GRADUATE UNIVERSITY SCIENCE AND TECHNOLOGY

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RESEARCH ON THE PRODUCTION OF (PHB)-RICH BIOMASS FROM DOMESTIC WASTEWATER USING CYANOBACTERIA

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The dissertation will be defended before the Academy-level Doctoral Dissertation Evaluation Council, convened at the Graduate University of Science and Technology – Vietnam Academy of Science and Technology at … hours … minutes, on the … day of …, 2026.

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ABSTRACT

1. The urgency of the dissertation

Domestic wastewater pollution is a major environmental challenge in Vietnam due to rapid urbanization and the low rate of wastewater treatment meeting standards. Domestic wastewater contains high levels of organic matter and nutrients (nitrogen, phosphorus), which, if not effectively treated, will cause eutrophication of water sources and degrade environmental quality. Traditional biological treatment technologies, while effective and widely applied, have limitations in terms of energy consumption, secondary sludge generation, and lack of added economic value, thus failing to meet the requirements of a circular economy and sustainable development. Alongside wastewater issues, plastic pollution from fossil fuels is increasing, creating an urgent need for biodegradable materials such as polyhydroxybutyrate (PHB). However, the current production cost of PHB remains high due to its dependence on pure raw materials and energy-intensive processes. In this context, cyanobacteria, with their photosynthetic capabilities, utilizing CO_2 as a carbon source, efficiently assimilating nitrogen and phosphorus in wastewater, and accumulating PHB in their biomass, create added economic value and are considered potential candidates for integrated wastewater treatment and bioplastic production technologies. Research into selecting suitable cyanobacteria strains, optimizing physicochemical conditions affecting growth and PHB accumulation, establishing the relationship between biomass and PHB content, and evaluating the effectiveness of domestic wastewater treatment are of urgent scientific and practical significance, contributing to the development of environmental technologies oriented towards a circular and sustainable economy..

2. Research Objectives

- Selecting cyanobacteria strains that exhibit rapid growth, good adaptability to phototrophic culture media, and high biomass yield and PHB accumulation content.

- The application of selected cyanobacteria strains for treating domestic wastewater combined with the production of PHB-rich biomass in a 30L outdoor tubular photobioreactor; simultaneously recovering and purifying PHB from the biomass for analysis of the basic properties of the PHB material, thereby confirming its potential to partially replace traditional plastics, contributing to the development of green biotechnology in line with the circular economy.

3. Main Research Contents

- The selection of cyanobacteria strains should demonstrate rapid growth rates, good adaptability to phototrophic culture media, and high biomass yield and PHB accumulation content.

- This study aims to optimize the physicochemical conditions affecting the growth and accumulation of PHB in the selected cyanobacteria strain. Physicochemical factors include pH, temperature, light intensity, aeration, carbon concentration, C:N:P ratio, etc., to improve biomass yield and PHB accumulation.

- This study investigates the influence of culture environmental conditions on the growth ability, PHB accumulation, and wastewater treatment efficiency of selected cyanobacteria strains under laboratory conditions.

- This experimental application of cyanobacteria strains in a 30 L outdoor tubular photobioreactor aims to integrate domestic wastewater treatment and PHB-rich biomass production, while also evaluating the system's efficiency and stability.

- The project involves recovering cyanobacteria biomass, extracting and purifying PHB, and studying the properties of the biomaterial to clarify the potential applications of PHB biodegradable plastics and gradually replace some traditional plastics.

CHAPTER 1. LITERATURE REVIEW

1.1. Overview of Cyanobacteria

1.1.1. Definition, Biological Characteristics, and Classification of Cyanobacteria

This section provides an overview of cyanobacteria, focusing on their growth and developmental mechanisms, morphological forms, and key biological characteristics, as well as their distribution across different aquatic and terrestrial ecosystems.

1.1.2. Factors affecting the growth and development of cyanobacteria

The growth of cyanobacteria is primarily governed by light intensity, temperature, pH, and nutrient availability, particularly nitrogen and phosphorus under tropical conditions. Photosynthetic efficiency and biomass accumulation are enhanced by CO_2 supplementation and by maintaining the pH within the range of 7–10. Certain halotolerant or alkaliphilic strains can be cultivated under non-sterile conditions, thereby reducing the risk of contamination. In addition, a two-stage batch cultivation strategy is commonly applied to maximize biomass production in the first stage and to induce PHB accumulation in the second stage, facilitating large-scale applications.

1.1.3. The potential applications of cyanobacteria

S. salina M8 is a salt-tolerant photosynthetic cyanobacterium capable of flexible growth in various environments, effectively treating nutrients and metals in wastewater, and accumulating valuable compounds such as PHB, lipids, and biopigments. Therefore, this strain shows great potential for integrated wastewater treatment systems that produce biomaterials and bioenergy in a circular economy. The secondary compounds recovered from the biomass have high biological activity, opening up prospects for widespread application in industrial, agricultural, medical, and pharmaceutical/cosmetic fields.

1.2. Cyanobacteria in domestic wastewater treatment

1.2.1. Current Status and Consequences of Pollution Caused by Domestic Wastewater

The proportion of domestic wastewater that is adequately treated worldwide, including in Vietnam, remains relatively low, leading to serious environmental pollution and an increased incidence of waterborne diseases. This situation underscores the urgent need to develop more efficient, sustainable, and environmentally friendly wastewater treatment technologies to meet current and future challenges.

1.2.2. Method of treating domestic wastewater using cyanobacteri

Traditional domestic wastewater treatment technologies primarily focus on pollutant removal but consume a lot of energy, generate sludge, and offer virtually no economic value. In contrast, wastewater treatment using microalgae and cyanobacteria allows for the direct conversion of pollutants (C, N, P) into economically valuable biomass containing proteins, lipids, biopigments, and biodegradable polymers such as PHB. This approach not only ensures effective environmental treatment but also creates a renewable source of biological materials, contributing to the economic efficiency of wastewater treatment systems in line with the circular economy.

1.3. Cyanobacteria in Bioplastic Production

1.3.1. Biodegradable Bioplastic Polyhydroxybutyrate (PHB)

Polyhydroxybutyrate (PHB) is a polymer belonging to the polyhydroxyalkanoate (PHA) family, a class of biopolyesters. PHB has attracted considerable attention due to its ability to be biosynthesized and extracted via biological pathways, while also being naturally biodegradable under environmental conditions.

1.3.2. Cyanobacteria in PHA/PHB Bioplastic Production

a. Cyanobacteria Spirulina sp.2 and Spirulina sp.4

Spirulina sp.2 and sp.4 are filamentous cyanobacteria strains capable of rapid growth, efficient assimilation of N and P in wastewater, and the potential to accumulate PHB under nutrient stress conditions. However, the PHB accumulation capacity of *Spirulina* is strongly dependent on the strain and culture conditions and requires specific experimental evaluation.

b. Cyanobacteria S. salina M8

S. salina M8 is a halotolerant cyanobacterium that grows well in coastal environments with salinities of 1–5% and is capable of accumulating intracellular storage compounds, particularly PHB. Owing to its photoautotrophic metabolism, which directly utilizes CO_2, light, and inorganic nutrients, this strain is well suited for PHB production from wastewater within a circular economy framework. Under conditions of carbon excess combined with nitrogen or phosphorus limitation, carbon flux through acetyl-CoA is preferentially directed toward the PHB biosynthetic pathway for carbon and energy storage. The PHB accumulation capacity of *S. salina* M8 is governed by both genetic characteristics and cultivation conditions, with nutrient balance (C, N, P and the C:N:P ratio), light intensity, temperature and pH playing decisive roles. Therefore, the integrated optimization of these parameters is essential to enhance PHB yield in combined wastewater treatment–bioplastic production systems.

1.4. Application of Statistical Methods for Screening and Optimization of Factors Affecting Cyanobacterial Biomass and PHB Production

The Plackett–Burman (PB) design was employed to identify the chemical, physical, and biological factors that significantly influence cyanobacterial growth and PHB accumulation. The significant variables identified during the screening stage were subsequently subjected to optimization using Response Surface Methodology (RSM), which enables the simultaneous evaluation of individual effects and interactions among factors, thereby determining the optimal cultivation conditions. In RSM,

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the relationship between independent variables and response variables (biomass concentration or PHB content) is commonly described by a generalized second-order polynomial regression equation as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum \beta_{ij} X_i X_j + \varepsilon \qquad (1.1)$$

Where: Y is the response variable measured from experiments designed according to the Response Surface Methodology; β represents the regression coefficients of the model; X_i and X_j are the uncoded levels of the ith and jth factors, respectively; k denotes the number of investigated factors; and ε is the random experimental error.

1.5. Studies on the application of cyanobacteria in bioplastic production from wastewater worldwide and in Vietnam

1.5.1. Worldwide studies on the application of cyanobacteria in bioplastic production from wastewater

In recent years, cyanobacteria have been extensively investigated for integrated wastewater treatment and polyhydroxybutyrate (PHB) bioplastic production. Numerous studies have demonstrated that genera such as *Synechocystis, Nostoc, Anabaena*, and *Spirulina* are capable of efficiently removing nitrogen and phosphorus from wastewater while accumulating PHB under nutrient-limited or carbon-excess conditions. The PHB content typically ranges from 6–20% of cell dry weight and can be further enhanced through optimization of cultivation conditions or the application of genetic engineering approaches. Pilot-scale and outdoor systems have confirmed the practical feasibility of the integrated wastewater treatment–bioplastic production model. Nevertheless, PHB accumulation efficiency and downstream recovery costs remain key challenges that require further investigation.

1.5.2. Studies on the application of cyanobacteria in bioplastic production from wastewater in Vietnam

The integration of wastewater treatment with PHB bioplastic production using cyanobacteria has recently attracted increasing attention

in Vietnam due to its combined environmental and economic benefits. However, domestic studies have mainly focused on heterotrophic bacteria, whereas research on cyanobacteria remains limited, particularly with respect to evaluations under real wastewater conditions and the development of complete technological processes. Therefore, further systematic studies are required to fully exploit the potential of cyanobacteria and to advance integrated wastewater treatment–PHB production systems in line with circular economy principles and sustainable development goals.

1.5.3. Rationale for further research

Domestic and international studies have shown a trade-off relationship between growth and PHB accumulation in cyanobacteria, but research aimed at optimizing both processes simultaneously remains limited. In Vietnam, research on cyanobacteria mainly focuses on isolation and morphological description, lacking integrated assessments of growth, environmental adaptability, and PHB content, especially in wastewater conditions. Furthermore, studies combining wastewater treatment and PHB recovery from cyanobacteria are scarce, lacking systematic data and technical solutions suitable for domestic conditions. These gaps highlight the need for research to select cyanobacteria strains that are well-adapted, efficiently accumulate PHB, and have potential applications in the treatment of domestic wastewater combined with bioplastic production.

CHAPTER 2. MATERIALS AND METHODS

2.1. Materials, chemicals, and equipment

2.1.1. Research materials

- Cyanobacteria: *S. salina* M8, Spirulina sp. 2, Spirulina sp. 4 were obtained from a collection of strains maintained and cultured at the Institute of Energy and Environmental Science and Technology.

- PHB biodegradable plastic material was extracted from cyanobacteria.

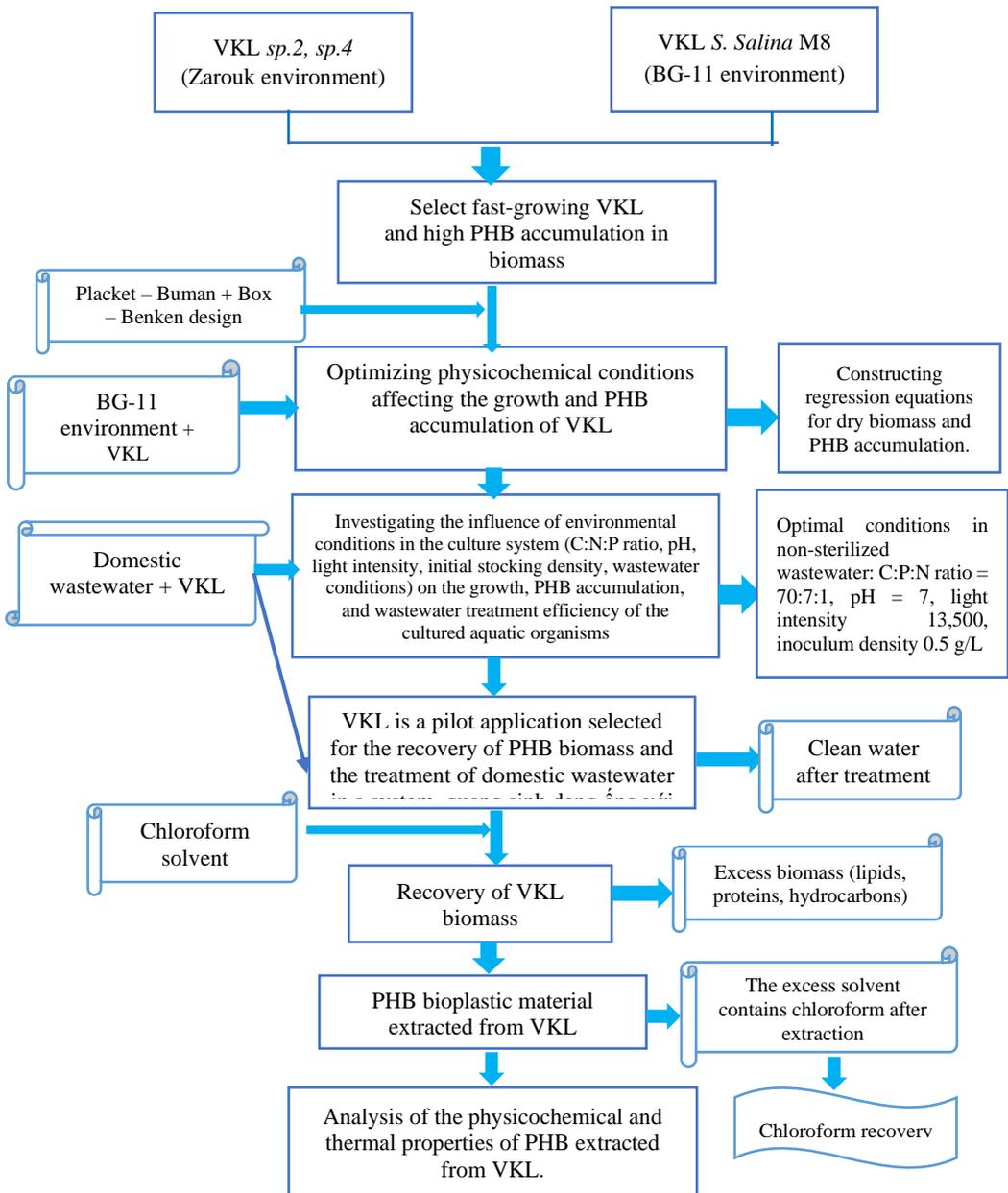- Domestic wastewater was collected in Thanh Xuan ward, Hanoi.

2.1.2. Chemicals and equipment

All chemicals, instruments, and laboratory equipment used in this study were standard analytical-grade materials and facilities available at the laboratories of the Institute of Chemistry and the Institute of Energy and Environmental Science and Technology.

2.2. Research methods

2.2.1. Experimental research method

A detailed flow chart of the experimental procedure is shown in Figure 2.1.

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┌─────────────────────────┐        ┌─────────────────────────┐
│   VKL sp.2, sp.4        │        │   VKL S. Salina M8      │
│ (Zarouk environment)    │        │ (BG-11 environment)     │
└─────────────────────────┘        └─────────────────────────┘
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VKL sp.2, sp.4 (Zarouk environment)

VKL S. Salina M8 (BG-11 environment)

Select fast-growing VKL and high PHB accumulation in biomass

Placket – Buman + Box – Benken design

BG-11 environment + VKL

Optimizing physicochemical conditions affecting the growth and PHB accumulation of VKL

Constructing regression equations for dry biomass and PHB accumulation.

Domestic wastewater + VKL

Investigating the influence of environmental conditions in the culture system (C:N:P ratio, pH, light intensity, initial stocking density, wastewater conditions) on the growth, PHB accumulation, and wastewater treatment efficiency of the cultured aquatic organisms

Optimal conditions in non-sterilized wastewater: C:P:N ratio = 70:7:1, pH = 7, light intensity 13,500, inoculum density 0.5 g/L

VKL is a pilot application selected for the recovery of PHB biomass and the treatment of domestic wastewater in a system, quang sinh dạng Ép ngoài

Clean water after treatment

Chloroform solvent

Recovery of VKL biomass

Excess biomass (lipids, proteins, hydrocarbons)

PHB bioplastic material extracted from VKL

The excess solvent contains chloroform after extraction

Analysis of the physicochemical and thermal properties of PHB extracted from VKL.

Chloroform recovery

Figure 2.1. Schematic diagram of biomass recovery and bioplastic PHB production integrated with domestic wastewater treatment using *S. salina* M8

2.2.2. Analytical methods

- Analysis of wastewater characteristics and evaluation of treatment performance.

- Methods for biomass harvesting and assessment of cyanobacterial growth.

- Methods for PHB extraction, quantification, and characterization.

2.2.3. Data processing methods

- Experimental data were processed using Excel and Minitab 18 software (Minitab, LLC, Pennsylvania, USA).

- Response surface analysis (RSM) was performed to determine the relationship between independent variables and output responses through a nonlinear regression model. The importance of the variables was assessed using the t-Student test.

- The Plackett–Burman and Box–Behnken experiments were performed with a single replication, while the validation experiments were conducted with two replications. Results are presented as mean ± standard deviation (Mean ± SD).

- Differences between conditions were analyzed using one-way ANOVA, and the differences were compared via p-values with $p < 0.05$, which were considered statistically significant.

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. Results of selecting fast-growing cyanobacterial strains with high PHB accumulation in biomass

The results indicated that *S. salina* M8 exhibited superior growth performance in the standard culture medium, achieving a dry biomass concentration of 1.986 g/L. This value was markedly higher than those obtained for *Spirulina* sp.2 (1.591 g/L) and *Spirulina* sp.4 (1.357 g/L) under the same cultivation period of 8 days. In terms of PHB accumulation, *S. salina* M8 reached 11.2%, approximately twice the PHB contents observed in *Spirulina* sp.2 (4.5%) and *Spirulina* sp.4 (5.6%). These results demonstrate that *S. salina* M8 outperformed the two *Spirulina* strains with respect to both biomass production and PHB accumulation, and was therefore selected as the most promising strain for subsequent experiments.
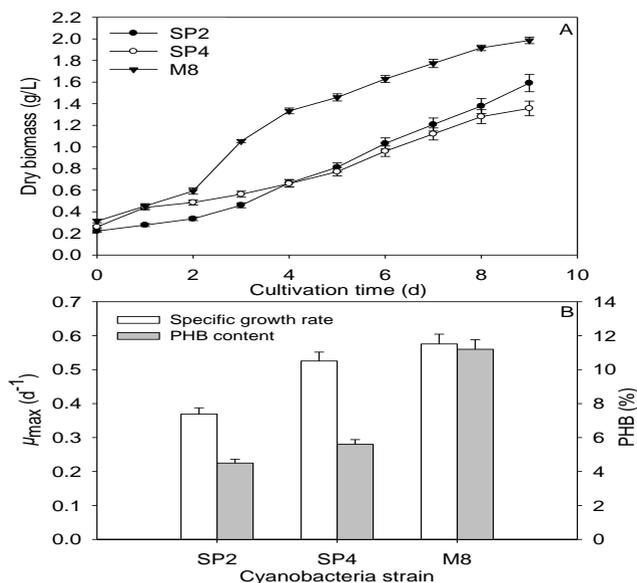
Figure 3.1. Biomass growth (A), specific growth rate, and PHB content (B) in the biomass of the three cyanobacterial strains

3.2. Results of screening for physicochemical factors significantly affecting biomass growth and PHB accumulation by *S. salina* M8

The effects of eight physicochemical factors on dry biomass concentration ($Y_{biomass}$, g/L) and PHB content (Y_{PHB}, %) are presented in Table 3.2.

Bảng 3.1. Coded variable matrix according to the Plackett–Burman design for the investigation of biomass and PHB production by *S. salina* M8 (n = 2)

No.	Variable level								$Y_{biomass}$ (g/L)	Y_{PHB} (%)
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**		
1	-1	1	1	1	-1	1	1	-1	0.95	8.56
2	0	0	0	0	0	0	0	0	1.38	15.50
3	1	-1	1	1	-1	1	-1	-1	1.53	11.50
4	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1.40**	**15.49**
5	**-1**	**1**	**-1**	**-1**	**-1**	**1**	**1**	**1**	**1.05**	**17.60**
6	1	1	-1	1	1	-1	1	-1	1.68	10.40
7	-1	1	1	-1	1	-1	-1	-1	1.01	13.60
8	-1	-1	-1	-1	-1	-1	-1	-1	0.23	5.60
9	**1**	**-1**	**1**	**-1**	**-1**	**-1**	**1**	**1**	**1.70**	**9.50**
10	0	0	0	0	0	0	0	0	1.39	15.70
11	-1	-1	-1	1	1	1	-1	1	0.44	5.89
12	**1**	**1**	**-1**	**1**	**-1**	**-1**	**-1**	**1**	**1.62**	**17.36**
13	**1**	**1**	**1**	**-1**	**1**	**1**	**-1**	**1**	**1.73**	**16.83**
14	-1	-1	1	1	1	-1	1	1	0.84	6.26

| 15	1	-1	-1	-1	1	1	1	-1	0.93	7.73
16	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1.38**	**15.44**
17	0	0	0	0	0	0	0	0	1.41	15.26

3.2.1. Results of screening for physicochemical factors that significantly affect biomass growth by S. salina M8

The results show that light (A), pH (B), and temperature (C) within the surveyed range have a clear and statistically significant effect on the biomass increase of *S. salina* M8.
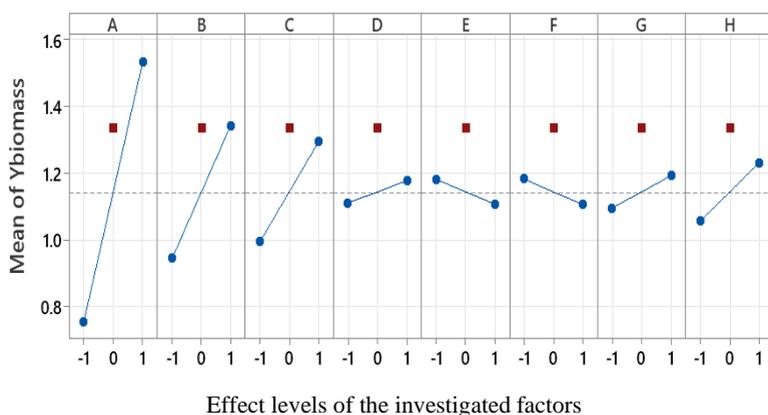
Figure 3.2. Effects of physicochemical factors on biomass growth of *S. salina M8*

3.2.2. Results of screening for physicochemical factors significantly affecting PHB accumulation by S. salina M8
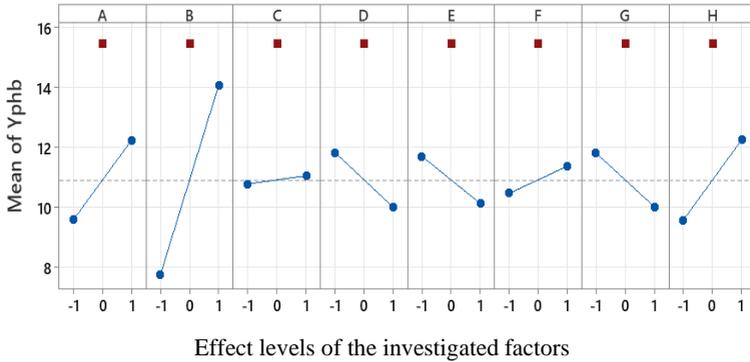
Figure 3.3. Effects of physicochemical factors on PHB content accumulated in the biomass of *S. salina* M8

The analysis results showed that pH was the only factor with a statistically significant influence on the accumulation of PHB in the biomass of *S. salina* M8 within the surveyed range.

3.3. Results on biomass optimization and PHB accumulation by *S. salina* M8

3.3.1. Optimizing dry biomass accumulation by S. salina M8

The regression equation describing biomass productivity as a function of three independent variables—light intensity, initial pH, and cultivation temperature - is presented in Equation (3.1):

$Y_{biomass} = 2.5567 + 0.1262A + 0.65B + 0.1537C - 0.5171A \times A - 0.5846B \times B$ $- 0.4971C \times C$ (3.1)

3.3.2. Optimizing PHB accumulation by S. salina M

$Y_{PHB} = 19.620 + 0.935A + 4.498B + 1.468C - 0.8921A \times A - 4.277B \times B - 1.397C \times C - 0.885A \times C$ (3.2)

The optimal conditions are light intensity of 9,800 Lux, pH = 8.0, temperature 32°C, dry biomass reaching 2.73 g/L, and PHB content above 21.4% **(Figure 3.5).**

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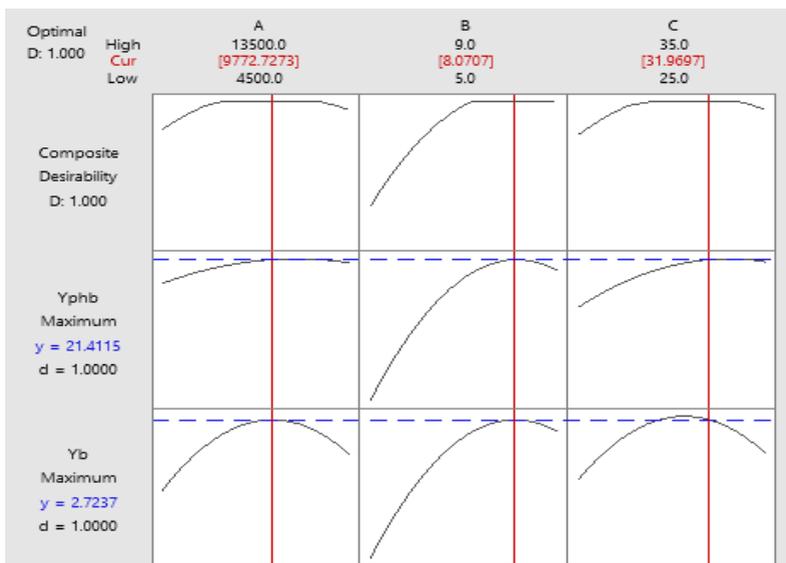
Figure 3.4. Graph of simultaneous optimization results of dry biomass and PHB content produced by *S. salina* M8

3.3.3. Validation of the optimal model and evaluation of the reliability of the quadratic regression models for dry biomass and PHB content

The results summarized in Table 3.2 show that the deviations between the predicted values obtained from the regression models and the experimentally measured values of dry biomass and PHB content of *S. salina* M8 were all below 5%. This indicates that both quadratic regression models ($Y_{biomass}$ and Y_{PHB}) exhibit high predictive accuracy and reliability in estimating dry biomass production and PHB accumulation by *S. salina* M8.

Bảng 3.2. Comparison between model-predicted and experimentally measured values of biomass and PHB content for *S. salina* M8 in the validation experiments

No.	Light intensity (Lux)	pH	Temperature (ºC)	Biomass ($Y_{biomass}$ g L^{-1})			(Y_{PHB}, %)		
				Predicted data	Measured data	Error (SD) (%)	Predicted data	Measured data	Error (SD) (%)
1	9,000	7	30	2.57	2.61±0.04	1.56	19.62	20.02±1.34	2.04
2	13,000	9	35	2.07	1.99±0.07	-3.86	20.23	20.98±0.86	3.71
3	10,000	8	30	2.74	2.71±0.11	-1.09	21.00	20.85±0.98	-0.71
4	10,000	8.5	30	2.73	281±0.09	2.93	20.81	21.07±1.22	1.25
5	10,000	8.5	35	2.35	2.42±0.05	2.98	21.19	20.98±0.78	-0.99

3.4. Effects Results on the influence of environmental conditions on the growth, PHB accumulation, and wastewater treatment efficiency of *S. salina* M8

3.4.1. Results on the effect of C:N:P ratio

The results showed that a C:N:P ratio of 70:10:1 was determined to be the optimal nutritional condition for the growth, PHB accumulation, and pollutant treatment efficiency of *S. salina* M8 in domestic wastewater, with a biomass concentration of 3.59 g/L; PHB accumulation content of 44.6%; and treatment efficiency of N-NH$_4^+$, T-N, PO$_4^{3-}$ exceeding 83%, COD reaching 98%, and T-P reaching 78%.
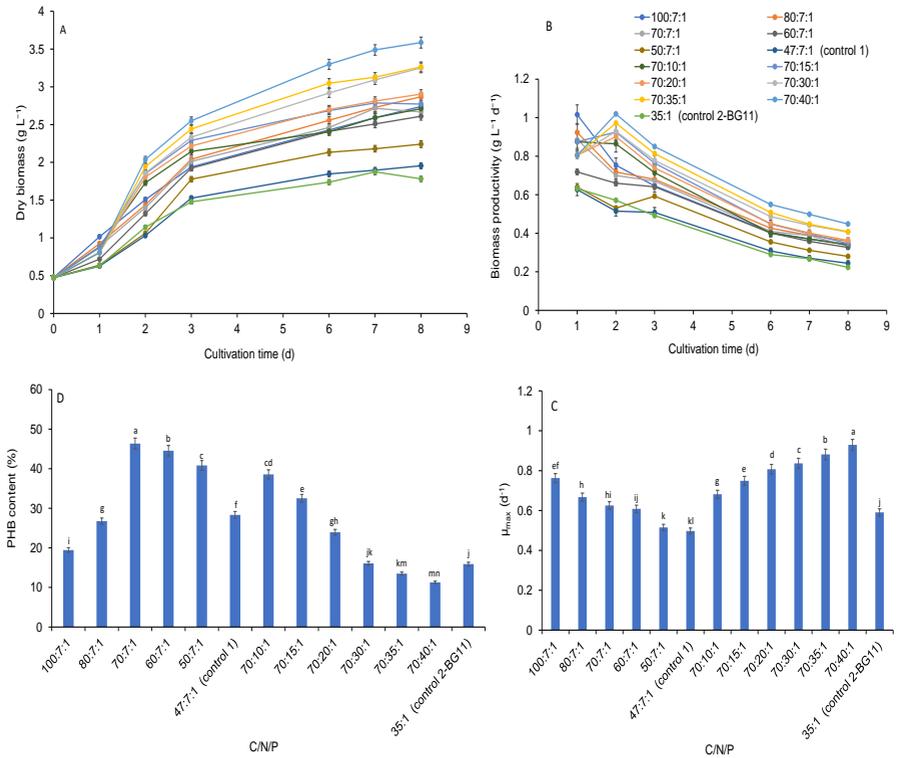
Figure 3.5. Growth of *S. salina* M8 in wastewater with different C/N/P ratios

3.4.2. Results regarding the effect of pH

The data in (Figure 3.15-A) shows that pH = 7 is the suitable environment for the growth and accumulation of PHB by *S. salina* M8, with the highest biomass (2.6 – 2.7 g/L) at pH = 7 – 9; the highest PHB content was at pH = 8 and 9 (corresponding to PHB = 46–51%), with an average level at pH = 7 (PHB = 44%). The pollutant treatment efficiency at pH = 7 reached over 80%, and COD reached 98.1%.

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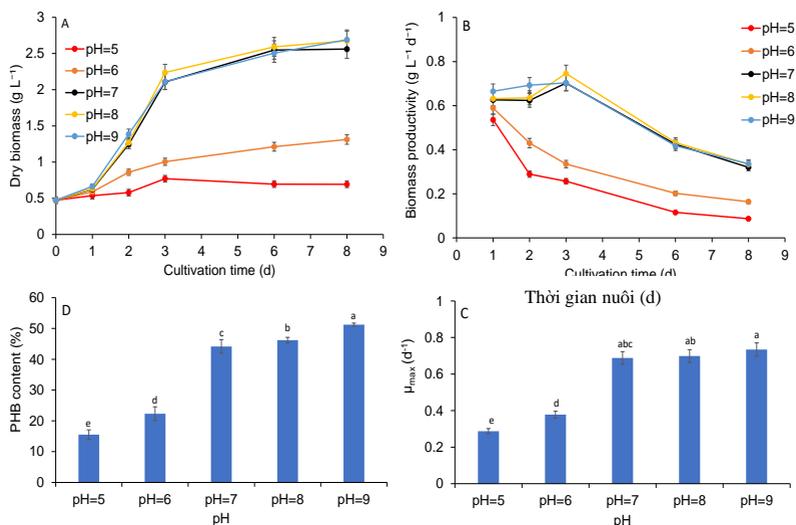
Figure 3.6. Growth of *S. salina* M8 in wastewater with different pH levels

3.4.3. Results regarding the effect of light intensity

The highest biomass was achieved at a light intensity of 13,500 Lux (3.1 g/L), with PHB content reaching 48%, while the pollutant treatment efficiency was over 82%, and COD reached 98.3%.
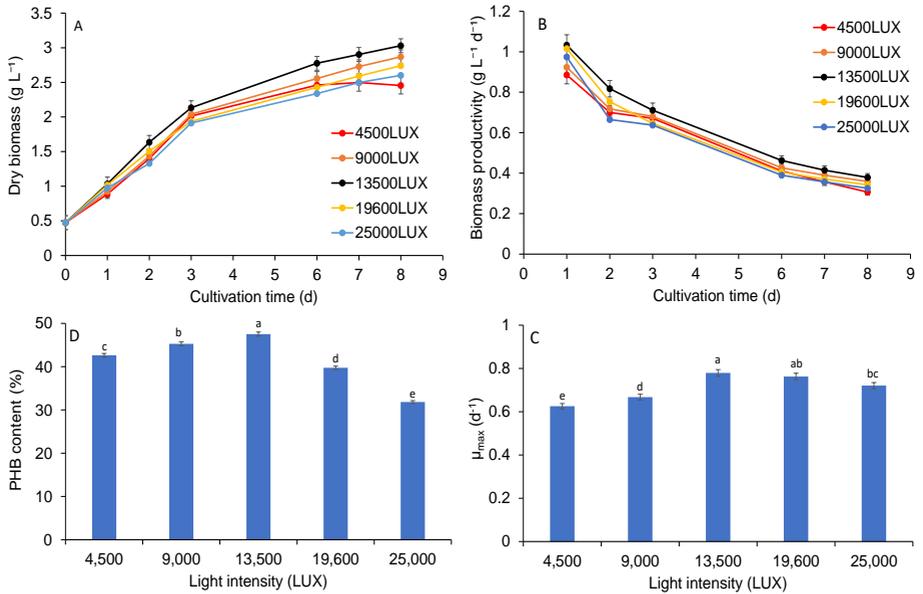
Figure 3.7. Growth of *S. salina* M8 in wastewater under different light
conditions

3.4.4. Results on the effect of initial S. salina M8 inoculum ratio on the growth of S. salina M8 in wastewater

An initial biomass concentration of 0.3 - 0.5 g/L resulted in the best
growth, high PHB accumulation, and stability. Therefore, an initial
inoculum concentration of 0.5 g biomass/L was chosen for subsequent
experiments.

3.4.5. Results on the growth potential of S. salina M8 in sterile and non-sterilized wastewater

S. salina M8 grew and developed well in non-sterilized
wastewater. Dry biomass in sterilized and non-sterilized wastewater
reached 2.8 and 3.0 g/L, respectively, while PHB content reached 43% in
sterilized wastewater and 40% in non-sterilized wastewater (Figure 3.8).
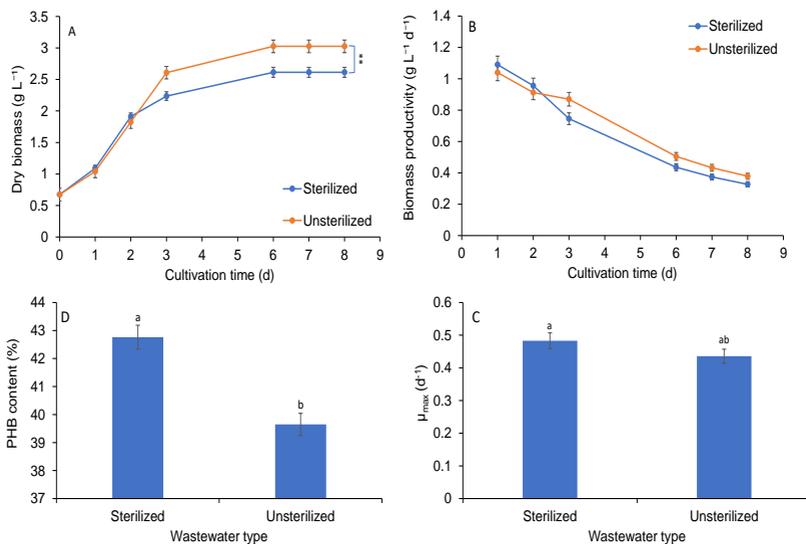
Figure 3.8. Growth of *S. salina* M8 in sterilized and non-sterilized wastewaters

The results showed that *S. salina* M8 grew well in non-sterilized domestic wastewater, achieving a biomass of 3.0 g/L, PHB 40%, and COD, N, and P treatment efficiency of over 80%, meeting QCVN 14:2025/BTNMT (column B). The study confirms the potential application of *S. salina* M8 in wastewater treatment combined with biomass recovery and PHB bioplastic production.

3.5. Pilot Experimental production of biomass and PHB from *S. salina* M8 cultured in domestic wastewater using a 30 L reactor syste

The results showed that the dry biomass reached its maximum of approximately 3.4 g/L on the 17th day before stabilization; biomass yield peaked at approximately 0.65 g/L/d (day 2) and then gradually decreased. The content increased from 10% dry weight (DCW) in the initial exponential phase to a maximum of 50% (DCW) on the 10th day, then gradually decreased to 17% (DCW) on the 20th day (Figure 3.9-D); the treatment efficiency of pollutants in wastewater reached over 90%.

Panel A: y = -0.0081x² + 0.2988x + 0.5347, R² = 0.9732

Panel B: y = 0.0015x² - 0.0519x + 0.6432, R² = 0.9202

Panel D: y = -0.3128x² + 6.3355x + 10.976, R² = 0.8569
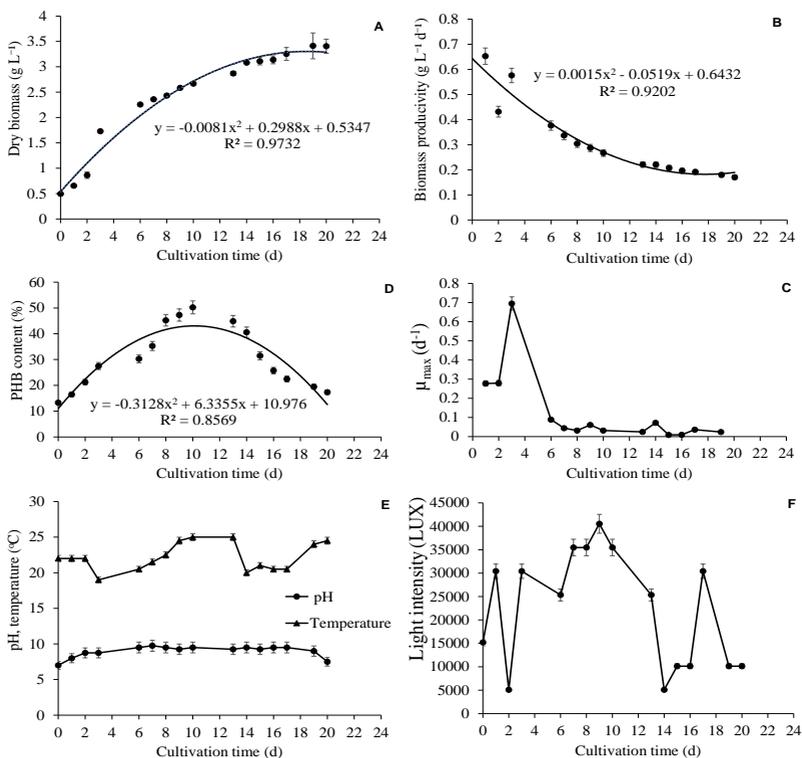
Figure 3.9. Growth performance and PHB accumulation of *S. salina* M8 cultivated in domestic wastewater using a 30 L vertical tubular photobioreactor

3.6. Results of the study on the extraction and characterization of PHB bioplastic material from *S. salina* M8 biomass

3.6.1. Results on the influence of extraction factors on PHB recovery efficiency

The optimal PHB recovery efficiency reached 27.65%, corresponding to the PHB content extracted above. The PHB recovery efficiency reached 92% when using 5 mL of solvent ($\approx$ 10 mL/g biomass), extraction temperature (T = 80°C), and extraction time (textraction = 60 minutes).

Table 3.2. Comparative summary of PHB content produced by selected *Synechocystis* strains

No.	Strain/system	Chế độ nuôi	PHB content (%)	Reference
1	*Synechococcus* sp. PCC 7942	Mixotrophic, acetate, nitrogen limitation	25,6	[93]
2	*Synechocystis* PCC 6803	Photoautotrophic, nitrogen limitation	26	[168]
3	*Synechocystis* PCC 6803	Heterotrophic, aceate	35	[168]
4	*Synechocystis* PCC 6803	Heterotrophic, acetate, fructose, phosphorus limitation	38	[121, 169]
5	*S. salina* M8	Mixotrophic, acetate, phosphorus limitation	27 – 31	This study

3.6.2. Characteristics of PHB bioplastic material extracted from S. salina M8 biomass

3.6.2.1. Material and molecular structure characteristics of PHB bioplastic

SEM images show that PHB has a porous structure with irregular micro-meso pores, which increases the surface area but can make it brittle and limit its mechanical properties.
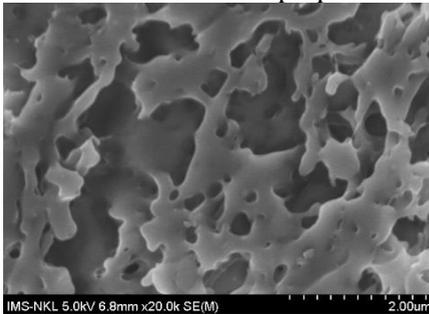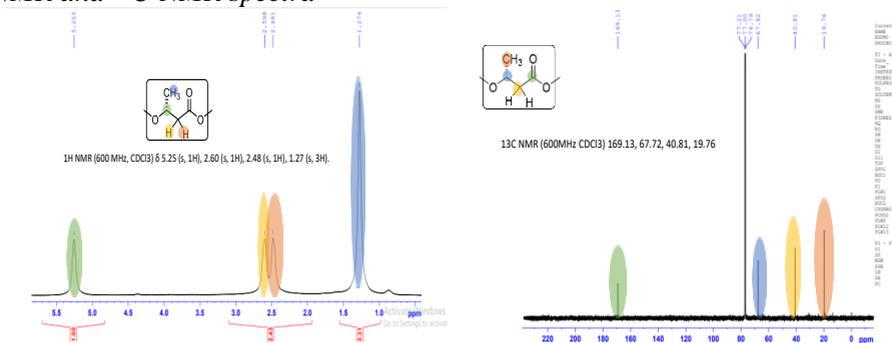

A. Surface morphology   B. FT-IR spectral structure
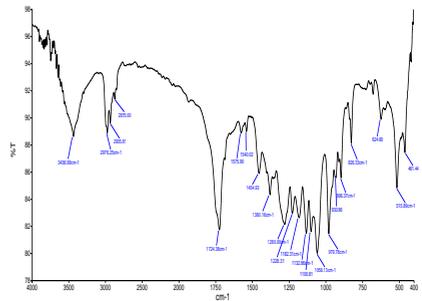
***Figure 3.10.*** Characteristics of PHB bioplastic extracted from *S. salina* M8 biomass

FT-IR spectroscopy of PHB extracted from *S. salina* M8 biomass showed prominent absorption peaks at 1724 cm⁻¹ and 1280 cm⁻¹, corresponding to the stretching vibrations of the carbonyl group (C=O) and the asymmetric C–O–C bond, respectively. These peaks are characteristic of ester linkages, confirming the typical molecular structure of PHB.

3.6.2.2. Structural characteristics of PHB bioplastic material through ¹H-NMR and ¹³C-NMR spectra

1H NMR (600 MHz, CDCl3) δ 5.25 (s, 1H), 2.60 (s, 1H), 2.48 (s, 1H), 1.27 (s, 3H).

13C NMR (600MHz CDCl3) 169.13, 67.72, 40.81, 19.76

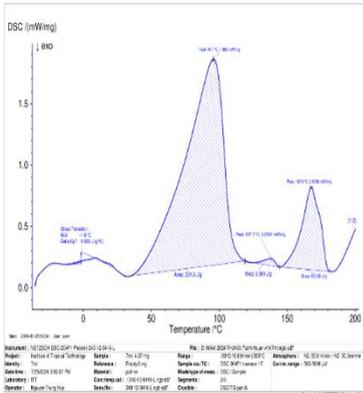***Figure 3.11.*** ¹H-NMR (A) and ¹³C-NMR (B) spectra of PHB bioplastic

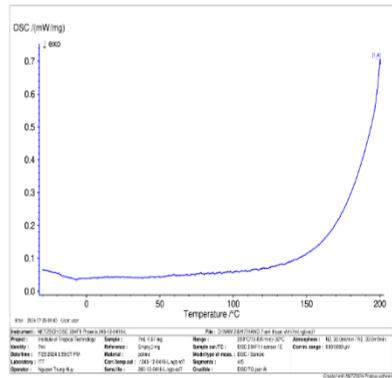*3.6.2.3. Thermogravimetric spectral characteristics of PHB bioplastic material through TGA and DSC analysis*
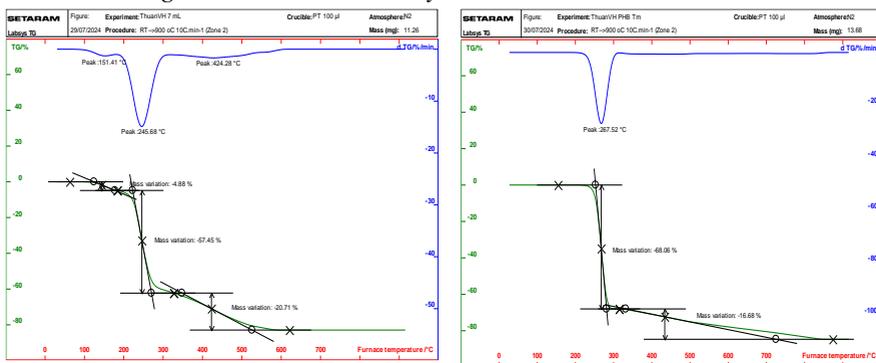
A- TGA/DTG thermograms of PHB extracted from *S. salina* M8 biomass
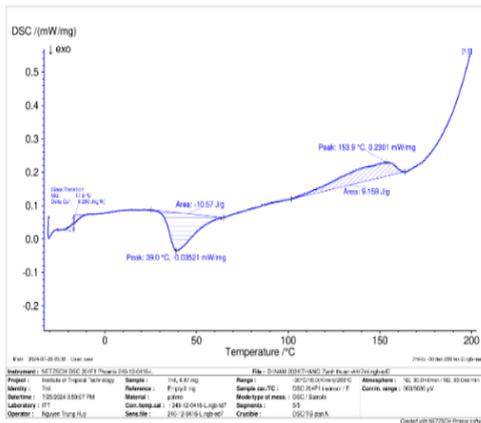
(B) - TGA/DTG thermograms of commercial PHB

Figure 3.12. TGA/DTG profiles of PHB bioplastic

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A- DSC thermogram of PHB
extracted from *S. salina* M8
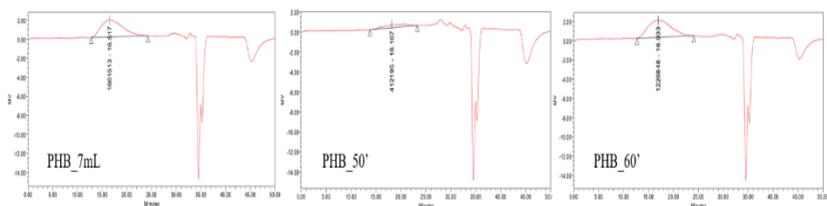biomass from (-30°C) to 200°C

B – DSC thermogram of PHB
extracted from *S. salina* M8
biomass from 200°C to (-30°C)

(C) - Second DSC heating scan of PHB extracted from *S. salina*
M8 biomass from -30°C to 200°C

Figure 3.13. DSC thermograms of PHB extracted from *S. salina* M8
biomass

3.6.2.4. Average molecular weight of purified PHB from S. salina M8

Sample	RT (min)	Mn	Mw	Mw/Mn	Area %
PHB_7mL	16.52	197393	856874	4.341	
PHB_50'	18.17	129495	492901	3.806	100
PHB_60'	16.93	193269	797780	4.129	

Figure 3.14. Molecular weight distribution of PHB extracted and
purified from *S. salina* M8 biomass

3.7. Integrated assessment of the circular model combining wastewater treatment - biomass recovery - PHB bioplastic production

The integrated model demonstrates the feasibility of exploiting a closed-loop value chain, including: reuse of treated effluent; utilization of nitrogen, phosphorus, and carbon in wastewater as nutrient sources; recovery of biomass for the production of biodegradable PHB; and reduction of CO_2 emissions through photosynthetic activity. Consequently, this model functions as a dual-benefit solution, simultaneously achieving effective domestic wastewater treatment and generating renewable resources from waste streams. The approach is well aligned with green technology, circular economy principles, and sustainable development goals, and shows strong potential for scaling up and application in municipal, industrial, and nutrient-rich agricultural wastewater treatment systems.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The main research results achieved in this thesis include:

- The identified strain *S. salina* M8 exhibits good growth rate, high adaptability, and superior PHB accumulation. This strain demonstrates biological stability and high potential for application in wastewater-based culture systems, and was therefore selected as the primary subject for further research.

- Determining the optimal conditions: light intensity of $\approx$ 9,800 Lux, pH $\approx$ 8.0 and temperature of $\approx$ 32°C, at which the highest biomass of 2.73 g/L and PHB content of 21.4% were obtained. A regression model predicting biomass and PHB was developed with high goodness of fit.

- The study demonstrated the feasibility of culturing cyanobacteria using domestic wastewater at laboratory scale, with optimal growth at C:N:P = 70:7:1, pH = 7, and light intensity of 13,500 lux, achieving a biomass of 3.0 g/L and PHB exceeding 40% of dry weight (DWC).

- Initial trials of the *S. salina* M8 strain were successfully conducted in a 30L photobiological system under outdoor cultivation conditions. The system operated under optimal conditions, resulting in good cyanobacteria growth with a dry biomass of 3.4 g/L and a PHB content of 50.2% of the dry cell weight. Simultaneously, the system achieved high pollutant removal efficiency, including 99.6% COD, 99.6% BOD_5, 90.3% total nitrogen, and 95.9% total phosphorus. This confirms the feasibility of the integrated model for treating domestic wastewater and producing PHB-rich biomass, paving the way for scaling up and practical application.

- A process for recovering, extracting, and purifying PHB was developed, along with a complete characterization of the material's structure, physicochemical properties, and thermodynamics. FT-IR, ^{1}H-NMR, ^{13}C-NMR, and GPC results confirmed that PHB possesses high purity, a large molecular weight (Mw = 856.9 kg/mol), and a broad polydispersity index (Mw/Mn = 4.34), making it suitable for biomaterial applications and demonstrating its potential as a replacement for fossil plastics.

Recommendations

- Continue researching more environmentally friendly and safe solvents for extracting PHB to replace chloroform.

- Expand testing of the *S. salina* M8 strain on other wastewater types (such as food, livestock, and seafood processing wastewater) to evaluate its adaptability, treatment efficiency, and PHB accumulation under diverse real-world conditions, while reducing the cost of supplementing pure carbon sources.

- Implement the cultivation of *S. salina* M8 in open photosynthesis systems operating under continuous flow conditions to assess the long-term stability of the system, its biomass maintenance capacity, and PHB accumulation, as a basis for scaling up and practical application.

NOVEL CONTRIBUTIONS OF THE DISERTATION

- Selecting and demonstrating the potential application of the cyanobacterium strain *S. salina* M8 in the treatment of domestic wastewater combined with the recovery of biodegradable PHB-rich biomass. Developing regression equations for dry biomass concentration and accumulated PHB content of *S. salina* M8. Determining the optimal culture conditions in a standard medium for *S. salina* M8 at light intensity $\approx 9,800$ lux, pH ≈ 8.0, and temperature $\approx 32°C$, achieving a maximum dry biomass concentration of 2.73 g/L and a maximum accumulated PHB content of 21.4% of dry biomass, contributing to clarifying the scientific basis for optimizing the cyanobacteria culture process for biomaterial recovery.

- The thesis demonstrated the effective treatment of domestic wastewater using the cyanobacterium *S. salina* M8, achieving COD and BOD_5 removal efficiency of 99.6%, total nitrogen removal efficiency of 90.3%, and total phosphorus removal efficiency of 95.9%. The quality of the treated water met the National Technical Standard QCVN 14:2025/BTNMT, showing the potential application of this cyanobacterium strain in biological wastewater treatment systems.

- The thesis successfully tested the *S. salina* M8 strain in a 30 L tubular photobiological system under optimal conditions, achieving a dry biomass concentration of 3.4 g/L and a maximum accumulated PHB content of 50.2% of the dry cell weight. The results confirmed the feasibility of the integrated model for treating domestic wastewater and producing PHB-rich biomass, while also providing a scientific basis for scaling up and practical application.

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