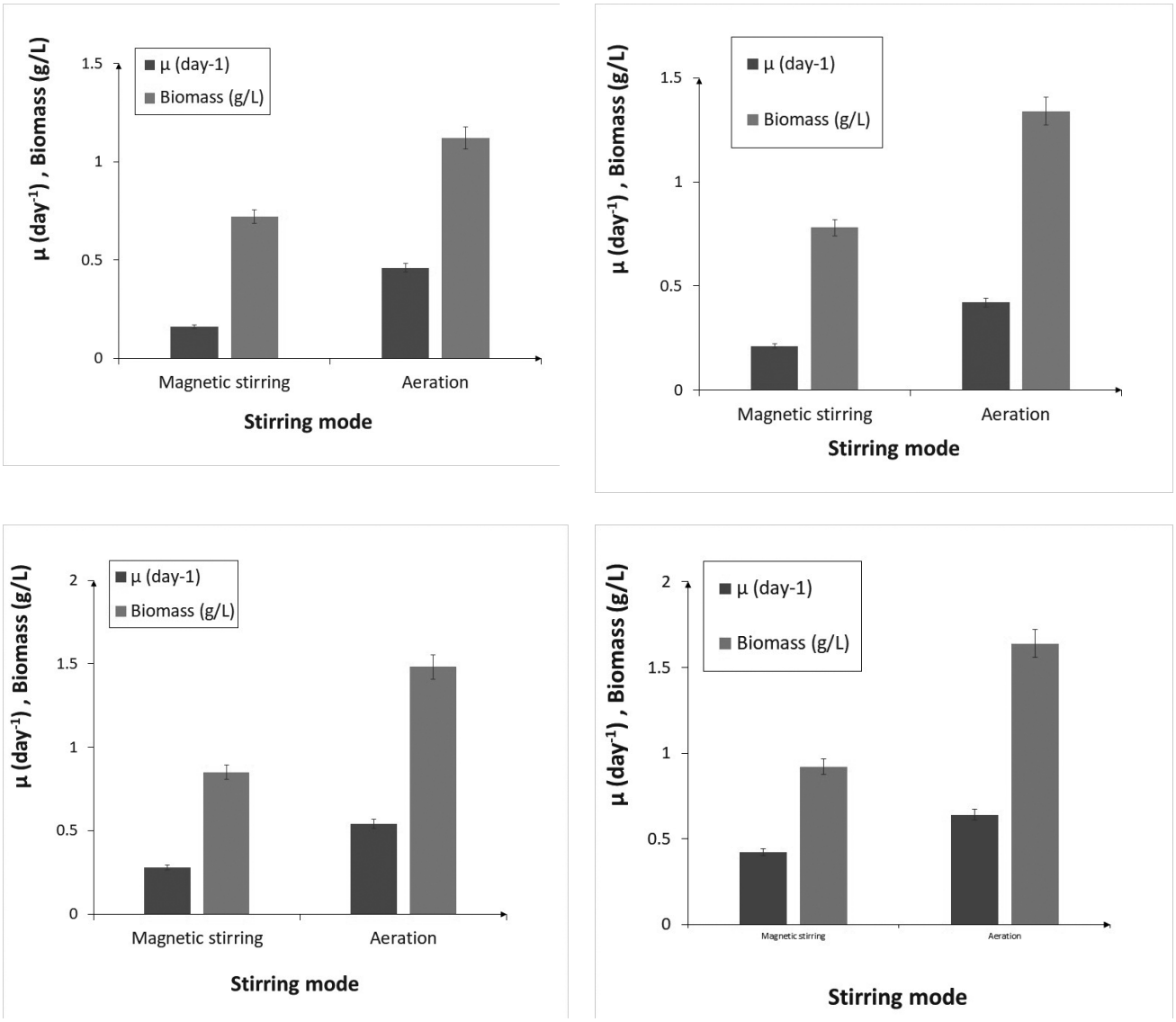

Figure 4. Specific Growth Rate and Biomass of S. salina M8 in Sterilized BG-11 (A), Non-sterilized BG-11 (B), Sterilized Wastewater (C), and Raw Wastewater (D) under Two Mixing Regimes: Magnetic Stirring and Aeration. Data were collected on the 8th day of cultivation under light intensity of 4.500 Lux and temperature of 27°C

0,015) day^{-1} and (1,48 ± 0,02) g/L (Figure 2C), and in the non-sterilized condition, these values increased to (0,64 ± 0,03) day $^{-1}$ and (1,64 ± 0,02) g/L (Figure 2D). These results indicate that aeration enhanced the specific growth rate of S. salina M8 by nearly threefold, and biomass productivity by approximately twofold, compared to magnetic stirring, regardless of the culture medium. Importantly, S. salina M8 exhibited robust growth not only in BG-11 but also in untreated domestic wastewater. This finding has significant implications for the practical application of S. salina M8 in wastewater treatment, as it eliminates the need for sterilization and thereby offers substantial energy savings. Moreover, aeration significantly enhanced the specific growth rate and biomass productivity of S. salina M8. This effect can be attributed to the fact that aeration not only improves nutrient exchange between the culture medium and cyanobacterial cells but also accelerates the diffusion and dissolution of CO_2 from the atmosphere. This process increases the availability of bicarbonate ions (HCO_3^-), which serve as an essential inorganic carbon source for photosynthesis. In contrast, magnetic stirring merely maintains cell suspension within the culture medium without significantly enhancing CO_2 transfer from the air [C.V.T. Do, et al., 2021]. Based on these results, all subsequent experiments were conducted using non-sterilized domestic wastewater under aeration conditions, in order to maximize the growth and biomass accumulation of S. salina M8 under conditions that closely simulate real-world applications.

Table 4. Effect of pH on the growth of S. salina M8

Day		pH=5	pH=6	pH=7	pH=8	pH=9
Biomass (g/L)	0	0,68 ± 0,06	0,76± 0,06	0,87± 0,01	0,89± 0,08	0,61± 0,25
	2	0,78± 0,02	0,87± 0,03	0,98± 0,02	1,01± 0,01	0,88± 0,04
	4	0,81± 0,12	0,94± 0,06	1,29± 0,03	1,23± 0,06	1,02± 0,02
	6	0,97± 0,01	1,03± 0,05	1,46± 0,04	1,36± 0,01	1,06± 0,13
	8	1,15± 0,03	1,25± 0,02	1,59± 0,02	1,49± 0,05	0,98± 0,09
Growth rate μ (day^{-1})	0	0,26±0,02	0,24±0,03	0,22±0,05	0,18±0,04	0,16±0,12
	2	0,30±0,01	0,34±0,02	0,38±0,02	0,39±0,01	0,34±0,45
	4	0,31±0,01	0,36±0,32	0,50±0,14	0,45±0,02	0,48±0,13
	6	0,38±0,02	0,40±0,31	0,57±0,06	0,53±0,3	0,41±0,02
	8	0,45±0,01	0,48±0,05	0,62±0,01	0,58±0,01	0,38±0,02

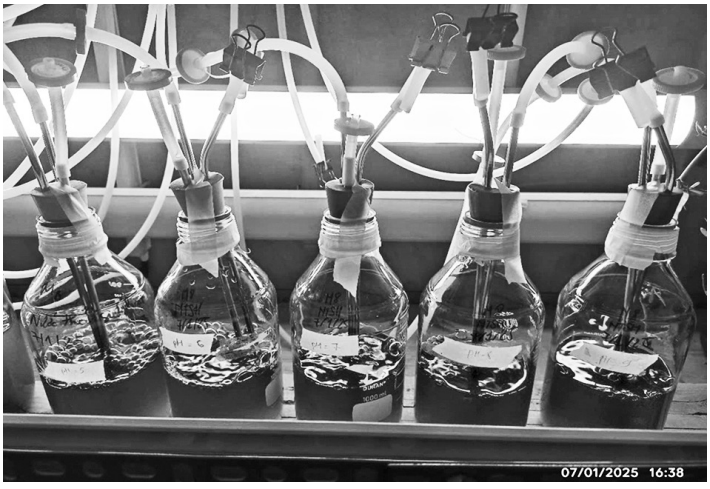

Figure 5. Experiment on culturing S. salina M8 in domestic wastewater at different pH values

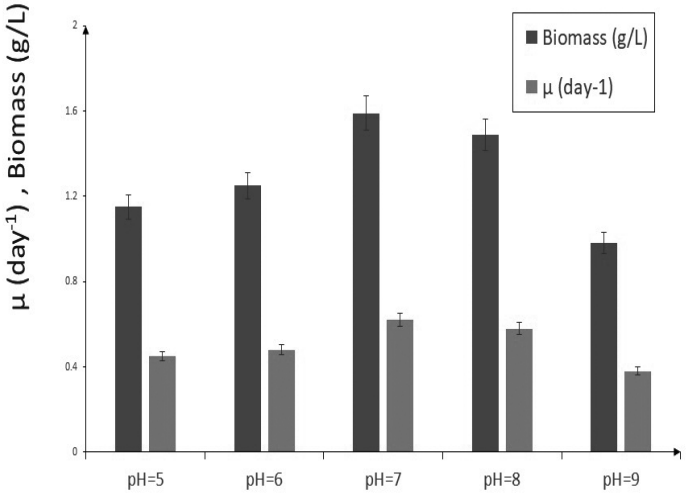

Figure 6. Specific growth rate and biomass of S. salina M8 in unsterilized domestic wastewater at different pH levels. Data were recorded on day 8 of the cultivation process under a light intensity of 4,500 Lux and a temperature of 27°C

3.3. Effect of pH on the growth of synechocystis salina M8 and the pollutant removal efficiency in domestic wastewater.

3.3.1. Effect of pH on the growth capacity of synechocystis salina M8

pH is an important factor that influences the photosynthetic process of microalgae by regulating the availability of dissolved carbon sources such as bicarbonate and CO_2 in the culture medium. In the cultivation of Synechocystis salina M8, pH directly affects nutrient uptake and growth rate, thereby playing a critical role in the efficiency of the cultivation process. The experiment was conducted in 1-liter Duran flasks at different pH values: 5, 6, 7, 8, and 9. The initial inoculum volume of S. salina M8 was set at 20% (v/v). Cultivation was carried out in non-sterilized domestic wastewater under aeration conditions (0.1 vvm), with continuous illumination at a light intensity of 4.500 Lux.

The results indicated that at neutral pH (pH = 7), S. salina M8 achieved the highest biomass of (1,59 ± 0,02) g/L on day 8, accompanied by the highest specific growth rate of (0,62 ± 0,01) day^{-1}. At pH 8, the biomass reached (1,49 ± 0,05) g/L, with a specific growth rate of (0,58 ± 0,01) day^{-1}. These results demonstrate that S. salina M8 grows best under neutral to slightly alkaline conditions. In contrast, under acidic conditions (pH = 5), biomass reached only (1,15 ± 0,03) g/L on day 8, and the specific growth rate remained the lowest among all treatments at (0,45 ± 0,01) day^{-1}. Similarly, under strongly alkaline conditions (pH = 9), although biomass increased over time, it only reached (0,98 ± 0,09) g/L on day 8,

Table 5. Pollutant concentrations in domestic wastewater before and after treatment with S. salina M8 at pH = 7

pH = 7.0	$N\text{-}NH_4^+$	TN	PO_4^{3-}	TP	COD
Before treatment (mg/L)	32,12 ± 0,42	36,12± 0,52	4,24 ± 0,15	5,2 ± 0,2	325,6 ± 0,3
After treatment (mg/L)	6,31 ± 0,22	7,23± 0,25	1,08± 0,05	1,27± 0,15	86,3± 0,5
Removal efficiency (%)	80,35± 0,33	79,98± 0,41	74,53± 0,51	75,58± 0,28	73,5± 0,12

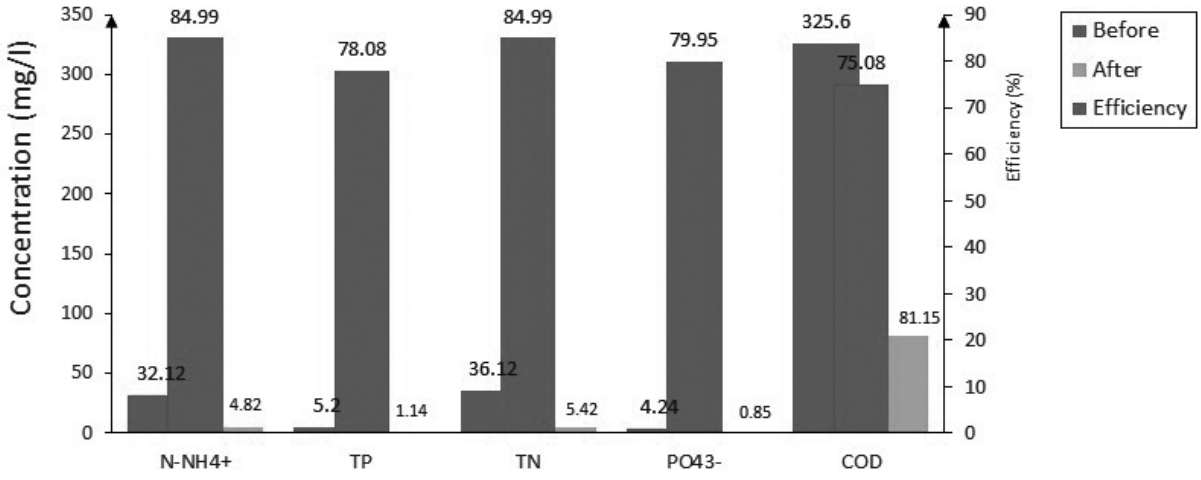

Figure 7. Concentrations of pollutants in domestic wastewater before and after treatment at pH 7, with 20% inoculum ratio, aeration at 0.1 vvm, and light intensity of 4,500 Lux

and the specific growth rate decreased from (0,48 ± 0,13) day $^{-1}$ to (0,38 ± 0,02) day $^{-1}$. These findings are consistent with those reported by Nguyen, B.T., et al., (2016), who observed optimal growth at pH 7.5, with a corresponding biomass of 0,355 ± 0,018 g/L and a growth rate of 0,136 ± 0,08 day $^{-1}$. (Nguyen, B.T., et al., 2016).

The pH of the culture medium for S. salina M8 tended to increase over time in all experimental conditions. This phenomenon can be explained by the photosynthetic process of the microalgae, in which CO_2 from the atmosphere dissolves into the medium and reacts as follows: $CO_2 + H_2O \rightarrow HCO_3^- + H^+$.

3.3.2. Evaluation of domestic wastewater treatment efficiency by S. salina M8 at pH = 7

The analysis results of pollutant concentrations and treatment efficiency by S. salina M8 at pH = 7 in domestic wastewater are presented in Table 5 and Figure 7.

The results showed that after 8 days of cultivation at pH 7, the concentrations of pollutants in the domestic wastewater significantly decreased. Specifically, the concentration of ammonium ($N\text{-}NH_4^+$) dropped sharply from (32,12 ± 0,42) mg/L to (6,31 ± 0,22) mg/L, corresponding to a removal efficiency of 80.35 ± 0.33%. This was the highest removal rate among the monitored parameters, reflecting the effective ammonium assimilation or biotransformation capability of S. salina M8. In addition, total nitrogen (T-N) was reduced from (36,12 ± 0,52) mg/L to (7,23 ± 0,25) mg/L, achieving a removal efficiency of 79,98 ± 0,41%, indicating a strong nitrogen uptake process, potentially through intracellular accumulation or biological nitrogen fixation. Similarly, $P\text{-}PO_4^{3-}$ and total phosphorus (T-P) exhibited removal efficiencies of 74,53 ± 0,51% and 75,58 ± 0,28%, respectively, demonstrating a high phosphorus removal capacity. For organic matter, COD decreased from (325,6 ± 0,3) mg/L to (86,3 ± 0,5) mg/L, corresponding to a removal efficiency of 73,5 ± 0,12%. Overall, S. salina M8 showed high simultaneous removal efficiencies of nitrogen, phosphorus, and COD under neutral pH conditions, indicating physiological stability and strong adaptability to domestic wastewater environments. These findings are consistent with the study by Doan Thi Oanh et al. (2020) on Chlorella vulgaris CNK, which achieved a 54% removal of $N\text{-}NH_4^+$ at pH 7 after 12 days of cultivation although lower than the efficiency observed for S. salina M8 in this study, highlighting the superior potential of the M8 strain for practical wastewater treatment application. (Doan Thi Oanh and partners, 2020).

3.4. Effects of C:N:P Ratio on growth and pollutant removal efficiency by S. salina M8

The experimental results investigating the effects of nutrient ratio C:N:P on the growth and pollutant removal efficiency in domestic wastewater by S. salina M8 after 8 days under the conditions of 20% (v/v) inoculum ratio, aeration at 0,1 vvm, and pH 7 are presented in Table 6 and Figure 9.

Table 6. Effect of C:N:P ratio on the biomass of S. salina

Day \ Ratio C:N:P		100:10:1	100:5:1	100:10:0.5	100:15:1
Biomass (g/L)	0	0,83±0,02	0,72±0,23	0,86±0,16	0,75±0,15
	2	1,16±,0,04	0,89±0,16	0,62±0,25	0,62±0,2
	4	1,47±0,01	1,17±0,24	1,17±0,45	0,95±0,23
	6	1,52±0,01	1,28±0,15	1,29±0,12	1,27±0,15
	8	1,64±0,05	1,46±0,02	1,26±0,02	1,12±0,01
Growth rate μ (ngày^{-1})	0	0,32±0,01	0,23±0,02	0,33±0,03	0,29±0,02
	2	0,45±0,02	0,34±0,02	0,24±0,15	0,24±0,01
	4	0,57±0,01	0,45±0,01	0,45±0,03	0,37±0,01
	6	0,59±0,02	0,50±0,01	0,50±0,25	0,49±0,02
	8	0,64±0,01	0,57±0,02	0,49±0,01	0,43±0,01

Figure 8. S. salina M8 experiments at different C:N:P ratios

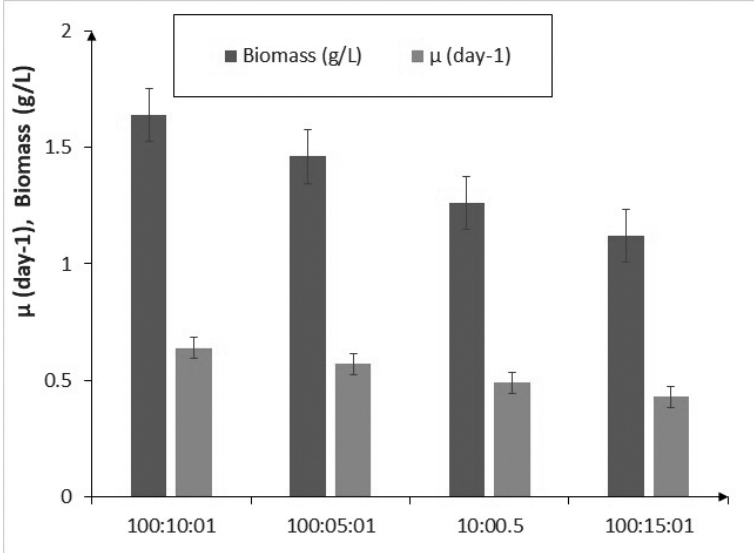

Figure 9. Specific growth rate and biomass of S. salina M8 in non-sterile wastewater at different C:N:P ratios. Data were measured on day 8 of the cultivation process under conditions of 4.500 Lux light intensity, 27°C temperature, pH 7, and aeration at 0,1 vvm

The results indicate a clear growth trend of S. salina M8 over the cultivation period across all experimental treatments. Notably, the treatment with a C:N:P ratio of 100:10:1 exhibited the highest biomass accumulation, increasing from (0,83±0,02) g/L at time point T0 to (1,64±0,05) g/L at T8. The specific growth rate (μ) also rose correspondingly from (0,32±0,01) day $^{-1}$ to (0,64±0,015) day $^{-1}$. This reflects the nutritional balance provided by this ratio, which optimally supports photosynthesis, carbon metabolism, and biomass synthesis in S. salina M8. In contrast, the C:N:P ratios of 100:5:1 and 100:10:0.5, while still supporting growth, resulted in lower biomass yields, reaching (1,46±0,02) g/L and (1,26±0,02) g/L at T8, respectively. The specific growth rates in these treatments were moderate, with μ = 0,57±0,02 and 0,49±0,01 day $^{-1}$. Nitrogen (100:5:1) or phosphorus (100:10:0.5) limitation likely constrained the synthesis of nucleic acids, proteins, ATP, and essential coenzymes required for growth. Interestingly, in the 100:15:1 treatment, despite a higher nitrogen content, biomass peaked at (1,27±0,15) g/L at T6 before slightly declining to (1,12±0,01) g/L at T8. This phenomenon is attributed to nitrogen excess, which not only fails to enhance growth but may exert inhibitory effects due to nutrient imbalance, possibly linked to ammonium accumulation or osmotic stress. These findings confirm that a balanced C:N:P nutrient ratio is a critical determinant of S. salina M8 growth performance. Phosphorus deficiency impairs nucleic acid and ATP synthesis, while nitrogen excess may alter intracellular pH, disrupt ion exchange, or lead to the accumulation of endogenous

Table 7. Pollutant concentrations in wastewater treated by S. salina M8 at different C:N:P ratios

Ratio C:N:P	NH_4^+ (mg/L)	TN (mg/L)	P-PO_4^{3-} (mg/L)	TP (mg/L)	COD (mg/L)
T1 (100:10:1)	4,82± 0,11	5,42± 0,14	0,85± 0,10	1,14± 0,56	81,15± 0,16
T2 (100:5:1)	9,64± 0,21	10,84± 0,43	1,19± 0,20	1,66± 0,47	97,38± 0,23
T3 (100:10:0.5)	11,24± 0,24	12,64± 0,16	1,70± 0,16	2,18± 0,31	113,61± 0,85
T4 (100:15:1)	8,03± 0,31	9,03± 0,62	1,06± 0,08	1,56± 0,12	90,89± 0,16

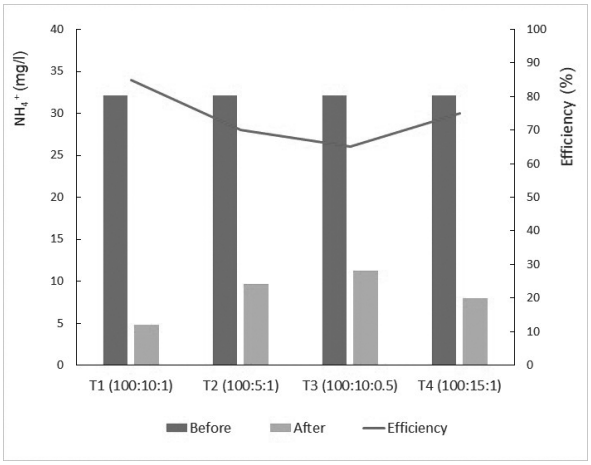

Figure 10. Ammonium nitrogen (N-NH4⁺) concentration in wastewater before and after treatment at different C:N:P ratios

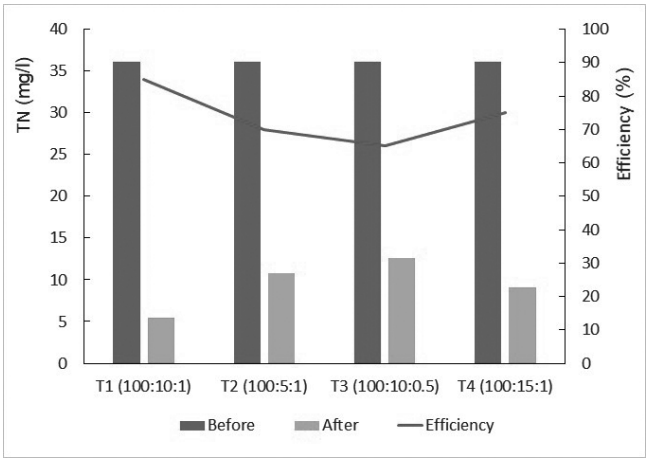

Figure 11. Total Nitrogen (T-N) concentration in wastewater before and after treatment at different C:N:P ratios

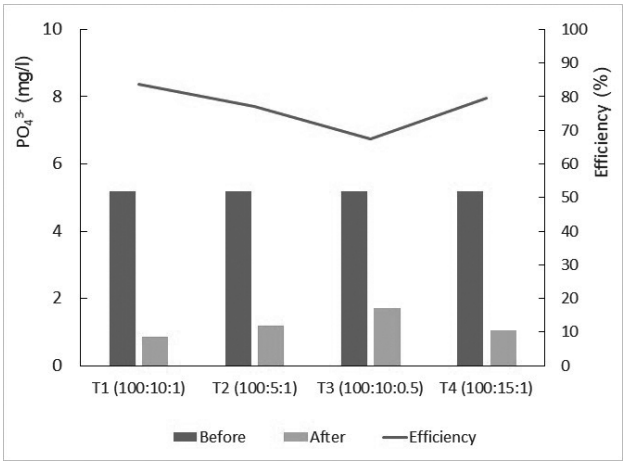

Figure 12. PO_4^{3-} concentration in wastewater before and after treatment at different C:N:P ratios

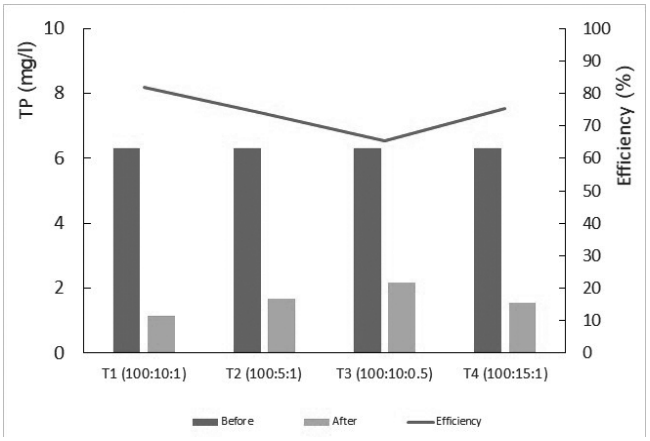

Figure 13. Total Phosphorus (T-P) concentration in wastewater before and after treatment at different C:N:P ratios

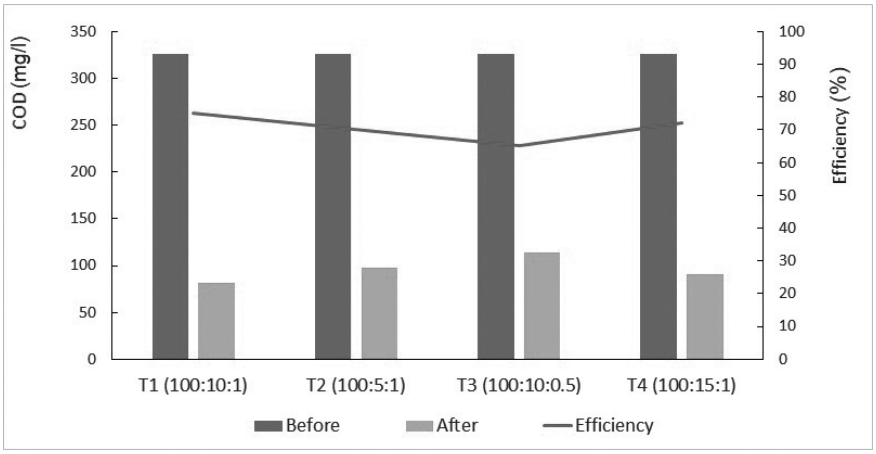

Figure 14. COD concentration in wastewater before and after treatment at different C:N:P ratios

Table 8. Pollutant removal efficiencies by S. salina M8 at C:N:P = 100:10:1

Ratio C:N:P = 100:10:1	$N\text{-}NH_4^+$	T-N	PO_4^{3-}	T-P	COD
Before (mg/L)	32,12 ± 0,42	36,12± 0,52	4,24 ± 0,15	5,2 ± 0,2	325,6 ± 0,3
After (mg/L)	4,82± 0,11	5,42± 0,14	0,85± 0,10	1,14± 0,56	81,15± 0,16
Efficiency (%)	84,99± 0,54	84,99± 0,62	79,95± 0,21	78,08± 0,2	75,08± 1,20

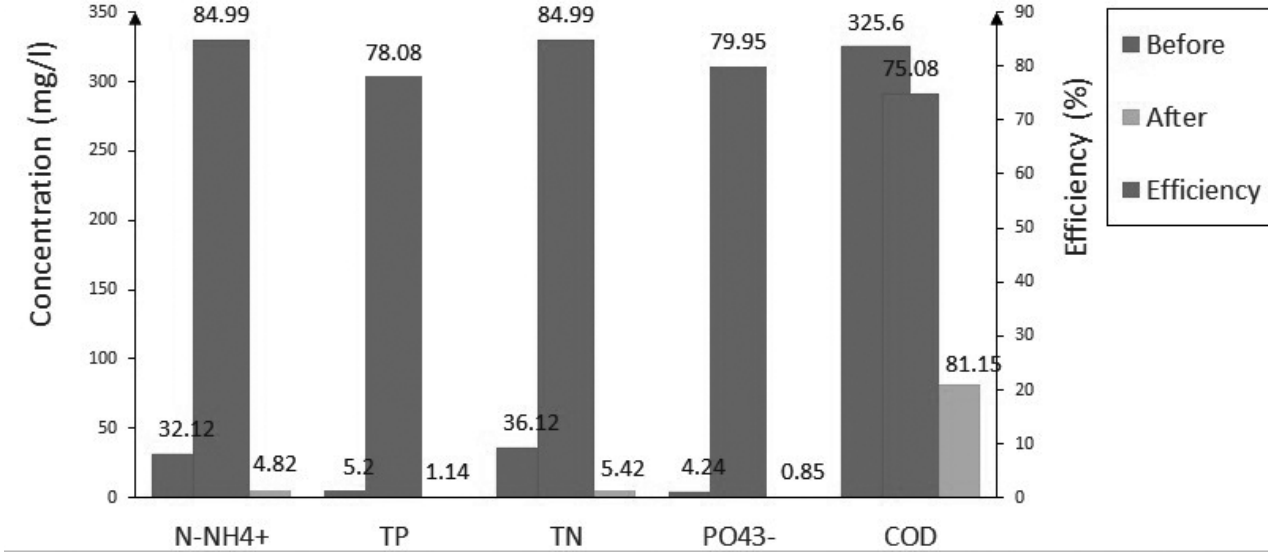

Figure 15. Pollutant concentrations before and after treatment at C:N:P = 100:10:1

inhibitors. These results are consistent with the study by Gonçalves et al. (2016), in which S. salina cultured in wastewater supplemented with 7% CO_2 achieved the highest average biomass productivity of (0,173 ± 0,009) g/L/day after seven days of cultivation. Although the nutrient conditions differ, the trend of optimal growth in CO_2 - rich and nutritionally balanced environments parallels the 100:10:1 treatment in this study.

The results showed that after 8 days of cultivation at a C:N:P ratio of 100:10:1, the concentrations of $N\text{-}NH_4^+$, total nitrogen (TN), total phosphorus (TP), phosphate (PO_4^{3-}), and chemical oxygen demand (COD) in the wastewater decreased significantly, indicating the high overall treatment efficiency of S. salina M8. Specifically, both $N\text{-}NH_4^+$ and TN reached a removal efficiency of 84,99 ± 0,54%, demonstrating the strain's capability to effectively eliminate both inorganic and organic nitrogen forms. PO_4^{3-} and TP were also removed with efficiencies of 79,95 ± 0,21% and 78,08 ± 0,20%, respectively. COD levels decreased from 325,6 ± 0,3 mg/L to 81,15 ± 0,16 mg/L, corresponding to a removal rate of 75,08 ± 1,20%, thereby meeting the discharge standard specified in QCVN 14:2008/BTNMT (column B). These findings are consistent with those reported by Nattawut Krasaesueb et al. (2019), where the strain Synechocystis sp. cultivated in ammonium and phosphate-rich wastewater achieved nutrient removal efficiencies of 96.99% for phosphate, 80,10% for nitrate, 67,90% for nitrite, and 98,07% for ammonium (N. Krasaesueb et al., 2019). Similarly, the results align with the study by Li et al. (2024), in which Chlorella vulgaris was co-cultivated with aerobic bacteria for the treatment of mixed wastewater at a C:N ratio of 15:1. This system achieved the highest removal efficiencies for COD, NH_4^+-N, and TP, which were 60,89 ± 1,80%, 43,38 ± 1,00%, and 68,55 ± 0,59%, respectively (Li, R et al., 2024).

Thus, the combination of a balanced C:N:P ratio of 100:10:1 with optimized culture conditions including 20% (v/v) algal inoculum, continuous aeration at 0,1 vvm, light intensity of 4.500 Lux, pH 7, and temperature at 27°C created a biologically favorable environment that enabled S. salina M8 to grow effectively, accumulate biomass, and simultaneously remove nitrogen-, phosphorus-containing compounds, and organic matter from wastewater. The ability to achieve removal efficiencies greater than 80% for most parameters under non-sterile wastewater conditions further confirms the potential of this strain for application in sustainable biological wastewater treatment systems.

4. CONCLUSION

This study conducted a preliminary investigation into the growth and domestic wastewater treatment efficiency of S. salina M8 in various nutrient environments, including BG-11 medium and domestic wastewater. The results demonstrated that S. salina M8 grew well in both BG-11 and domestic wastewater under aerated conditions. Biomass productivity reached 1,34 g/L in non-sterile BG-11 medium and 1,64 g/L in domestic wastewater under identical conditions: pH 7, light intensity of 4.500 Lux, a C:N:P nutrient ratio of 100:10:1, and continuous aeration at 0,1 vvm over 8 days. Pollutant removal efficiencies were as follows: N-NH_4^+ (85%), total nitrogen (85%), phosphate (PO_4^{3-}) (79,95%), total phosphorus (78,08%), and chemical oxygen demand (COD) (75,08%). The treated wastewater met the discharge standards specified in QCVN 14:2008/BTNMT, column B. Although the findings were obtained at laboratory scale, further research and practical implementation of S. salina M8 in small-scale wastewater treatment systems such as those for households, residential areas, schools, or public institutions are needed. Moreover, future studies should focus on reducing operational costs, and recovering and reusing S. salina M8 biomass. This includes the extraction and purification of high-value products such as polyhydroxyalkanoates (PHA), biofertilizers, biofuel feedstock, or phycocyanin, thereby contributing to the development of a sustainable green and circular economy■

REFERENCES

1. Anh, T. T. D. (2022, July 28). Current status of environmental pollution in Vietnam https://doi.org/10.31219/osf.io/9hwca].

2. Urgently solve environmental problems in Hanoi (n.d.). Nhandan.Vn. Retrieved April 17, 2025, from http://secical.nhandan.vn/cap-bach-giai-bai-toan-moi-truong-o-Ha-Noi.

3. Dang Thuan Tran, Thi Cam Van Do, Quang Trung Nguyen, Truong Giang Le. Simultaneous remival of pollutants and high value biomaterials production by Chlorella variabilis TH03 from domestic wastewater Clean Technologies and Environmental Policy. http://dio.org/10/1007/s10098-020-01810-5.

4. N. Krasaesueb, A. Incharoensakdi, W. Khetkorn, Utilization of shrimp wastewater for poly-β-hydroxybutyrate production by Synechocystis sp. PCC 6803 strain ΔSphU cultivated in photobioreactor, Biotechnology Reports, 23 (2019) e00345.

5. Santos, C. A., & Costa, J. A. V. (2020). Cultivation of microalgae in the Brazilian scenario: A review. Clean Technologies and Environmental Policy, 22(6), 1325–1338. https://doi.org/10.1007/s10098-020-01810-5.

6. Voulvoulis, N., Arpon, K. D., & Giakoumis, T. (2017). The EU Water Framework Directive: From great expectations to problems with implementation. Science of the Total Environment, 575, 358–366. https://doi.org/10.1016/j.scitotenv.2016.09.228.

7. M. I. B. Pereira et al., "Mixotrophic cultivation of Spirulina platensis in dairy wastewater: Effects on the production of biomass, biochemical composition and antioxidant capacity," PLOS ONE, vol. 14, no. 10, p. e0224110, 2019, doi: 10.1371/journal.pone.0224110.

8. Trentin, G., Bertucco, A., & Sforza, E. (2019). Mixotrophy in Synechocystis sp. for the treatment of wastewater with high nutrient content: effect of CO2 and light. Bioprocess and Biosystems Engineering, 1-9. https://doi.org/10.1007/s00449-019-02162-1.

9. D.T. Tran, T.C. Van Do, Q.T. Nguyen, T.G. Le, Simultaneous removal of pollutants and high value biomaterials production by Chlorella variabilis TH03 from domestic wastewater, Clean Technologies and Environmental Policy, 23 (2021) 3-17

10. C.V.T. Do, N.T.T. Nguyen, T.D. Tran, M.H.T. Pham, T.Y.T. Pham, Capability of carbon fixation in bicarbonate-based and carbon dioxide-based systems by Scenedesmus acuminatus TH04, Biochemical Engineering Journal, 166 (2021) 107858.

11. Nguyen, B.T., & Rittmann, B. (2016). Effects of inorganic carbon and pH on growth kinetics of Synechocystis sp. PCC 6803. Algal Research, 19, 363–369.

12. Doan Thi Oanh, Duong Thi Thuy, Nguyen Thanh Trung (2020), Evaluation of the effect of initial pH on the growth and treatment of nitrogen compounds in domestic wastewater by microalgae Chlorella vulgaris CNK.

13. Gonçalves A.L., Rodrigues C.M., Pires J.C., Simões M., The effect of increasing CO¬2 concentrations on its capture, biomass production and wastewater bioremediation by microalgae and cyanobacteria, Algal research, 14, 127-136, 2016.

14. N. Krasaesueb, A. Incharoensakdi, W. Khetkorn, Utilization of shrimp wastewater for poly-β-hydroxybutyrate production by Synechocystis sp. PCC 6803 strain ΔSphU cultivated in photobioreactor, Biotechnology Reports, 23 (2019) e00345.

15. Li, R., Guo, D., Li, T., Zhao, J., & Pan, J. (2025). Effect of C:N ratio on treatment of mixed industrial-domestic wastewater by microalgae-bacteria consortium. Biomass Conversion and Biorefinery, 15, 10829–10837. https://doi.org/10.1007/s13399-024-05871-0.