Effects of nutrients and photoperiod on production of polyhydroxyalkanoates (PHAs) by thermophilic cyanobacteria

Alicia Lee Jia Wong
Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia

Anthony Nyangson Steven
Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia

Shamila Azman
Department of Water and Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia

Nur Izzati Mohd Noh
Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia

https://doi.org/10.5109/7395624

出版情報：Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 11, pp.933-938, 2025-10-30. International Exchange and Innovation Conference on Engineering & Sciences
バージョン：

Effects of nutrients and photoperiod on production of polyhydroxyalkanoates (PHAs) by thermophilic cyanobacteria

Alicia Lee Jia Wong[1], Anthony Nyangson Steven[2,3], Shamila Azman[3], Nur Izzati Mohd Noh[1]
[1]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, [2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, [3]Department of Water and Environmental Engineering. Faculty of Civil Engineering, Universiti Teknologi Malaysia.
izzati@utm.my

Abstract: *Bioplastic production using microorganisms offers a sustainable alternative to synthetic plastics. Among these microorganisms, cyanobacteria have shown significant potential for producing polyhydroxyalkanoates (PHA) due to their minimal requirement for growth. Light/dark cycles are important for cell division, metabolic reactions and reserve accumulation in cyanobacteria. Under environmental stress and nitrogen-limited conditions with excess carbon, cyanobacteria store carbon in the form of PHA in granules. This study explores the effects of nitrogen-limited condition with excess carbon under different photoperiods on biomass production and PHA accumulation in three thermophilic cyanobacteria strains isolated from Malaysia hot springs. The results showed various effects of the C:N ratio and photoperiods on biomass production and PHA accumulation, depending on the strain. FTIR analysis confirmed the presence of PHA in the strains, which exhibited characteristic components similar to those of commercial poly(3-hydroxybutyrate) (PHB). However, further research is needed to significantly enhance PHA accumulation under optimized conditions and to characterize the molecular structure of PHA.*

Keywords: Polyhydroxyalkanoates; Thermophilic cyanobacteria; Nutrients; Photoperiod

1. INTRODUCTION

The accumulation of plastic trash in oceans and land has driven a search for eco-friendly alternatives, such as bioplastics [1]. The development of biodegradable materials from non-feedstock autotrophic organisms ensures a sustainable supply of biomaterials [2]. Bioplastics, produced from renewable raw materials, offer a promising solution to address the environmental issues caused by petrochemical plastics. Several bio-based polymers, including polynucleotides, polyamides, polysaccharides, polyoxoesters, polythioesters, polyanhydrides, polyisoprenoids, and polyphenols, have emerged as potential substitutes because they are derived from natural, renewable resources like plants, animals, and microorganisms [3]. Among these, polyhydroxyalkanoates (PHAs), a group of polyoxoesters, have drawn significant focus due to their biodegradable and thermoplastic properties [4]. These materials are degradable by depolymerase enzymes produced by bacteria and fungi, which break down PHAs, and they also possess high melting points [5, 6]. PHAs are unique as they are directly synthesized by bacteria under nutrient-limited conditions in the presence of a carbon source [1, 7]. Under these conditions, microorganisms convert the available carbon sources into PHAs, which accumulate as insoluble granules within the cytoplasm, serving as intracellular energy reserves for long-term storage to support the bacteria's survival [8]. Poly-3-hydroxybutyrate (PHB), a widely recognized type of PHA, is known for its biodegradability and biocompatibility, positioning it as a viable eco-friendly substitute for traditional plastics [9].

PHAs are composed of hydroxyalkanoic acid monomers, which each unit features a hydroxyl and a carboxyl group forming ester bonds, with an R group side chain that varies between saturated and unsaturated alkyl groups [10]. PHAs can be categorized as homopolymers or copolymers. Homopolymers are composed of a single type of monomer, with poly(3-hydroxybutyrate) P(3HB) being the most extensively studied. PHB is a linear, isotactic thermoplastic polyester with high crystallinity and stiffness [11]. Copolymers contain two or more monomer types, such as P(3HB-co-4HB) and P(3HB-co-3HV-co-3HH) [12]. These exhibits enhanced flexibility and mechanical properties compared to homopolymers. PHAs are the only polymers fully synthesized by microorganisms, and more than 150 types of monomers have been identified [13].

Reportedly, production of PHAs relies primarily on heterotrophic microbes such as *Cupriavidus necator*, *Pseudomonas aeruginosa* or *Bacillus* sp. [7, 14, 15]. Continuous supply of carbon sources remains the main challenge in producing industrial yield of PHA from heterotrophic microbes. On the other hand, cyanobacteria offer a possible alternative for PHA production. These photosynthetic microorganisms synthesize PHAs autotrophically by utilizing inorganic carbon sources such as CO_2 or HCO_3^- along with sunlight, eliminating the need for intensive aeration [7]. Their minimal space and water requirements, combined with their dependence on sunlight for energy, make cyanobacteria a cost-effective and sustainable option for PHA production.

Previous studies have demonstrated that PHA accumulation in cyanobacteria can be significantly enhanced under nutrient-stress conditions, such as when carbon is in excess and nitrogen is limited [16, 17]. Similarly, variations in photoperiod, 16/8 h and 12/12 h light/dark cycles, have been suggested to improve photosynthetic efficiency which could further boost PHA production in the photosynthetic microbe [18]. Nitrogen concentration in growth media is also an important factor to increase production of fatty acids, the precursors of PHA, in cyanobacteria [19].

In this study, we cultivated thermophilic cyanobacterial strains in growth media consisting of different carbon:nitrogen (C:N) ratio under different photoperiods. Effects of the nitrogen-limited condition with excess carbon under different photoperiods on biomass production and PHA accumulation were evaluated. Our results are a contribution to understanding the capacity of thermophilic cyanobacteria as sustainable resource of bioplastic materials under the optimal nutrient supply and photoperiod condition.

2. MATERIALS

2.1 Cyanobacteria strains

Thermosynechococcus elongatus isolated from Ulu Slim hot spring, *Fischerella* sp. isolated from Kg. Cherana hot spring, and *Mastigocladus* sp. isolated from Ulu Kuang hot spring, Malaysia.

2.2 Chemicals for growth media

Cyanobacteria were maintained in 1xBG11 medium according to Rippka et al. [20] with modification (from 10xBG11 consists of 176 mM $NaNO_3$, 2.3 mM K_2HPO_4, 3.0 mM $MgSO_4.7H_2O$, 2.4 mM $CaCI_2.2H_20$, 0.3 mM Citric acid, 0.4 mM $(NH_4)_5[Fe(C_6H_4O_7)_2]$, 0.03 mM Na_2EDTA, 1.9 mM Na_2CO_3, 0.01 mM H_3BO_3, 2.3 µM $MnCI_2.4H_2O$, 0.19 µM $ZnSO_4.7H_2O$, 0.4 µM $CuSO_4.5H_2O$, 0.08 µM $Na_2MoO_4.2H_2O$, and 0.06 µM $Co(NO_3)_2.6H_2O$).

3. METHODS

3.1 Growth condition

Cyanobacteria strains were grown in 1xBG-11 at 40± 2ºC under continuous light photoperiod (24L:0D). The stock cultures were added with 5 mL of 10X BG11 at every 5 days throughout 21-day and 56-day of cultivation for unicellular and filamentous species, respectively. New subcultures were made after the 21-day or 56-day of cultivation for the respective species.

For analysis of biomass and PHA yield, cultures were grown in 1XBG-11 media (labelled as control onwards) and modified 1xBG-11 consists of carbon:nitrogen (C:N) ratio, 13:1 and 32.4:1 [17], under photoperiods of 24L:0D and 8L:16D as well as 12L:12D light/dark cycles. The C:N ratio was prepared by adjusting the amount of $NaNO_3$ used in the media. 1×BG-11 with a 13:1 C:N ratio contains 0.0124 g/L $NaNO_3$, whereas the 32.4:1 C:N ratio contains 0.0050 g/L $NaNO_3$. The culture were also grown under continuous light (24L:0D), 12 h light and 12h dark (12L:12D) or 8 h light and 16h dark (8L:16D).

3.2 Analysis of Biomass

Cell pellet was collected by centrifugation at 13,000 rpm and washed twice with distilled water. The cell pellet was weighed and dried in an oven at 70°C until a constant weight was achieved. The dried biomass was then weighed to determine the dry cell weight.

3.3 Extraction and quantification of PHA

Extraction of PHA was done according to the procedure by Nguyen et al. [17]. The cells were collected by centrifugation at 3,500 rpm for 20 min and washed twice with distilled water. The cells were then treated with 5% (v/v) sodium hypochlorite (NaClO) and incubated at 37 °C for 1 hour. Chloroform (10 mL) was added and the mixture was agitated for 1 h. Following centrifugation at 3,500 rpm for 20 min, the lower phase containing PHA in chloroform was transferred to a clean glass vial and evaporated to a volume of 2 mL. Cold methanol was added to the chloroform solution at a 1:4 (v/v) ratio, and the mixture was stored at −20°C overnight to precipitate the PHA. The precipitated PHA was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The PHA pellet was dried to a constant weight and weighed. PHA accumulation (%) was calculated using the formula below:

$$\% \text{ PHA} = ((\text{Mass of PHA in grams})/(\text{Mass of dry biomass in grams}))\times 100$$

3.3 Fourier transform infrared (FT-IR) analysis

FT-IR spectroscopy was employed for qualitative analysis of functional groups in the extracted PHA samples, based on the protocol by [21]. The PHA extracted functional groups characterization was performed using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy with a PerkinElmer Frontier FT-IR spectrometer (PerkinElmer, England). Spectral data were collected over a range of 4000 to 650 cm^{-1}. Samples were scanned 30 times at a 4 cm^{-1} resolution. The resulting pattern of functional groups were compared to the PHB standards (CAS number: 26744-04-7 and 26063-00-3).

4. RESULTS AND DISCUSSION

4.1 Effects of C:N ratio and photoperiod on biomass production

The nutrient requirement and photoperiod exposure to enhance biomass production were varied among the cyanobacteria species studied (Fig. 1). The experiments were conducted using non-enriched BG-11 media and inorganic carbon-enriched BG-11 media with different C:N ratios to identify the effects of nitrogen-limited conditions with excess carbon under various photoperiod conditions on biomass production of the filamentous *Mastigocladus* sp. and *Fischerella* sp., as well as the unicellular *Thermosynechococcus elongatus*.

The biomass production of *Mastigocladus* sp. was significantly highest under 8L:16D in 1xBG-11 with 13:1 C:N ratio compared to other photoperiod conditions when using the same media ($P< 0.05$) (Fig. 1a). A similar pattern was observed when *Mastigocladus* sp. was grown in the control media, although lower biomass production was obtained compared to when the 13:1 C:N ratio was used. The biomass production of *Mastigocladus* sp. was slightly increased when cultivated under constant light in 1xBG-11 with 32.4:1 C:N ratio, with no significant difference between all photoperiods. The results suggest that prolonged dark period can enhance biomass production of *Mastigocladus* sp. especially when nitrogen concentration is low. This filamentous and heterocyst-forming genus of cyanobacteria conducts oxygenic photosynthesis during the day and nitrogen fixation at night. During oxygenic photosynthesis, carbon fixation occurs which turning inorganic carbon into organic molecules that cells can use for energy storage and biosynthesis [22]. Thus, a significant increase of biomass production was observed in inorganic carbon-enriched media with low nitrogen concentration under prolonged dark, indicating that the dark period may be necessary for cell maintenance [23] in *Mastigocladus* sp. to ensure nighttime survival until the light present. Noteworthy, biomass production of *Mastigocladus* sp. in

1xBG-11 with 32.4:1 C:N ratio was limited in some manner by very high inorganic carbon and low nitrogen concentrations in these cultures.

On the other hand, the biomass production of *Fischerella* sp. was significantly highest under constant light (24L:0D) in non-enriched 1xBG-11 media (control) compared to when the cultures were grown under constant light (24L:0D) in inorganic carbon-enriched 1xBG-11 media (Fig. 1b). The results showed that C:N ratio was not a significant factor for biomass production of *Fischerella* sp. in the presence of light/dark cycles (P> 0.1). This filamentous genus is a heterocyst-forming cyanobacteria, which might favor the cells to conduct nitrogen fixation even in the presence of light. Heterocysts accommodate the oxygen-sensitive process of nitrogen fixation that occurs at nighttime (Kumar 2010). The slight increase in biomass production under constant light (24L:0D) in inorganic carbon-enriched 1×BG-11 medium may indicate the role of heterocysts in metabolic activity, although the difference was not significant when *Fischerella* sp. grew under 12L:12D and 8L:16D.

Light/dark cycles play important roles in enhancing the biomass production of *Thermosynechococcus elongatus* (Fig. 1c). The significant increase of biomass production of *Thermosynechococcus elongatus* (P<0.05) in the presence of light/dark cycles corroborate with the fact that this freshwater unicellular species are non-heterocyst species which are known to be able to conduct nitrogen fixation predominantly at night [24, 25]. The results also showed that C:N ratio was not a significant factor for biomass production of *Thermosynechococcus elongatus* in the presence of light/dark cycles (P> 0.1).

4.1 Effects of C:N ratio and photoperiod on PHA accumulation

Cyanobacteria can naturally synthesize PHA as carbon storage for survival under environmental stress conditions. This study investigates the hypothesis that high carbon concentration and light stress can induce PHA biosynthesis. The results showed high PHA accumulation, between 0.52% of PHA in its dried biomass (DCW) to 3.79% DCW, in *Thermosynechococcus elongatus* compared to *Mastigocladus* sp. (0.22% to 0.46% DCW) and *Fischerella* sp. (0.06% to 0.28% DCW) (Fig. 2).

Mastigocladus sp. has the potential to produce PHA, however, studies on PHA accumulation in this filamentous species are limited. The highest PHA accumulation occurred in *Mastigocladus* sp. grew in 1xBG-11 with 32.4:1 C:N ratio under 12L:12D, although no significant difference to the cultures grew in non-enriched BG-11 (control) and 1xBG-11 with 13:1 C:N under the same photoperiod (Fig. 2a). The results showed that PHA accumulation was enhanced in *Mastigocladus* sp. when grown under 12L:12D and 8L:16D, indicating the roles of light/dark cycles in cellular process and PHA accumulation. Moreover, previous studies have shown that alternating light and dark cycles, instead of constant illumination, enhance PHA production due to cell division that predominantly occurs during the light phase and reserve accumulation during the dark phase [26, 27]. Noteworthy, nitrogen-limited conditions with excess carbon may not be a significant factor to increase PHA accumulation in *Mastigocladus* sp. as there is no significant difference between cultures in non-enriched BG-11 and inorganic carbon-enriched BG-11 media.

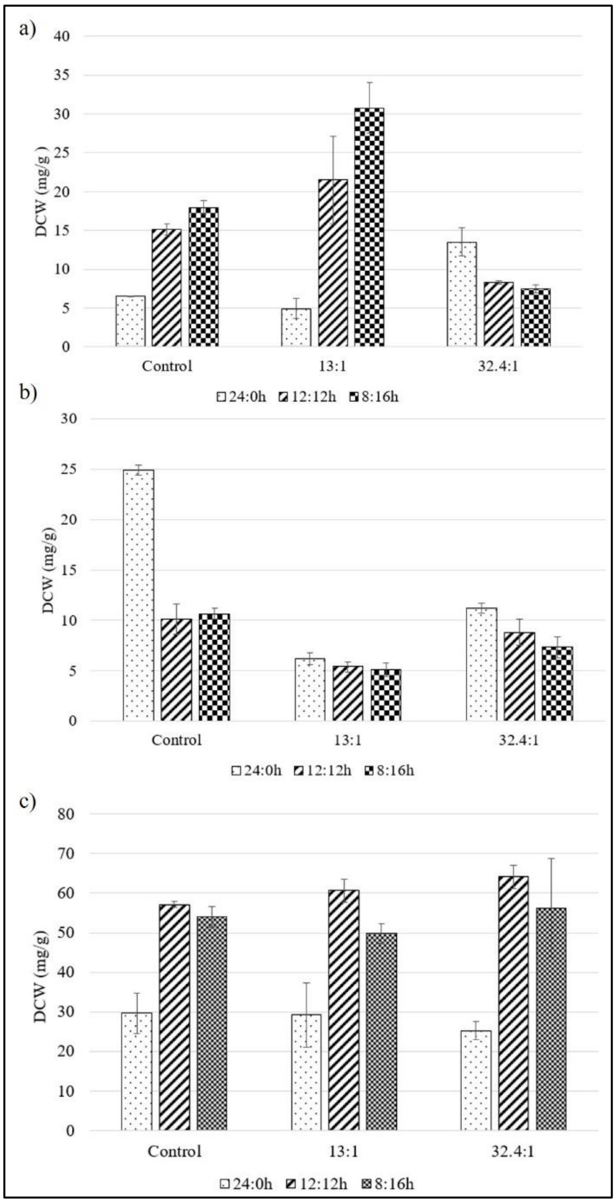

Fig. 1. Biomass production of a) *Mastigocladus* sp., b) *Fischerella* sp. and c) *Thermosynechococcus elongatus* in different nutrient and photoperiods. The photoperiods were 24:0h (24L:0D), 12:12h (12L:12D) and 8:16h (8L:16D). Control: Culture grown in 1xBG11 media

Another genus of cyanobacteria that are not well-studied for their potential to produce PHA is *Fischerella* sp. The results showed that PHA accumulation in *Fischerella* sp. was the lowest in all media and photoperiod used for growth compared to *Mastigocladus* sp. and *Thermosynechococcus elongatus* (Fig. 2b). The nitrogen-limited conditions with excess carbon cannot enhance PHA production in *Fischerella* sp.

PHA accumulation in *Thermosynechococcus elongatus* was significantly enhanced in 1xBG-11 with 13:1 C:N ratio when grown under constant light (24L:0D) compared to 12L:12D and 8L:16D (P<0.05) (Fig. 2c). The result indicates that constant light stress and nitrogen-limited BG-11 promote PHA accumulation in *Thermosynechococcus elongatus*. In prolonged light conditions, cell division rate may increase and result in nutrient starvation in cyanobacteria thus enhances carbon storage in the form of PHA granules [28, 29]. Besides, PHA accumulation in cyanobacteria typically occurs under nutrient-limited conditions [17]. However, the growth media with highly excessive carbon and very low nitrogen (32.4:1 C:N ratio) did not favor PHA accumulation in the unicellular species. Photoperiods also did not result in significant increase of PHA accumulation in non-enriched BG-11 and BG-11 with 32.4:1 C:N ratio. In previous studies, PHA accumulation of 14.5% DCW and 55% DCW was reported in *Thermosynechococcus elongatus* and *Synechococcus* MA19, respectively, under optimized growth conditions [30, 10]. Such differences in PHA accumulation between the studied species and literature data suggest that further optimization of other growth conditions, such as light intensity and temperature, may enhance PHA accumulation. Reportedly, excess light intensity and low temperature during the dark phase enhance PHA accumulation in cyanobacteria [28, 31].

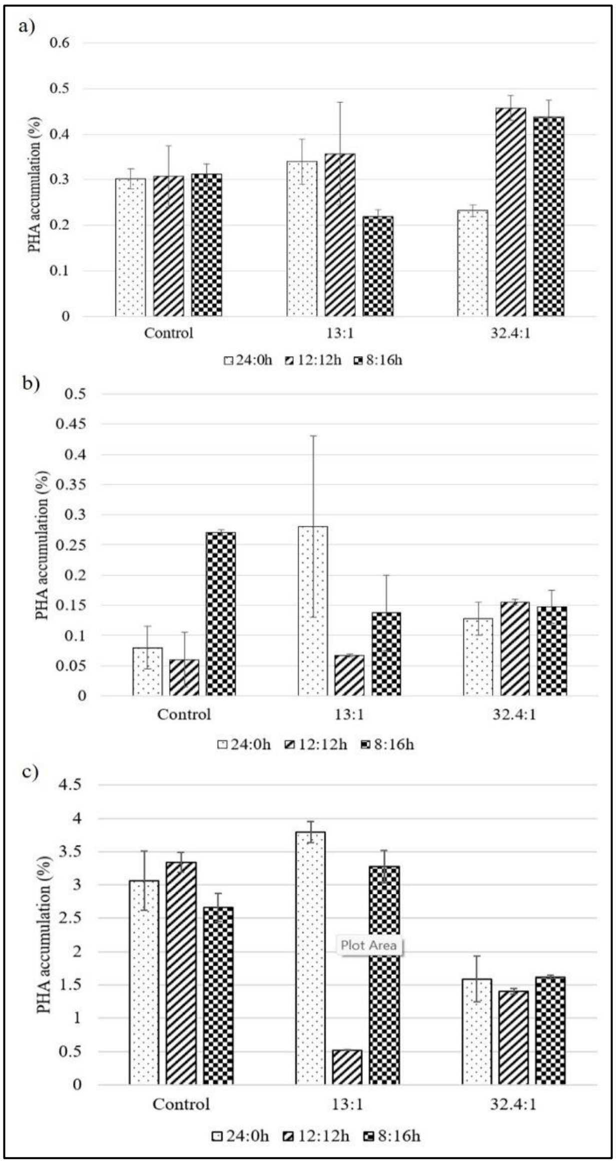

Fig. 2. PHA production (%) of a) *Mastigocladus* sp., b) *Fischerella* sp. and c) *Thermosynechococcus elongatus* in different nutrient and photoperiods. The photoperiods were 24:0h (24L:0D), 12:12h (12L:12D) and 8:16h (8L:16D). Control: Culture grown in 1xBG11 media

4.1 Characterization of PHA from *Mastigocladus* sp., *Fischerella* sp. and *Thermosynechococcus elongatus*

The FTIR spectra in Fig. 3 demonstrates the prominent peaks at 2956, 2922 and 2848 cm^{-1} for PHA extracted from *Mastigocladus* sp., *Fischerella* sp. and *Thermosynechococcus elongatus*. A previous study reported that the presence of the peaks in the range of 2848–2976 cm^{-1} indicated the C–H stretching vibration of the methyl ($-CH_3$) and methylene ($-CH_2$) groups of the PHA extracted from *Anabaena platensis* (*A. platensis*) NLNA2, *A. platensis* NLHT3, *A. platensis* NNBQN1, and *Microcystis aeruginosa* DTB1 [32]. These groups are characteristic components of the PHA polymer backbone. The PHA from *Mastigocladus* sp., *Fischerella* sp. and *Thermosynechococcus elongatus* possess similar characteristic components with the commercialized PHB standards (CAS number: 26063-00-3 and CAS number: 26744-04-7). Specifically, the peaks at 1456, 1389, 1224, 1131, 1094, 1050 and 828 cm^{-1} were observed for all the cyanobacterial species studied and the commercialized PHB standards. Similar to this study, peak at 1457 cm^{-1} and 1380 cm^{-1} was also presence in the PHA extracted from *Bacillus megaterium* which are attributed to the stretching vibration of the $-CH_2$ and $-CH_3$, respectively [33]. The commercialized PHB standards are synthesized from bacteria, thus suggest similar characteristic components of PHA produced by cyanobacteria and heterotrophic bacteria, for example, *Bacillus megaterium*. Other characteristics components observed at the peak 1094 cm^{-1} and 828 cm^{-1} the symmetric and asymmetric stretching modes of the –C-O and –C-C groups, which also observed in the PHA obtained from *Pseudomonas aeruginosa* TISTR 1287 [34]. Since FTIR analysis is still only an indirect method for PHA characterization in bacterial cells, other types of compounds and biopolymers may show peaks at wavenumbers similar to PHA. Further analysis using gas chromatography-mass spectrometry (GC-MS) worth to be used to confirm the molecular components of PHA from *Mastigocladus* sp., *Fischerella* sp., and *Thermosynechococcus elongatus*.

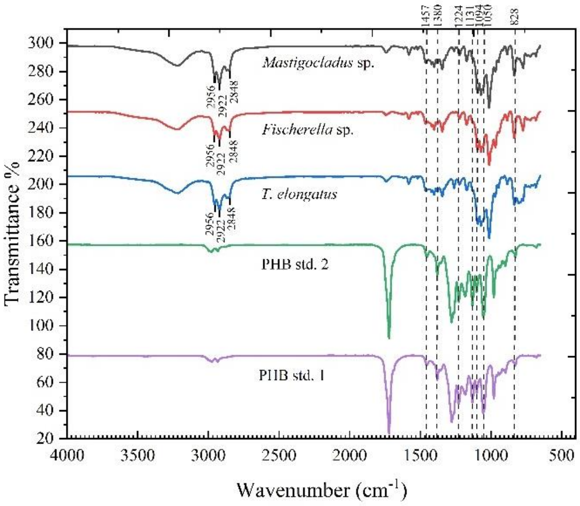

Fig. 3. FTIR spectra of PHA from *Mastigocladus* sp., *Fischerella* sp., *Thermosynechococcus elongatus*, PHB standard CAS number: 26063-00-3 (PHB std. 2) and PHB standard CAS number: 26744-04-7 (PHB std. 1).

5. REFERENCES

[1] G. García, J. E. Sosa-Hernández, L. I. Rodas-Zuluaga, C. Castillo-Zacarías, H. Iqbal, R. Parra-Saldívar, Accumulation of PHA in the microalgae Scenedesmus sp. under nutrient-deficient conditions, Polymers 13 (2020) 131.

[2] M. C. Alvarado, S. G. N. Polongasa, P. D. C. Sanchez, A preliminary evaluation on the development of edible drinking straw from Guso (Eucheuma cottonii) seaweeds, Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES), 9 (2023) 51-58.

[3] Z. Aziz, Investigating microbial quorum sensing potential for enhanced production of biodegradable polymers, PhD thesis, University of Westminster, 2021.

[4] S. Shah, A. Kumar, Production and characterization of polyhydroxyalkanoates from industrial waste using soil bacterial isolates, Braz J Microbiol 52 (2021) 715-726.

[5] S. A. Acharjee, P. Bharali, B. Gogoi, V. Sorhie, B. Walling, Alemtoshi, PHA-based bioplastic: a potential alternative to address microplastic pollution. Water Air Soil Pollut. 234 (2022) 21.

[6] P. Basnett, S. Ravi, I. Roy, Natural bacterial biodegradable medical polymers: Polyhydroxyalkanoates, in: X. Zhang (Ed.), Science and Principles of Biodegradable and Bioresorbable Medical Polymers, Cambridge, Woodhead Publishing 2017, pp. 257-277.

[7] E. Rueda, E. Gonzalez-Flo, S. Mondal, K. Forchhammer, D. M. Arias, K. Ludwig, B. Drosg, I. Fritz, C. R. Gonzalez-Esquer, S. Pacheco, J. García, Challenges, progress, and future perspectives for cyanobacterial polyhydroxyalkanoate production, Rev. Environ. Sci. Biotechnol. 23 (2024) 321-350.

[8] A. Alsaadi, S. S. K. Ganesen, T. S. M. Amelia, R. Moanis, E. Peeters, S. Vigneswari, K. Bhubalan, Polyhydroxyalkanoate (PHA) biopolymer synthesis by marine bacteria of the Malaysian coral triangle region and mining for PHA synthase genes, Microorganisms 10 (2022) 2057.

[9] D. Vicente, D. Proença, P. Morais, The role of bacterial polyhydroalkanoate (PHA) in a sustainable future: A Review on the biological diversity. Int. J. Environ. Res. Public Health 20, (2023) 2959.

[10] A. K. Singh, L. Sharma, N. Mallick, J. Mala, Progress and challenges in producing polyhydroxyalkanoate biopolymers from cyanobacteria, J. Appl. Phycol. 29 (2017) 1213-1232.

[11] R. G. Martins, I. Severo Gonçalves, M. G. de Morais, J. A. V. Costa, Bioprocess engineering aspects of biopolymer production by the cyanobacterium spirulina strain LEB 18, Int. J. Polym. Sci. (2014) 895237.

[12] X. Liang, D. K. Cha, Q. Xie, Properties, production, and modification of polyhydroxyalkanoates, Resour. Conserv. Recycl. Adv. 21 (2024) 200206.

[13] J. Mai, K. Kockler, E. Parisi, C. M. Chan, S. Pratt, B. Laycock, Synthesis and physical properties of polyhydroxyalkanoate (PHA)-based block copolymers: A review, Int. J. Biol. Macromol. 263 (2024) 130204.

[14] R. E. Martínez-Herrera, M. E. Alemán-Huerta, O. M. Rutiaga-Quiñones, E. d. J. de Luna-Santillana, T. O. Elufisan, A comprehensive view of Bacillus cereus as a polyhydroxyalkanoate (PHA) producer: A promising alternative to petroplastics, Process Biochem. 129 (2023) 281-292.

[15] R. Chandra, A. Thakor, T. H. Mekonnen, T. C. Charles, H.-S. Lee, Production of polyhydroxyalkanoate (PHA) copolymer from food waste using mixed culture for carboxylate production and Pseudomonas putida for PHA synthesis, J. Environ. Manag. 336 (2023) 117650.

[16] A. Kaewbai-Ngam, A. Incharoensakdi, T. Monshupanee, T., Increased accumulation of polyhydroxybutyrate in divergent cyanobacteria under nutrient-deprived photoautotrophy: An efficient conversion of solar energy and carbon dioxide to polyhydroxybutyrate by Calothrix scytonemicola TISTR 8095, Bioresour. Technol. 212 (2016) 342-347.

[17] T. L. A. Nguyen, H. Thi Thu Tran, T. M. T. Quach, Y. H. Dao, H. C. Duong, O. Thi Doan, T. Thi Duong, L. Thi Thu Tran, Biopolymer polyhydroxyalkanoate production from Arthrospira platensis NLHT3 cyanobacterium isolated in Vietnam, Environ. Technol. Innov. 36 (2024) 103841.

[18] L. H. Sipaúba-Tavares, M. G. Tedesque, D. C. Fenerick, R. N. Millan, B. Scardoeli-Truzzi, Effect of light/dark cycles on the growth of Haematococcus pluvialis in mixotrophic cultivation with alternative culture media, Biotechnol. Res. Innov. 6 (22022) e2022202.

[19] M. S. Islam, M. M. Rahman, B. B. Saha, Full factorial experimental design for growth of Spirulina platensis and valuable products enhancement, Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES) 6 (2020) 299-304.

[20] R. Rippka, J. Deruellas, J.B. Waterbury, M. Herdman, R.Y. Stainer, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, Microbiology 111 (1979) 1-61.

[21] A. Kettner, M. Noll, M., C. Griehl, Leptolyngbya sp. NIVA-CYA 255, a promising candidate for Poly(3-hydroxybutyrate) production under mixotrophic deficiency conditions, Biomolecules 12 (2022) 504.

[22] D. Noreña-Caro, M. G. Benton, Cyanobacteria as photoautotrophic biofactories of high-value chemicals, J. CO_2 Util. 28 (2018) 335–366.

[23] D. G. Welkie, B. E. Rubin, S. Diamond, R. D. Hood, D. F. Savage, S. S. Golden, A Hard day's night: Cyanobacteria in diel cycles, Trends Microbiol. 27 (2019) 231-242.

[24] P. Fay, Oxygen relations of nitrogen fixation in cyanobacteria, Microbiol Rev. 56 (1992) 340-73.

[25] A. A. Issa, M. Hemida, T. Ohyam, T., Nitrogen Fixing Cyanobacteria: Future Prospect, in Advances in Biology and Ecology of Nitrogen Fixation, InTech. 2014.

[26] D. M. Arias, J. García, E. Uggetti, Production of polymers by cyanobacteria grown in wastewater: Current status, challenges and future perspectives, New Biotech. 55 (2020) 46-57.

[27] E. Jacob-Lopes, S. Revah, S. Hernández, K. Shirai, T. T. Franco, Development of operational strategies to remove carbon dioxide in photobioreactors, Chem. Eng. J. 153 (2009) 120–126

[28] L. H. Gracioso, A. Bellan, B. Karolski, L. O. B. Cardoso, E. A. Perpetuo, C. A. O. do Nascimento, R. Giudici, V. Pizzocchero, M. Basaglia, T. Morosinotto, Light excess stimulates Poly-beta-hydroxybutyrate yield in a mangrove-isolated strain of Synechocystis sp., Bioresour. Technol. 320 (2021) 124379.

[29] D. G. Gradíssimo, L. P. Xavier, A. V. Santos, Cyanobacterial polyhydroxyalkanoates: A sustainable alternative in circular economy, Molecules 25 (2020) 4331.

[30] J. O. Eberly, R. L. Ely, Photosynthetic accumulation of carbon storage compounds under CO2 enrichment by the thermophilic cyanobacterium Thermosynechococcus elongatus, J. Ind. Microbiol. Biotechnol. 39 (2012) 843–850.

[31] K. Tanaka, M. Kishi, H.Assaye, T. Toda, Low temperatures in dark period affect biomass productivity of a cyanobacterium Arthrospira platensis, Alg. Res. 52 (2020) 102132,

[32] T. T. H. Pham, T. L. A. Nguyen, T. T. Duong, O. T. Doan, H. T. T. Tran, L. T. T. Tran, Selection of microalgae and cyanobacteria to produce polyhydroxyalkanoates (PHAs) - A case study in Vietnam, Case Stud. Chem. Environ. Eng. 10 (2024) 100808.

[33] M. A. Porras, M. A. Cubitto, M. A.Villar, Quantitative determination of intracellular PHA in Bacillus megaterium BBST4 strain using mid FTIR spectroscopy, XIV Latin American Symposium on Polymers, 2014.

[34] P. Tanikkul, G. L. Sullivan, S. Sarp, N. Pisutpaisal, Biosynthesis of medium chain length polyhydroxyalkanoates (mcl-PHAs) from palm oil, Case Stud. Chem. Environ. Eng. 2 (2020) 100045.