Vietnam Journal of Earth Sciences, 47(4), 507–526, https://doi.org/ 10.15625/2615-9783/23664

Vietnam Journal of Earth Sciences
https://vjs.ac.vn/index.php/jse

The potential of microalgae for wastewater nutrient removal combined with polyhydroxybutyrate (PHB) production

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Received 31 July 2025; Received in revised form 23 August 2025; Accepted 22 October 2025

ABSTRACT

Poor management of domestic wastewater and dependence on synthetic plastics have been placing a heavy burden on the natural environment, endangering both the ecosystem and human well-being. Microalgae technology has demonstrated enormous potential in simultaneously addressing both problems through wastewater remediation and nutrient recovery, while also facilitating the production of bioplastics. This study evaluated the growth performance, polyhydroxybutyrate (PHB) production, and the ability of four microalgae strains, including Scenedesmus sp., to treat domestic wastewater. SCE, *Chlorella* sp. HB, *Chlorella* sp. CNK, and *Chlorella* sp. CNA, cultivated in the standard artificial medium (Blue Green-11 - BG11), the standard artificial medium (BG11) with sodium acetate, and the real domestic wastewater, and aerated at an aeration rate of 1.2 v v^{-1} m^{-1} for 10 hours/day, at temperature 25°C, light intensity of 5 klux, a light: dark cycle of 10 hours: 14 hours for 12 test days. After 12 experimental days, the study results demonstrated that four microalgae achieved their highest biomass productivities and PHB contents in domestic wastewater, ranging between 7.0±0.4 and 25.7±0.5 mg L^{-1} d^{-1}, and between 6.3±0.2 and 13.0±0.3% by weight, respectively. The addition of organic carbon (sodium acetate) to the standard medium could increase the growth and PHB accumulation capacity of microalgae, particularly in their mixotrophic growth. The microalgae also demonstrated good adaptability in domestic wastewater, achieving high efficiencies, with COD removal ranging from 53.6±4.7 to 78.1±1.1%, TN removal from 56.6±2.6 to 84.5±1.5%, and TP removal from 43.2±11.0 to 70.0±4.2%. Notably, the removal of nitrogen and phosphorus was primarily due to the assimilation of microalgae, further confirming their potential in nutrient recovery from wastewater. The two microalgae that performed the best were *Scenedesmus* sp. SCE and *Chlorella* sp. HB, with the estimated PHB productivities of 2.5±0.1 and 3.3±0.1 mg L^{-1} d^{-1}, respectively. Future studies should focus on optimizing operational conditions to enhance performance, particularly in larger-scale cultivation systems.

Keywords: Microalgae, domestic wastewater, polyhydroxybutyrate, nutrient removal.

1. Introduction

The world population has increased by almost 3 billion people, from 5.33 to 8.2 billion, during the period from 1990 to 2024 (United Nations, 2024). In 2018, more than half, 55.3%, of the world population lived in urban areas, which was expected to reach 60.4% by 2030. Among different income groups, the highest urbanization rate,

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measured by the relative increase in population in urban areas, was observed in low- and middle-income countries, at 1.24% and 1.28% per year, respectively, during the 1990–2018 period (United Nations, 2019). Consequently, overpopulation and urbanization have placed significant burdens on the water environment, especially in developing nations worldwide. An estimated 380 billion m^3 of wastewater is generated globally each year, of which 63% is collected and 52% is treated (Jones et al., 2021; Pratap et al., 2023). Domestic wastewater accounts for the largest proportion of total wastewater flow, with 267.5 billion m^3/year globally, of which 63% is collected in sewers and septic tanks, and 54.7% is treated (Qadir et al., 2025). Domestic wastewater primarily consists of human feces, urine, and wastewater generated during daily activities such as washing, bathing, and cooking at home, also known as "grey water" (Gesba et al., 2015). Improving water quality is one of the most crucial objectives for sustainable development in all United Nations (UN) member nations, according to the 2030 Agenda (United Nations, 2018). Therefore, wastewater treatment before discharge is mandatory in many countries, as it often contains inorganic and organic compounds that can adversely affect aquatic flora and fauna, and can interact indirectly with the food chain, leading to serious health problems for humans. Furthermore, pollutants can have adverse effects on the physical and chemical properties of water, leading to damage to coastal waters, lakes, rivers, and aquifers (Pacheco et al., 2020). Poor management of wastewater from urban areas has been polluting river systems worldwide, with a forecasted increase of at least 30% in the coming years, particularly in the most severe regions, including Europe, Southeast Asia, and North America (Strokal et al., 2021). In addition, the expensive conventional centralized urban water management systems can hardly be afforded by many developing urban areas. At the same time, little effort has been made to recover the energy and nutrients from the wastewater (Larsen et al., 2016). Within the scope of sustainable development, nature-based technologies, which utilize natural processes to remediate wastewater, have shown significant benefits in enhancing the resilience of ecosystems against climate change while improving water management. It was demonstrated that these nature-based systems, such as constructed wetlands or microalgae technology, could reduce greenhouse gas emissions by 2 to 5 times, while requiring significantly lower capital investment and thus being more suitable for small communities compared to conventional wastewater treatment technologies (Garfí et al., 2017). On the other hand, domestic wastewater also contains a significant amount of organic matter, nitrogen, and phosphorus nutrients, which can be considered a source of nutrients for microalgae growth (Chavan and Mutnuri, 2018; Sátiro et al., 2025). Various microalgae strains have been successfully cultivated and have demonstrated excellent efficiency in domestic wastewater. Applications of *Chlorella sorokiniana* JD1-1 to domestic wastewater treatment have presented treatment efficiencies of up to 96.5–97.1% for total nitrogen and more than 99% for total phosphorus (Lee et al., 2022). Six freshwater algae species, including *Scenedesmus* sp. LX1, *R. subcapitata*, *T. obliquus*, *Chlorella* sp. HL, *Chlorella* sp. (A) and *Chlorella* sp. (B) also showed high nutrient removal and value-added production in urban domestic wastewater. NH_4-N and TP removal efficiency of 93.81% and 87.72%, respectively, were demonstrated by *Scenedesmus* sp. LX1 (Q. Wang et al., 2022). *Desmodesmus* sp. KNUA024 emerged as the promising strain, removing 99.10% of ammonia nitrogen, 91.31% of total nitrogen, and 95.67% of total phosphate (Do et al., 2019). Nowadays, the use of microalgae for

recovering nutrients from wastewater combined with numerous valuable compound production, such as biofuels, pharmaceuticals, or feeds, has been attracting serious consideration recently due to their simple, year-round cultivation, good adaptation to various wastewater types, and high productivity (Abdelfattah et al., 2023).

The consumption of plastics has generated a massive amount of plastic waste, which was estimated to be 6300 million metric tons in 2015 (Geyer et al., 2017). Besides, plastic wastes, once left unattended, are likely exposed to numerous physicochemical (irradiation, heat, or pH) and/or biological (animals and microorganisms) factors, thus triggering the release of toxic paints or additives, and the formation of smaller plastic fractions such as microplastics and nanoplastics (Le et al., 2023). It was estimated that from 1.5 to 5.2 million tons of microplastics ended up in the natural environment in 2015 (Ryberg et al., 2019), thus posing serious risks of penetration into the food web and threatening natural life and human well-being (Doan et al., 2023, 2021; Nam et al., 2022, 2019; Nguyen et al., 2025). Efforts have been made to address the global plastic waste problem, primarily through recycling or incineration. Yet, issues such as toxic gases and substances generated by plastic waste incineration (Verma et al., 2016) or financial and technological difficulties in recycling plastics (Garcia and Robertson, 2017) have prevented them from wider application. Together, these solutions could only handle around 21% of all plastic waste, leaving 4900 million metric tons of this waste piling up in landfills or accumulating in the environment (Geyer et al., 2017). Bioplastics, derived from renewable sources with biodegradable properties, have been identified as a promising alternative to traditional non-biodegradable plastics derived from petroleum (Mastropetros et al., 2022). The global biodegradable bioplastic production increased from 0.88 to 1.6 million tons between 2017 and 2021, and was expected to reach 5.33 million tons by 2026 (Ali et al., 2023). Recently, bioplastics derived from microalgae have garnered considerable attention due to their relatively simple cultivation, which can occur year-round, with high productivity, a low land footprint, and a high capacity for bioplastic accumulation. Microalgae have demonstrated promising biopolymer accumulation capacities, ranging from 12 to 77% of the cellular dry weight, primarily in the form of polyhydroxyalkanoates (PHAs) and their short-chain type, poly(3-hydroxybutyrate) (PHB) (Chong et al., 2022). These polymers are easy to process, non-toxic, and exhibit great biodegradability, with applications ranging from materials for various medical devices, drug carriers, and food packaging (Behera et al., 2022). Besides, wastewater can be used as substrate for microalgae cultivation, hence achieving the dual purpose of wastewater remediation and biopolymer production. Promising nutrient removal performance, as high as 95–100% of ammonium nitrogen and 95–99% of phosphate, and good PHB accumulation, from 5.5 to 65% of dry cell weight, were achieved by some microalgae (Mastropetros et al., 2022).

Although the potential of microalgae for wastewater treatment or the production of high-value biopolymers is well-recognized, a significant research gap remains in evaluating the performance of locally sourced microalgae strains for this dual-purpose application, particularly using undiluted domestic wastewater. Most existing studies focus on either biomass production or nutrient removal rather than a combination of both in a single, cost-effective system (Dammak et al., 2023). Therefore, the novelty of this study lies in its focused assessment of four indigenous Vietnamese microalgae strains to identify their potential for a dual system that couples

effective nutrient removal from raw domestic wastewater with high-efficiency PHB production. This approach provides crucial, context-specific data essential for the practical and scalable application of this technology within the region (Amadu et al., 2021).

This study aims to assess the growth and PHB production capacities of different local microalgae strains in domestic wastewater by comparing their performances in the standard artificial culturing medium. The potential of these strains in biomass production and nutrient recovery from wastewater, combined with PHB production, was then discussed.

2. Materials and methods

2.1. Microalgae strains and domestic wastewater

Four microalgae strains used in this study were *Scenedesmus* sp. SCE, *Chlorella* sp. HB, *Chlorella* sp. CNK, and *Chlorella* sp. CNA, which were coded as SCE, HB, CNK, and CNA, respectively. They were known for their good adaptability in wastewater (Amorim et al., 2021; Pham et al., 2022) and their ability to produce PHB (Arora et al., 2023; Chong et al., 2022). These strains were previously collected from different locations in northern Vietnam and maintained at the Institute of Science and Technology for Energy and Environment, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The axenic cultures of the four microalgae were obtained and enriched in Blue Green 11 (BG-11) medium from the exponential phase to the logarithmic phase before the study.

The domestic wastewater was obtained from the collection tank of a residential wastewater treatment plant in Hanoi City, Vietnam. After collection, the domestic wastewater was allowed to settle for 60 minutes, followed by a filtration step using a nylon filter bag (NMO filter bag, 25 μm, China) to remove coarse suspended matter. This filtered wastewater was then used as the growth medium for cultivating microalgae in its undiluted and raw form. The main characteristics of initial domestic wastewater included chemical oxygen demand (COD, 313.67±6.81 mg O_2 L^{-1}), total nitrogen (TN, 55.07±0.64 mg N L^{-1}), ammonium nitrogen (NH_4-N, 50.32±0.23 mg N L^{-1}), nitrate nitrogen (NO_3-N, 0.05±0.02 mg N L^{-1}), nitrite nitrogen (NO_2-N, 0.09±0.02 mg N L^{-1}), total phosphorus (TP, 10.20±0.18 mg P L^{-1}), orthophosphate (PO_4-P, 8.23±0.15 mg P L^{-1}), and pH (7.21±0.39).

2.2. Experimental conditions

In this study, BG-11 medium was used as the standard medium due to its wide application in the growth of freshwater microalgae (Jiang et al., 2018). The medium compositions included: (1) $NaNO_3$ 1.5 g L^{-1}; (2) 10 mL per every liter of culture medium with the following chemicals: $K_2HPO_4.3H_2O$ (4 g L^{-1}), $MgSO_4.7H_2O$ (7.5 g L^{-1}), $CaCl_2.2H_2O$ (3.6 g L^{-1}), $C_6H_8O_7.H_2O$ (0.6 g L^{-1}), $Fe(NH_4)_3(C_6H_5O_7)_2$ (0.6 g L^{-1}), $EDTA.Na_2$ (0.1 g L^{-1}), Na_2CO_3 (2 g L^{-1}), and (3) 1 mL per liter of culture medium with the following chemicals: $Na_2MoO_4.2H_2O$ (0.39 g L^{-1}), H_3BO_3 (2.86 g L^{-1}), $CuSO_4.5H_2O$ (0.08 g L^{-1}), $MnCl_2.4H_2O$ (1.81 g L^{-1}), $ZnSO_4.7H_2O$ (0.22 g L^{-1}).

Four microalgae strains, SCE, HB, CNK, and CNA, were cultivated in the standard BG-11 medium, BG-11 medium supplemented with an external source of organic carbon in the form of sodium acetate (SA), and domestic wastewater were used to assess their growth and PHB production capacities, denoted as BG-11, BG-11 + SA, and WW, respectively. It was reported that organic carbon is beneficial for PHB production by microalgae (Mastropetros et al., 2022); therefore, the microalgae were cultured in BG-11 medium with an additional SA concentration of 0.6375 g L^{-1} (Mourão et al., 2020).

Each experiment was performed in triplicate with an initial biomass concentration of 0.15 g dry weight L^{-1} (Franchino et al., 2016). An initial biomass concentration of 0.15 g L^{-1} was selected to ensure a sufficient inoculum for rapid growth while minimizing self-shading effects and providing a representative starting point for efficient nutrient uptake. The tests were monitored regularly to assess the development of microalgae. The pH of each mixed culture was measured every 2 days in the morning, which was used to evaluate the photosynthesis of microalgae during the culturing period, showing a general increasing trend with values ranging between 7.2 and 8.6. All tests were conducted in 1000 mL transparent glass flasks, with a working volume of 500 mL. All flasks were continuously aerated at an aeration rate of 1.2 v v^{-1} min^{-1} for 10 hours per day under a controlled room temperature of 25°C. Illumination was ensured using white LED lamps with an average light intensity of 5 kilolux (klx), measured at the outer surface of each flask by a digital light meter (HT603, China), and a light: dark cycle of 10:14 hours (Ansari and Fatma, 2016; Rana and Prajapati, 2021). The culture period was 12 days.

2.3. Determination of microalgae growth

A 5 mL sample was collected every 2 days to analyze the growth of microalgae in all tests. Growth was monitored gravimetrically and expressed as dry cell weight by a high-precision analytical microbalance with a readability of 0.01 mg (ME204, Mettler-Toledo GmbH, Switzerland). Microalgal cells were harvested by centrifugation (8000 rpm, 10 min) using a Hettich D-78532 Tuttlingen centrifuge (Germany), and the biomass was dried at 105°C for 24 h in a Memmert Universal Oven (Germany). The biomass productivities ($P_{biomass}$) of the studied microalgal strains after 12-day cultivation were calculated as follows (Samadhiya et al., 2022):

$$P_{\text{biomass}}\ (mg\ L^{-1}d^{-1}) = (m_n - m_0)/(t_n - t_0)$$

Where: m_0 and m_n were the dry biomass concentrations (mg L^{-1}) on the final day (t_n) and on the initial day (t_0) of the culturing period, respectively.

2.4. Chlorophyll a extraction and analysis

Every 2 days, 10 mL of the solution containing the microalgae biomass was withdrawn from all tests, subsequently filtered using glass microfiber filter paper (Whatman, UK, 0.45 µm pore size). The sample was then soaked in 10 mL of 90% acetone for 24 hours, followed by centrifugation at 7000 rpm for 10 minutes at 4°C. In the next step, the supernatant is collected and measured for absorbance at 665 nm and 750 nm wavelengths using a spectrophotometer (Shimadzu UV-2450, Japan), which were denoted as B665 and B750, respectively. Then the sample was acidified with HCl solution (1 mol L^{-1}). After 1 minute, the acidified solution was measured again for absorbance at 665 nm and 750 nm wavelengths, marked as A665 and A750, respectively. The volumes of the filtrate sample and the extraction volume are denoted as V_f and V_e, respectively. Finally, chlorophyll a (Chl-a) can be determined by the following equation (Shanmuganathan et al., 2023):

$$Chl - a\ (\mu g\ L^{-1}) = [(B665 - B750) - (A665 - A750)] \times 26.7 \times (V_e/V_f)$$

The productivity of Chl-a of the microalgae after 12-day cultivation was calculated as:

$$Chl - a\ producivity\ (\mu g\ L^{-1}d^{-1}) = (X_{Chl-a,n} - X_{Chl-a,0})/(t_n - t_0)$$

Where $X_{Chl\text{-}a,n}$ and $X_{Chl\text{-}a,0}$ were the Chl-a concentration measured at the final (t_n) and the beginning (t_0) of each test, respectively.

2.5. PHB extract method

After completing the cultivation process, microalgal biomass from each experimental condition was collected by settling for 60 min

and then filtering through a 25 µm nylon mesh (NMO filter bag, same type as previously described). This method was chosen to facilitate the rapid collection of the majority of biomass for subsequent analyses. The retained biomass was rinsed twice with a phosphate buffer at neutral pH (pH 7.0) and then dried in an oven set at 55°C until the mass stabilized, indicating complete dehydration. To initiate PHB extraction, the dried biomass was treated with a 4% sodium hypochlorite solution and subjected to sonication in a 40°C water bath for 30 minutes to achieve both decolorization and cellular disruption. Subsequently, 50 mL of chloroform was added to the treated biomass, and the sonication process was repeated under the same temperature conditions for an additional hour. The chloroform-containing extract was centrifuged at 8000 rpm for 10 minutes to allow phase separation. The lower organic phase, presumed to contain PHB, was collected and mixed with pre-chilled methanol at a volumetric ratio of 1:5 (chloroform: methanol). This mixture was incubated at -20°C overnight to induce PHB precipitation. Following incubation, the precipitated material was separated by centrifugation at 12,000 rpm for 10 minutes. The supernatant was discarded, and the resulting white PHB pellet was dried in an oven at 50°C. To further purify the biopolymer, the dried pellet was washed two to three times with acetone to remove any residual impurities before downstream analyses (Kumari et al., 2022; Pham et al., 2024).

The PHB content achieved after 12-day cultivation was calculated based on the dry biomass weight by the gravimetric method, used in each extraction as follows:

$$Y\ (\%) = M_P \times 100/M_B$$

Where Y is the PHB accumulation expressed as a percentage, M_P is the dry mass of PHB obtained in grams, and M_B is the dry biomass used for extraction, in grams.

2.6. Fourier transform infrared spectroscopy (FTIR) analysis

The samples were analyzed using an ATR-FTIR system, specifically a Nicolet iS10 Fourier transform infrared (FTIR) spectrometer (Thermo Fisher Scientific, USA), equipped with a diamond single-bounce ATR crystal and a focusing lens made of zinc selenide (ZnSe). First, the background spectrum - the absorption spectrum of the pure ZnSe crystal - was recorded. Then, the sample was placed directly on the diamond crystal surface (ensuring that the sample completely covered the contact surface), and the FTIR spectrum of the sample was acquired. The spectra were recorded in the range of 4000–400 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans. The resulting spectrum was determined by subtracting the background spectrum from the sample spectrum. Finally, the spectral data were processed and analyzed using OMNIC software (Thermo Scientific).

2.7. Domestic wastewater quality determination

The compositions of wastewater at the beginning and the end of each experimental process were analyzed. Wastewater samples were separated from microalgae biomass by settling, filtering with a 25 µm NMO membrane, and centrifuging (8000 rpm, 10 min) before any measurement. The supernatant will be used to analyze the components of the wastewater. The pH level was analyzed using a multiparameter meter (HI98194, Hanna Instruments, Hungary). The concentration of NH_4-N was analyzed by the manual spectrometric method (ISO-7150-1:1984), NO_2-N concentration was measured by the improved Griss Satlman method (ISO-6768:1998), and NO_3-N parameter was determined by the spectrometric method using sulfosalicylic acid reagent (ISO 7890-3:1988); Determination of Kjeldahl nitrogen analyzed

TN-Method after mineralization with selenium, ISO-5663:1984. In terms of phosphorus, the level of PO_4-P was measured by the ammonium molybdate spectrometric method (ISO-6878:2004), while TP was analyzed by the ascorbic acid method (SMEWW 4500P C:2012). The COD concentration in the wastewater sample was analyzed by the titration method (ISO-6060:1989).

The removal efficiency and removal rate achieved by the microalgae were calculated as follows (Lee et al., 2022):

$$Efficiency\ (\%) = \left(\frac{C_0 - C_t}{C_0}\right) \times 100\%$$

$$Removal\ rate\ (mg\ L^{-1}d^{-1}) = \frac{C_0 - C_t}{t - t_0}$$

Where C_0 and C_t are the concentrations in the mixed liquor expressed in mg L^{-1} at initial time t_0 and at measurement time t (day).

2.8. Statistics

The concentrations of different wastewater constituents, microalgae biomass, chlorophyll a, removal rates, removal efficiencies, and the productivities were reported in average values with standard deviations. Comparisons of multiple data sets were conducted using the Kruskal-Wallis test, followed by the post-hoc Dunn test for pairwise comparisons. The significance level was set at $p \leq 0.05$. The analysis and visualization of data were conducted using Microsoft Excel 2016 and R software, version 4.4.1 (R Core Team, 2016).

3. Results and Discussion

3.1. Microalgal growth

All four studied microalgae exhibited better growth in BG-11 medium supplemented with SA and in domestic wastewater compared to the original BG-11 medium (Fig. 1a and b). Microalgae SCE, CNK, and CNA generally grew better in domestic wastewater than BG-11 + SA, which was observed in biomass productivity, final Chl-a concentration, and Chl-a productivity. The opposite was true in the case of microalgae HB, where better growth was obtained in the BG-11+SA medium. Overall, the biomass productivities of the studied microalgae ranged between 3.7±0.3 and 27.8±1.0 mg L^{-1} d^{-1}. In contrast, the final Chl-a concentrations and Chl-a productivities varied from 1148.5±145.5 to 4045.1±341.5 µg L^{-1} and from 24.2±12.3 to 233.5±28.9 µg L^{-1} d^{-1}, respectively. The highest biomass productivities were achieved by microalgae HB cultured in BG-11+SA medium and in domestic wastewater, at 27.8±1.0 and 25.7±0.5 mg L^{-1} d^{-1}, respectively. In contrast, microalgae CNK had the lowest biomass productivity, at 3.7±0.3 mg L^{-1} d^{-1}, which was obtained in BG-11 culture media ($p < 0.05$). In terms of Chl-a concentration, starting from a general level of 1030.1±148.3 µg L^{-1} in all experiments (Figure b), the highest final Chl-a level was achieved by microalgae SCE, at 4045.1±341.5 µg L^{-1} in domestic wastewater, respectively. The lowest Chl-a level of 1148.5±145.5–1292.1±85.7 was obtained by microalgae CNK in all culture media ($p < 0.05$). A similar trend was observed in the Chl-a productivities, with the highest value achieved by microalgae SCE in domestic wastewater. In contrast, the lowest value was observed for microalgae CNK in BG-11 medium ($p < 0.05$). There were significant differences in biomass productivity and Chl-a productivity between the two strains, SCE and HB, and the other two microalgae strains, CNK and CNA. This study observed that the cell size of SCE and HB was significantly larger than that of the other two strains. The increased cell size of microalgae may result in a higher dry weight of microalgal biomass. In the study by Wang et al. (2022), a correlation was also reported between the size and dry weight of the microalgae strains. *Scenedesmus* sp. LX1 outperformed *Chlorella* sp. HL in terms of dry biomass weight, reaching a maximum of 0.55±0.02 g/L, which may be related to their

cell size. With a microscope micrometre, the diameter of *Chlorella* sp. HL was measured at 3.02±0.48 µm, while the cell size of *Scenedesmus* sp. LX1 were measured at 8.63±1.20 µm in length and 5.36±0.69 µm in width, respectively (Q. Wang et al., 2022). Under ideal circumstances, the fitness (growth rates) of larger cells was higher, whereas smaller cells fared better at lower resource availability (Fae Neto et al., 2023).

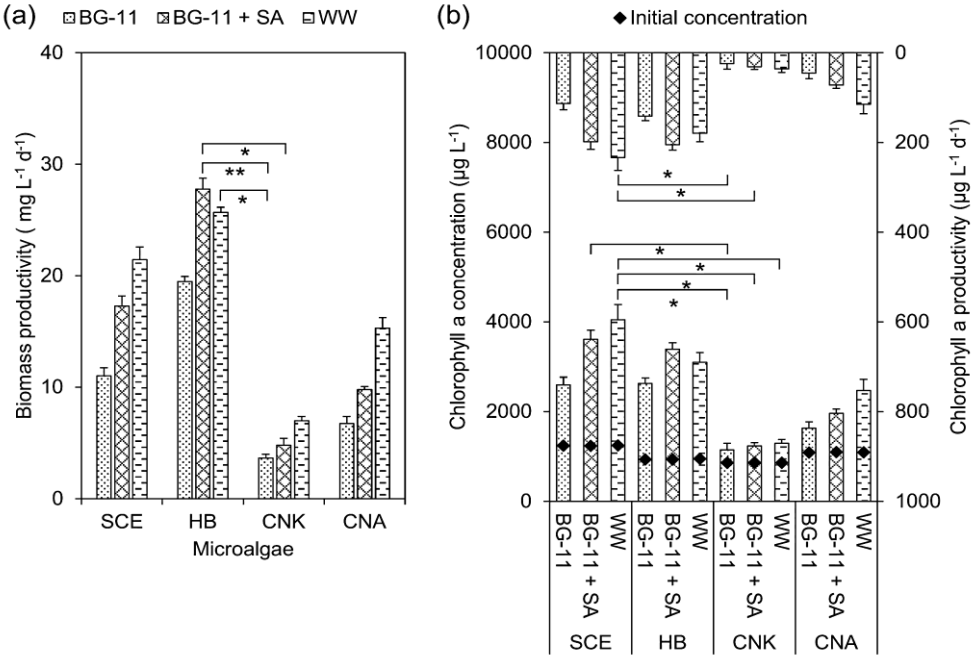

Figure 1. The growth of microalgae in different experiments, assessed by (a) biomass productivities and (b) final concentrations (bottom columns) and productivities (top columns) of chlorophyll a. The brackets indicated values with significant difference, and the asterisks showed the level of p values (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

The results showed that sodium acetate supplement enhanced the growth of all four microalgae, observed in both biomass and Chl-a productivities. A similar conclusion was reported by different studies, which observed significant increases in the growth of microalgal strains such as *Synechococcus elongatus*, *Anabaena*, *Chlorella vulgaris*, *Chlorella minutissima*, and *Chlorella pyrenoidosa*, with the addition of external organic carbon sources consisting of glucose, sodium acetate, sucrose, or glycerol (Parthiban and Jambulingam, 2023; Sharma et al., 2016). It was indicated that the presence of an organic carbon source, along with available inorganic carbon sources, would trigger the mixotrophic growth of microalgae, resulting in higher biomass production (Sharma et al., 2016). Moreover, all tested microalgae demonstrated good acclimation in undiluted domestic wastewater, as evidenced by high levels of biomass and Chl-a production, which is advantageous for large-scale applications. Previous studies have also reported that certain microalgae strains, such as *Chlorella sorokiniana*, *Chlorella zofingiensis*, and *Scenedesmus* sp., exhibit higher growth potential when cultured in domestic wastewater compared to artificial growth medium (BG-11) (Lee et al., 2022; Shu et al., 2018). This may be explained by the fact that domestic wastewater has a suitable initial N/P nutrient ratio ranging from 5:1 to

8:1 for microalgae growth, while BG11 medium with an N/P nutrient ratio of 34.7:1 may contain nitrogen-rich leading to phosphorus limitation for microalgae cultivation (Lee et al., 2022; Xin et al., 2010). As shown in Table 1, the biomass productivities achieved in this study are modest compared to others, which ranged between 35 and 480 mg L^{-1} d^{-1}. Further study should focus on optimizing the culturing process to enhance the biomass production of these microalgae.

Table 1. Wastewater remediation, biomass production, and PHB content of different microalgae (all values are expressed in mean ± SD, if available)

Microalgae	**Wastewater type**	**COD removal (%)**	**TN removal (%)**	**TP removal (%)**	**Biomass productivity (mg L^{-1} d^{-1})**	**PHB content (%)**	**PHB productivity * (mg L^{-1} d^{-1})**	**References**
Synechocystis sp.	Centrate wastewater	n.a.	71.8	67.4	n.a.	22.7±2.5	n.a.	(Grivalský et al., 2024)
Synechococcus leopoliensis	liquid biogas digestate	n.a.	97.7	n.a.	300	0.9	2.7	(Mariotto et al., 2023)
	aquaculture wastewater	n.a.	95.9	n.a.	250	0.2	0.5	
Mixture of filamentous microalgae and cyanobacteria, dominated by *Synechococcus* sp. at the end	Wastewater from an agricultural drainage channel	n.a.	95	99	35–86	4.5	0.3–1.6	(Rueda et al., 2020)
Phormidium sp.	Cheese whey	42.9–47.9	27.5–37.1	41.2–48.3	290–480	0.4–1.4	1.2–6.7	(Sventzouri et al., 2025)
Synechocystis sp.		49.4–57.9	18.4–59.0	19.1–44.6	270–380	0.5–2.8	1.9–7.6	
Chlorogloeopsis fritschii		51.4–52.3	30.4–53.1	32.8–41.2	240–380	0.6–10.7	2.3–25.7	
Arthrospira platensis		11.1–17.1	40.0–55.9	11.6–41.6	170–360	0.6–0.9	1.0–3.2	
Microalgae consortium including cyanobacteria and filamentous microalgae	Aquaculture effluent	0–46.2	25.6–32.8	0–65.4	190–280	0.001–0.014	0.002–0.012	(Wicker et al., 2022)
Synechocystis sp.	Wastewater from the shrimp pond	n.a.	35.6–80.1	71.2–97.0	35.5–57.8	32.5±1.7	11.5±0.7	(Krasaesueb et al., 2019)
Synechocystis cf. salina	Digestate from anaerobic tank	81.6–86.1	25.5–40.4	> 60.5	90–110	4.8–6.3	2.2	(Meixner et al., 2016)
Scenedesmus spp. SCE	Domestic wastewater	74.6±1.2	84.5±1.5	65.0±3.2	21.5±1.1	11.7±0.4	2.5±0.1	This study
Chlorella vulgaris HB		78.1±1.1	81.0±0.2	70.0±4.2	25.7±0.5	13.0±0.3	3.3±0.1	
Chlorella vulgaris CNK		53.6±4.7	56.6±2.6	43.2±11.0	7.0±0.4	8.2±0.6	0.6±0.1	
Chlorella vulgaris CNA		62.8±2.1	61.0±0.4	54.6±2.2	15.3±0.9	6.3±0.2	1.0±0.1	

n.a.: not available; *: calculated from biomass productivities and PHB contents

3.2. Polyhydroxybutyrate (PHB) production

Higher PHB levels were detected in microalgae growing in domestic wastewater in comparison to other media (Fig. 2Figure 2). The highest PHB contents were obtained in microalgae HB and SCE, at 13.0±0.3 and 11.7±0.4%, respectively, while the lowest values, at 4.8±1.0 and 4.9±0.7%, were observed in microalgae CNK and CNA in BG-11 medium ($p < 0.05$). It should be noted that the addition of SA in BG-11 medium improved the PHB production in all studied microalgae, although the changes were statistically insignificant.

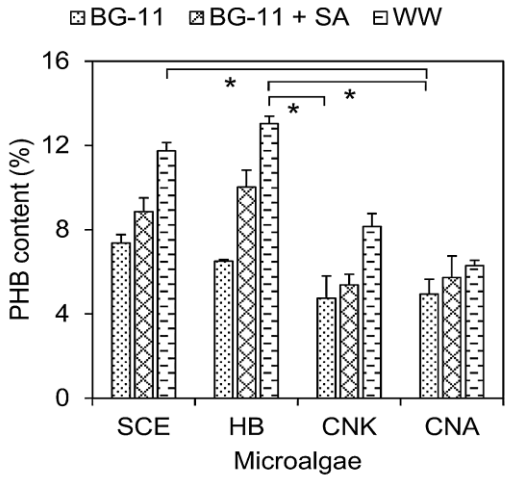

Figure 2. The PHB contents of microalgae in different experiments. The brackets indicated values with significant difference, and the asterisks showed the level of p values (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

Supplementation of organic carbon, primarily acetate, was shown to enhance PHB production in microalgae (Arias et al., 2020). Significant increases in PHB content were observed in microalgae *Chlorella vulgaris*, at 20.0%, and in cyanobacteria *Spirulina platensis*, at 16.2%, as a result of sodium acetate addition and nitrogen starvation (Zhila et al., 2025). Similarly, the supplementation of organic carbon in the form of sodium acetate in this study increased 18.8–54.0% of the PHB content in the tested microalgae. In addition, all four microalgae exhibited the highest PHB accumulation capacities in domestic wastewater, with increases of 29.4–100.4% compared to the original BG-11 medium, thereby reconfirming their potential for combining wastewater nutrient removal, biomass production, and PHB production. Notably, the PHB contents obtained in the studied microalgae were at a high level compared to other similar studies, which typically ranged from below 1 to 32.5±1.7% (Table 1).

Fourier transform infrared spectroscopy (FTIR) analysis was conducted to verify the presence of polyhydroxybutyrate (PHB) in the biomass of the microalgae strains, *Scenedesmus* sp. SCE, *Chlorella* sp. HB, *Chlorella* sp. CNK, and *Chlorella* sp. CNA growing in domestic wastewater. The results presented in Fig. 3 demonstrated the presence of functional groups in the obtained polymer samples. The FTIR spectra of the polymers extracted from the biomass of these microalgae strains were similar to those of the standard PHB reported in previous studies (Duangsri et al., 2020; Sooksawat et al., 2023). The most notable peaks of PHB were recorded at the absorption bands 3344–3414 cm^{-1}; 2854–2978 cm^{-1}; 1722–1734 cm^{-1}; 1381–1423 cm^{-1}, 1281–1292 cm^{-1}; 1057–1059 cm^{-1}; 964–972 cm^{-1}. The strong absorption band in the region 3344–3414 cm^{-1} is assigned to the stretching vibration of the hydroxyl group (–OH) bonded to the carbonyl (Balan et al., 2025). The stretching vibrations of the C–H bond in the methyl ($–CH_3$) and methylene ($–CH_2$) functional groups were detected in the absorption band 2854–2978 cm^{-1}, indicating the presence of characteristic hydrocarbon components in the polymer backbone of PHB (Kavitha et al., 2016). Notably, the sharp peak at 1722–1734 cm^{-1} is characteristic of the

stretching vibration of the C=O bond in the carbonyl ester group, a typical structural feature of PHB (Hassan et al., 2024). The strong vibration of the C=O ester group in the 1735–1745 cm^{-1} region of PHB, obtained from *Arthrospira platensis* biomass, was also observed, demonstrating the reliability of the current analytical results (Duangsri et al., 2020). In addition, the absorption bands in the range of 1381–1423 cm^{-1} represent the symmetric and asymmetric stretching vibrations of the –CH, $–CH_2$, and $–CH_3$ groups (Kumari et al., 2022). The absorption peaks at 1281 cm^{-1} and 1292 cm^{-1} in the microalga *Scenedesmus* sp. SCE, *Chlorella* sp. HB can be attributed to the deformation vibrations of the $–CH_3$ group and the stretching vibrations of C–O–C, as indicated by the broadening of this band due to the vibrational overlap of these groups. The absorption band at 1128 cm^{-1} can be attributed to the stretching vibrations of the carbonyl group (Hernández-Núñez et al., 2019). In the FTIR spectrum (*Figure 3*Fig. 3), the absorption bands at 1057–1059 cm^{-1} are attributed to the stretching vibrations of the –C–O– bond. The deformation vibrations of the C–H bond are observed at the peaks 964 cm^{-1}, 968 cm^{-1}, and 972 cm^{-1} (Zhila et al., 2025). The results shown in Fig. 3 show characteristic signals of PHB, confirming the presence of this compound in four microalgae strains.

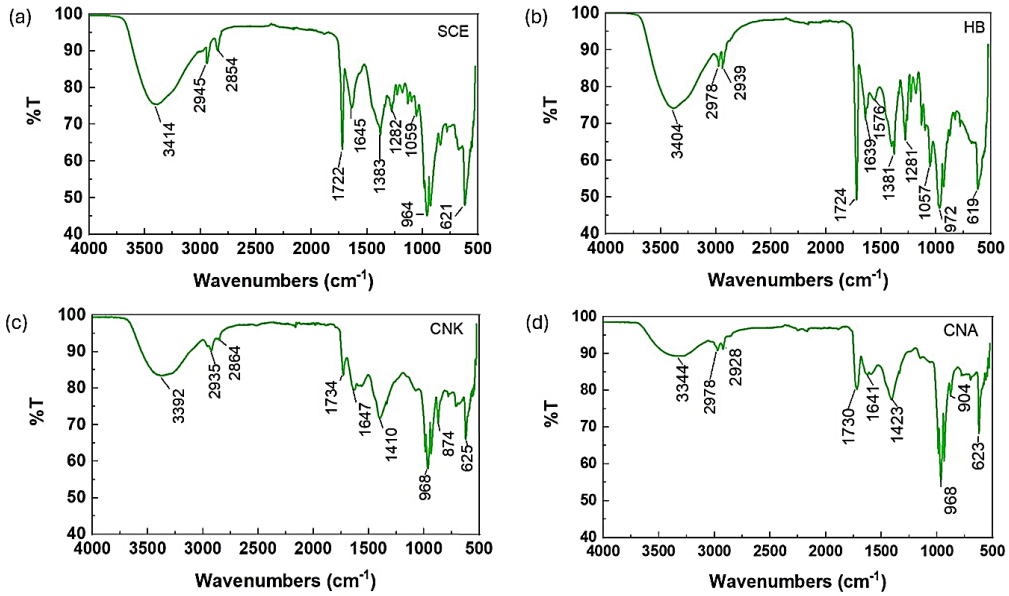

Figure 3. FTIR spectra of polymers obtained from the biomass of microalgae (a) SCE, (b) HB, (c) CNK, and (d) CNA growing in domestic wastewater, showing functional groups representing PHB

3.3. Wastewater nutrient removal

In this study, most of the nitrogen in wastewater was in the form of ammonium (NH_4-N), accounting for 90.7–91.8% and 80.3–87.7% of TN measured at the beginning and the end of all experiments applied domestic wastewater (Fig. 4a). Phosphate (PO_4-P) was the dominant form of phosphorus detected in domestic wastewater at the beginning (80.2–81.2% of TP) and the end (58.8–80.2% of TP) of all experiments (Fig. 4d). The lowest NH_4-N final concentration was obtained in the experiment culturing microalgae SCE in domestic wastewater. In contrast, the highest concentration was detected in the test

employing CNK ($p < 0.05$), corresponding to NH_4-N removal efficiencies of 86.4±0.2% and 59.2±0.3%, with NH_4-N removal rates of 3.6±0.0 and 2.5±0.0 mg N L^{-1} d^{-1}, respectively. These values were comparable to TN removal efficiencies and removal rates of microalgae SCE and CNK (Fig. 5b). Similarly, the low PO4-P concentration measured at the end of the experiment, employing microalgae HB for domestic wastewater remediation, resulted in a high phosphate removal efficiency of 72.3±1.6% and a removal rate of 0.5±0.0 mg P L^{-1} d^{-1}, which was in line with the TP removal performance of this microalgae (Fig. 5c).

TN and TP observed in domestic wastewater were primarily due to reductions in NH_4-N and PO_4-P, thus highlighting the significant role of microalgae in nutrient recovery from wastewater. Bacterial nitrification made a minor contribution to nitrogenous conversion in the experiments, as nitrogen in the forms of nitrate (NO_3-N) and nitrite (NO_2-N) was present at low concentrations during the test (0.02–0.91 mg N L^{-1}, Fig. 4b and c). Together, both forms accounted for 0.2–0.3% of TN detected at the beginning and 3.0–9.1% of TN measured at the end of all experiments. The results confirmed the dominant role of microalgae in removing nitrogen and phosphorus from domestic wastewater.

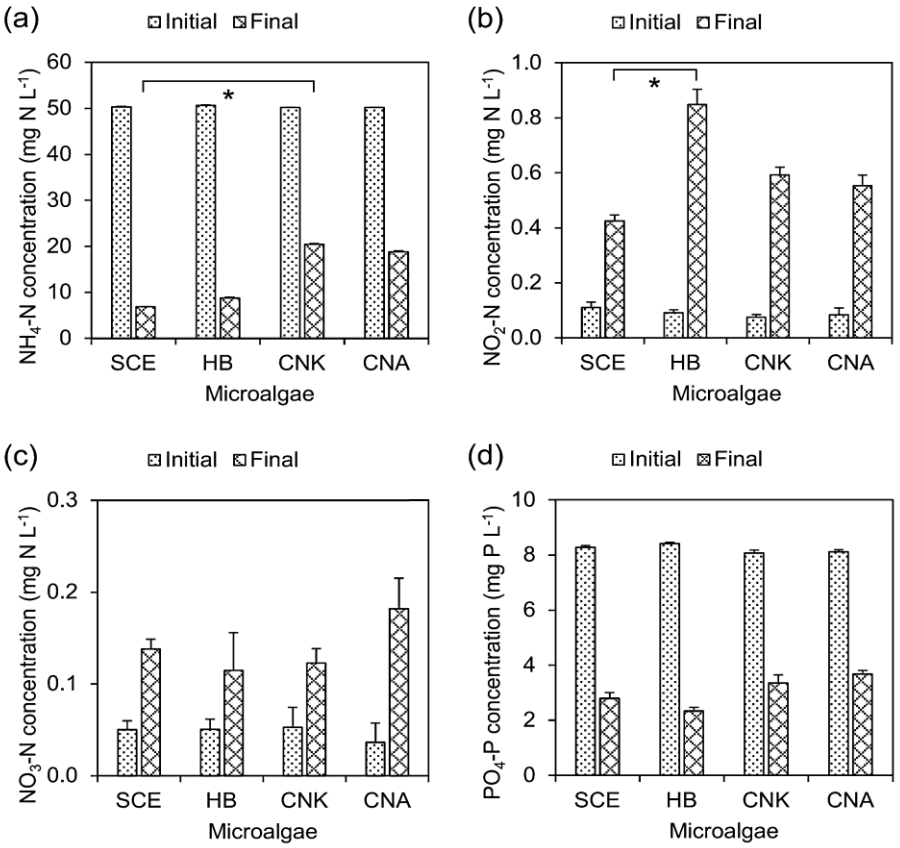

Figure 4. Initial and final concentrations of (a) NH_4-N, (b) NO_2-N, (c) NO_3-N, and (d) PO_4-P in wastewater remediated by different microalgae. The brackets indicated values with significant difference, and the asterisks showed the level of p values (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

The results indicate that the removals of microalgae HB and SCE were more effective in wastewater remediation. HB achieved the highest removal efficiencies and removal rates of COD and TP, while SCE showed the highest TN removal efficiency and removal rate (Figure). In comparison, microalgae CNK showed the poorest remediation performance ($p < 0.05$). The COD removal efficiencies and COD removal rates obtained in this study varied between 53.6±4.7 and 78.1±1.1% and between 14.0±1.5 and 20.9±0.3 mg O_2 L^{-1} d^{-1}, respectively. The studied microalgae removed from 56.6±2.6 to 84.5±1.5% of TN and from 43.2±11.0 to 70.0±4.2% of TP in domestic wastewater, equivalent to the TN and TP removal rates of 2.6±0.1–3.9±0.1 mg N L^{-1} d^{-1} and 0.4±0.1–0.6±0.0 mg P L^{-1} d^{-1}, respectively.

The wastewater remediation performance achieved by the studied microalgae was at a high level compared to other similar studies (Table 1). It was noted that harmonious cooperation between microalgae and native bacteria in wastewater is crucial for the successful microalgae-based wastewater treatment system (Dinh et al., 2022). The bacterial oxidation process is responsible for COD removal, utilizing oxygen supplementation from the photosynthesis of microalgae. Meanwhile, microalgae assimilate inorganic nitrogen and phosphorus in wastewater, thereby achieving effective nutrient recovery (Pham et al., 2022). Additionally, microalgal photosynthesis also triggers an increase in pH in the solution, which favors the volatilization of ammonia (Pham et al., 2021). Ammonia stripping at a pH level of 8.5 was estimated to account for around 13% of the total nitrogen in the microalgae-based wastewater treatment system (Álvarez-González et al., 2023). Moreover, the aeration applied during the experiment could be beneficial for both COD removal by bacteria and ammonia volatilization via additional oxygen supplementation and increased gas transfer rate, respectively.

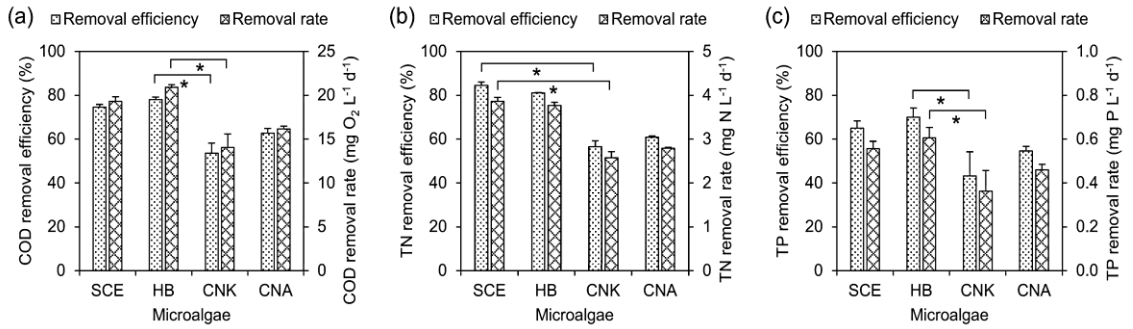

Figure 5. Wastewater remediation by different microalgae, assessed by removal efficiencies and removal rates of (a) COD, (b) TN, and (c) TP. The brackets indicated values with significant difference, and the asterisks showed the level of p values (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

3.4. Potential of microalgae for PHB production combined with biomass growth and wastewater nutrient recovery

The potential of four microalgae strains tested in this study in PHB production, combined with wastewater nutrient recovery and biomass production, was evident. The obtained results enabled the estimation of the PHB production rates of different microalgae, which were calculated as the product of biomass productivity and PHB content (Price et al., 2020). The calculated PHB production rates of microalgae SCE, HB, CNK, and CNA in domestic wastewater were 2.5±0.1, 3.3±0.1,

0.6±0.1, and 1.0±0.1 mg L^{-1} d^{-1}, respectively (Table 1). The PHB productivities of cyanobacteria *Synechococcus leopoliensis* in biogas digestate and aquaculture wastewater at pilot scale, achieving the calculated productivities from 0.5 to 2.7 mg L^{-1} d^{-1} (Mariotto et al., 2023). Obviously, the overall PHB productivity is governed by both biomass productivity and the microalgae's capability for PHB accumulation. Wicker et al. (2022) investigated the PHB production by a consortium of different filamentous microalgae and cyanobacteria in aquaculture wastewater, obtaining a high biomass productivity of 190–280 mg L^{-1} d^{-1}, yet low PHB content of 0.001–0.014 % (Wicker et al., 2022). Therefore, their results lead to an estimated PHB productivity of only 0.012 mg L^{-1} d^{-1} (Table 1). Besides organic carbon supplementation, which was demonstrated in these studies, other strategies have been proposed for increasing cellular PHB content in microalgae, such as nutrient deficiency, optimal irradiance, or pH regulation (Arias et al., 2020). A two-phase cultivation was applied, and the cyanobacterium Synechocystis sp. was allowed to grow in the first phase. During the second phase, different stressors, including salinity, phosphorus content, and light intensity, were applied to stimulate PHB accumulation in the microalgal cells. The authors indicated that phosphorus deficiency, high light intensity (up to 500 µmol m^{-2} s^{-1}), and salinity (up to 60 mS cm^{-1}) could enhance the production of PHB in the cyanobacteria (Rueda et al., 2022).

In addition, optimizing large-scale cultivation systems also requires further insights to ensure cost-effective and reliable operation. Issues, including open versus closed culturing systems, batch versus continuous operations, or materials used and reactor configurations, need in-depth investigations, which are often case-by-case considerations (Mata et al., 2010). As the systems are primarily operated in outdoor conditions, operational conditions such as mixing, retention time, aeration, nutrient or contamination control should be optimized to cope with the dynamic variation of external factors like light, temperature, or precipitation (Yashavanth et al., 2021). A hybrid cultivation system was proposed by Narala et al. (2016), which combined a pressure air lift-driven bioreactor that provided relatively controlled conditions for microalgal exponential growth with an open raceway pond that offered nutrient-depleting conditions for the lipid accumulation process. The hybrid system demonstrated a significant improvement in microalgal growth rate, with more effective light utilization, while also decreasing the likelihood of biomass contamination by grazers (Narala et al., 2016).

High costs remain a significant barrier to the commercialization of PHB and other microalgae products. Currently, estimates of the cost of microalgae bioplastics are significantly higher compared to the costs of bioplastics from conventional microbial producers (2–6 \$ kg^{-1}) and conventional plastics derived from petrochemicals (1–2 \$ kg^{-1}) (Levett et al., 2016; López-Pacheco et al., 2022). The cost of the carbon source is a significant contributor, accounting for 30–50% of the total production cost of bioplastics. Therefore, developing an economically viable method for bioplastic production requires careful selection of an appropriate carbon source (Borges et al., 2024). Utilizing wastewater as a nutrient source for microalgae to produce PHB is an economic move, contributing to a significant reduction in input costs (K. Wang et al., 2022). The choice of large-scale cultivation systems was also of great importance, with open raceway ponds and closed photobioreactors being the most common types.

In comparison, a closed photobioreactor allows for better control of contamination, resulting in 3–5 times higher biomass productivity and 4–20 times lower land requirement, while the financial investment in the system is 3–10 times higher than that of open ponds (Mata et al., 2010). A techno-economic analysis revealed that the cost of microalgae biomass production ranged from 1.6 € kg^{-1} (open raceways) to 12.4 € kg^{-1} (closed photobioreactors), reflecting the significant difference in initial investment costs between these systems (Llamas et al., 2021). Using wastewater as a nutrient source could reduce the cost of biomass production in open ponds to 0.60 € kg^{-1}. Furthermore, PHB production from microalgae in wastewater and flue gases could reduce costs from 3 € kg^{-1} to 1.8 € kg^{-1} (Saratale et al., 2024). This highlights the practical and economic relevance of these findings, positioning microalgae as a cost-effective alternative for PHB production, especially in the context of resource recovery from waste streams. In the next step, further studies should focus on optimizing the growth and PHB production of microalgae in wastewater for large-scale applications. Moreover, the influence of different contaminants found in wastewater, such as predators and heavy metals, on the system's performance must also be investigated.

4. Conclusions

This study investigated the production of PHB by four microalgae, specifically *Scenedesmus* sp. SCE, *Chlorella* sp. HB, *Chlorella* sp. CNK, and *Chlorella* sp. CNA, and their potential in combining wastewater nutrient removal, biomass growth, and PHB production. All four microalgae tested were capable of accumulating PHB, with the highest capacities achieved by *Scenedesmus* sp. SCE and *Chlorella* sp. HB. These two strains also performed better in terms of biomass and Chl-a productivities. An organic carbon supplement in the form of sodium acetate showed an improvement in both biomass growth and PHB accumulation capacity, likely due to the mixotrophic growth of microalgae. The highest growth and PHB production were obtained from microalgae cultivated in domestic wastewater. All microalgae exhibited high wastewater remediation performance, with the best results observed in the case of *Scenedesmus* sp. SCE and *Chlorella* sp. HB. Wastewater analysis results showed that nutrient assimilation was the primary removal process, suggesting effective nutrient recovery by the tested microalgae. The potential of using microalgae SCE and HB for PHB production, coupled with wastewater nutrient recovery and biomass production, was evident. Further studies should focus on optimizing the larger-scale cultivation system.

Acknowledgements

The Ministry of Agriculture and Environment funded this work for the project TNMT.2023.562.05, and the Institute of Science and Technology for Energy and Environment, Vietnam Academy of Science and Technology (VAST), for the project No. CS.02/2024-2025.

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