symmetry

MDPI

Article

Complete Epoxy Phosphonate Conversion to Dimethyl (1*E*)-3-Hydroxyprop-1-Enylphosphonate with Photobiocatalysts' Assistance

Sunday Ocholi Samson, Monika Serafin-Lewańczuk *, Małgorzata Brzezińska-Rodak, Magdalena Klimek-Ochab and Ewa Żymańczyk-Duda *

Department of Biochemistry, Molecular Biology and Biotechnology, Faculty of Chemistry, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland; sunday.samson@pwr.edu.pl (S.O.S.); malgorzata.brzezinska-rodak@pwr.edu.pl (M.B.-R.); magdalena.klimek-ochab@pwr.edu.pl (M.K.-O.)

* Correspondence: monika.serafin-lewanczuk@pwr.edu.pl (M.S.-L.); ewa.zymanczyk-duda@pwr.edu.pl (E.Ż.-D.); Tel.: +48-713202597 (E.Ż.-D.)

Abstract: Phosphonates derivatives are compounds of interests and are applied as drugs of, e.g., antibacterial antiviral activities, connected with their inhibitory activity towards different enzymes, which is related to the configuration of particular compound isomers. The biological synthesis of such molecules is the method of choice and can be carried out using enzymes or whole cells from organisms. Photobiocatalysts employed in the bioconversion of epoxymethyl dimethyl phosphonate are able to convert this substrate into a pure geometric isomer of the unsaturated product, dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate, which is a rare and expensive compound of high added value. Six different strains were screened towards dimethyl epoxy phosphonate and in the case of *Synechococcus bigranulatus*, over 99% conversion was achieved. The product structure was confirmed with Mass Spectroscopy (MS); ^{1}H, ^{13}C, ^{31}P, and 2D Nuclear Magnetic Resonance (NMR); and Infrared Spectroscopy (IR).

Keywords: biotransformation; cyanobacteria; high value-added compounds; phosphonates; photobiocatalysts

check for updates

Citation: Samson, S.O.; Serafin-Lewańczuk, M.; Brzezińska-Rodak, M.; Klimek-Ochab, M.; Żymańczyk-Duda, E. Complete Epoxy Phosphonate Conversion to Dimethyl (1*E*)-3-Hydroxyprop-1-Enylphosphonate with Photobiocatalysts' Assistance. *Symmetry* **2024**, *16*, 1676. https://doi.org/10.3390/sym16121676

Academic Editor: György Keglevich

Received: 11 November 2024
Revised: 12 December 2024
Accepted: 13 December 2024
Published: 18 December 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Organophosphorus compounds, especially phosphonates, are significant in a wide array of industrial, agricultural, and pharmaceutical applications due to their unique chemical and biological activities. The stable carbon–phosphorus (C–P) bond in phosphonates imparts excellent resistance to both chemical hydrolysis and enzymatic degradation, making these compounds highly valuable. This stability is important for improving biologically active molecules, including enzyme inhibitors (by imitating the transition state of phosphorylated intermediates), antimicrobial agents, and antiviral drugs [1]. The synthesis and application of phosphonates in pharmaceuticals influence their ability to mimic natural phosphate esters, thereby inhibiting a wide range of enzymes and initiating pathways for treating several ailments, including infectious diseases, cancer, and inflammatory disorders [2–5].

Epoxymethyl dimethyl phosphonate (EMDP) represents a crucial intermediate in organophosphorus chemistry as its unique structure, characterized by an epoxide group and a phosphonate moiety, provides extensive reactivity and versatility in synthetic applications. The epoxide group is exceptionally remarkable for its nucleophilic ring-opening reactions, while the phosphonate group contributes to the stability and bioactivity of the resulting molecules [6]. EMDP can be exploited as a precursor in the synthesis of several phosphonates, including hydroxyalkyl and vinyl phosphonates, which are fundamental in the development of pharmaceuticals and agricultural chemicals [7–9]. The availability of

the epoxide group in phosphonates can enhance the formation of new stereocenters, a feature that is vital for drug discovery and development [10]. Conventional chemical synthesis of phosphonates from EMDP often requires harsh conditions, such as strong acids or bases, elevated temperatures, and environmentally hazardous solvents. These conditions can lead to undesired side reactions and low selectivity [11,12], prompting the current research for greener, more feasible approaches. Hence, biocatalysis, which employs enzymes or whole-cell systems, has emerged as a promising alternative to conventional chemical synthesis. Biocatalysis renders the benefits of ecofriendly reaction conditions, higher selectivity, and fewer by-products, aligning with the principles of green chemistry [13–15].

The final obtained product, dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate (DMHP), synthesized from EMDP via photobiocatalytic processes, is a phosphonate of significant interest due to its biological and pharmacological prospectives (Figure 1). The obtained product is a precursor of dimethyl (1*E*)-3-oxoprop-1-enylphosphonate used in the synthesis of β-lactams with antibacterial properties [16]. This compound presents a hydroxyl group adjacent to an unsaturated carbon–phosphorus bond which is relatively stable and resistant to hydrolysis, distinguishing it from more common carbon–oxygen–phosphorus (C–O–P) bonds available in phosphates, making it a versatile intermediate for further functionalization and drug development [17,18]. The structural properties of dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate allow its application as a scaffold for designing enzyme inhibitors and other biologically active molecules, particularly in antiviral and anticancer research [3,5,19]. The availability of the hydroxyl group conveys the compound with potential reactivity in condensation reactions, allowing for the formation of hydrogen bonds, influencing its solubility and interaction with biological targets such as the active sites of enzymes, potentially inhibiting key biological processes. For instance, it can act as a transition-state analog in the inhibition of phosphatases or other enzymes that process phosphate esters [20,21]. The presence of a double bond between the second and third carbon atoms signals a degree of unsaturation, making the molecule more reactive in certain types of chemical reactions, including addition and oxidation reactions. The phosphonate group imparts several vital properties, including acidity (due to the presence of acidic protons on the phosphonic acid moiety), coordination ability with metal ions, and potential bioactivity due to its imitation of phosphate esters, which are common in biological systems.

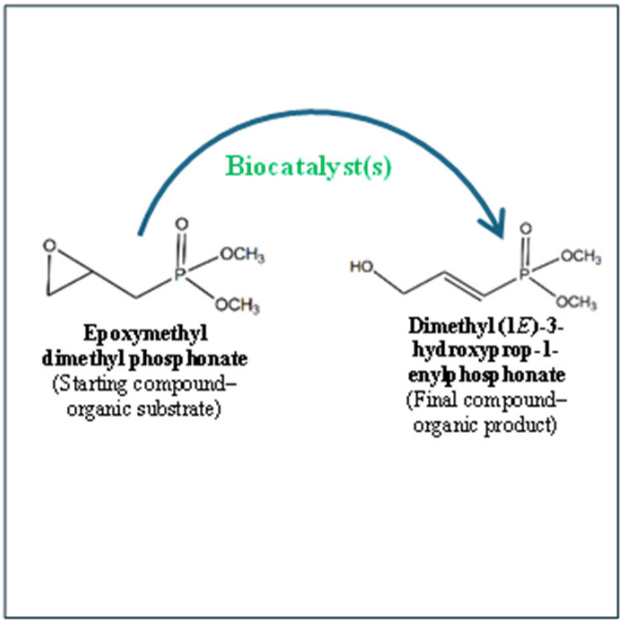


Figure 1. Cascade of photobiocatalysis.

Applications of DMHP in pharmaceuticals, agrochemicals, and research and development highlight its economic value. DMHP as a phosphonate derivative, could serve

as a precursor or intermediate in producing bioactive compounds, especially for antiviral and anticancer drugs. Its structure and configuration make it valuable in synthesizing analogs of natural metabolites, contributing to the development of enzyme inhibitors and potential therapeutic agents. It could also be useful in producing herbicides and pesticides, improving crop yields and addressing food security challenges. DMHP's potential role in chemical synthesis makes it indispensable in creating novel compounds, and enhancing advancements in biotechnology. Despite the high value of dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate, its large-scale production utilizing standard chemical methods is quite challenging. Problems associated with stereoselectivity and yield make it difficult to achieve high purity and efficiency [22–24]. This challenge has drawn attention to biocatalysis as an alternative production method.

Cyanobacteria are photosynthetic microbes that have received attention for their potential in biotechnological applications as biocatalysts due to their ability to convert light energy into biochemical processes. The strains investigated in this study include *Synechococcus bigranulatus*, *Nostoc cf-muscorum*, *Limnospira indica*, *Limnospira maxima*, *Leptolyngbya foveolarum*, and *Nodularia moravica*, and represent a diverse selection of cyanobacteria, each with unique metabolic characteristics influencing their usefulness as photobiocatalysts. *Synechococcus* species are well-known for their fast growth rates, effective photosynthetic systems, and metabolic flexibility. *Synechococcus bigranulatus* demonstrated a high potential for photobiocatalysis in this study [25]. This strain's photocatalytic potential can be ascribed to its efficient light-harvesting competences and robust metabolic pathways, which optimize energy transfer for enzymatic reactions and reinforce the transformation of several substrates [26,27]. In addition, genetic engineering research has improved the photosynthetic efficiency and biocatalytic capacities of *Synechococcus* strains, making them attractive microorganisms for industrial applications [28,29]. *Nostoc cf-muscorum* is a filamentous cyanobacterium known for its nitrogen-fixing abilities and flexibility under low light, nutrient availability, and robust environmental adaptability [30]. The lower biocatalytic activity observed in this study may result from its slower growth rates and reduced photosynthetic efficiency under laboratory conditions [31,32]. *Nostoc* species often distribute significant metabolic resources to nitrogen fixation, which may constrain their capacity for other biocatalytic processes [30]. Thus, *Nostoc* strains might be appropriately suited for applications requiring stress tolerance and nitrogen fixation rather than high-rate chemical conversions. *Limnospira* species, earlier classified under the Spirulina genus, are identified for their high biomass productivity and adaptability. Both *Limnospira indica* and *Limnospira maxima* achieved significant conversion rates as *Synechococcus bigranulatus* in this study. Their high efficiency in photobiocatalysis is probably due to their effective photosynthetic machinery and ability to grow well under diverse controlled intensities of light [33–36]. *Limnospira* species are generally employed in industrial applications for biofuel production, nutraceuticals, and high-value chemicals due to their photosynthetic efficiency and scalability [37]. *Leptolyngbya foveolarum* is a filamentous cyanobacterium identified for its resistance in extreme environments, such as thermal springs and hypersaline conditions [38]. Although less studied in the context of biocatalysis, *Leptolyngbya* species have demonstrated promise in environmental applications, including the bioremediation of heavy metals and organic pollutants [39,40]. In this study, *Leptolyngbya foveolarum* demonstrated efficient conversion competence for EMDP, implying that its metabolic pathways assist organophosphorus transformations, although at a lower rate compared to *Synechococcus* and *Limnospira* strains. Future research could focus on optimizing reaction conditions and potentially engineering *Leptolyngbya* strains to boost their photobiocatalytic performance [41,42]. *Nodularia* species are primarily acknowledged for their nitrogen-fixing capacities and symbiotic relationships with plants in nutrient-deficient environments [43,44]. *Nodularia* sp., a filamentous strain, has been investigated for ecological applications, such as biofertilization and soil conditioning [45,46]. In the context of biocatalysis, *Nodularia* sp. exhibited reasonable conversion rates for EMDP, while not as efficient as *Synechococcus* strain or *Limnospira* strains in photobiocatalytic processes, *Nodularia*'s unique metabolic attributes, including

nitrogen fixation and tolerance to low-nutrient environments, could make it valuable for applications requiring multifunctional biocatalysis [47].

Comparative analysis of the cyanobacterial strains in this study features significant variations in their competencies for the photobiocatalytic conversion of EMDP to dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate. *Synechococcus bigranulatus* and *Limnospira* species exhibited great conversion rates and limited side reactions, attributed to their efficient photosynthetic systems and metabolic flexibility [48–53]. In contrast, *Nostoc cf-muscorum*, *Leptolyngbya foveolarum*, and *Nodularia* sp. exhibited moderate conversion efficiencies, possibly due to their metabolic focus on nitrogen fixation or resilience in extreme environments rather than high-rate biocatalysis of phosphonates [25,54–56]. In addition, previous research emphasizes the capabilities of *Leptolyngbya foveolarum*, *Nostoc cf-muscorum*, and *Nodularia* sp. as promising biocatalysts, particularly in the enantioselective synthesis of chiral phosphonates [25]. *Nodularia* species in previous investigation demonstrated a significant degree of conversion and optical purity in producing diethyl (*S*)-2-hydroxy-2-phenylethylphosphonate, indicating its potential in biocatalysis despite metabolic adaptations toward resilience in extreme conditions like nitrogen fixation [25]. Recent developments in synthetic biology and metabolic engineering can offer promising prospects for advancing the performance of cyanobacteria in biocatalytic applications, enabling the feasible production of high-value products like dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate. For instance, research has revealed that engineered *Synechococcus elongatus* can efficiently produce C3 platform chemicals like glycerol, dihydroxyacetone, and 3-hydroxypropionic acid, which serve as precursors for biofuels and biodegradable materials [57]. This integration has the potential to minimize carbon footprints while generating valuable chemicals through photosynthesis [57–59]. In another development, the integration of Green Chemistry Technologies (such as cyanobacterial systems) with other sustainable technologies, such as enzyme immobilization or continuous flow reactors will further advance process efficiency and scalability of high-value organophosphorus compounds in a sustainable means [59–62].

This study aims to harness photobiocatalytic processes to facilitate the complete conversion of epoxymethyl dimethyl phosphonate into dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate. This is a rare compound with remarkable value due to its unique chemical structure and versatile potential in advanced pharmaceutical, agricultural, and industrial applications. By exploiting this innovative technique, future research could significantly advance the green synthesis of valuable phosphonates.

2. Materials and Methods

The organic substrate employed in the photobiocatalysis technique was epoxymethyl dimethyl phosphonate, sourced from SPECIFIC POLYMERS (Castries, France). Growth media components were purchased from Sigma Aldrich (St. Louis, MI, USA), Chempur (Śląskie, Poland), Avantor Performance Materials (Gliwice, Poland). The biomass of cyanobacterial strains and their growth media used include *Synechococcus bigranulatus*—CCALA 187 (BG-11 medium), *Nostoc cf-muscorum*—CCALA 129 (BG-10 medium), *Limnospira indica*—PCC 8005 (Spirulina medium), *Limnospira maxima*—CCALA 27 (Spirulina medium) *Leptolyngbya foveolarum*—CCALA 76 (BG-11 medium), and *Nodularia moravica*—CCALA 114 (BG-11 medium). These strains were obtained from the Culture Collection of Autotrophic Organisms (CCALA) at the Institute of Botany, Czech Academy of Sciences. The analytical instruments used include Mass Spectrometry (MS), Infrared Spectroscopy (IR), High-Performance Liquid Chromatography (HPLC), and Nuclear Magnetic Resonance (NMR). NMR spectra were measured on a Bruker Avance™ 600 (Billerica, MA, USA) at 600.58 MHz for ^{1}H and 243.12 MHz for ^{31}P or on a Jeol ECZ 400 S (Tokyo, Japan) at 161.92 MHz for ^{31}P. Chemical shifts (δ) were reported in ppm and coupling constants (J) were given in Hz. MS spectra were obtained using high-resolution mass spectrometer with analyzer time-of-flight (TOFMS) from Waters LCT PremierTM XE (Milford, MA, USA).

Growth media were prepared according to standard laboratory protocols [63]. Each strain was inoculated into separate 250 mL Erlenmeyer flasks containing 100 mL of the appropriate growth medium. The growth of the strains was monitored under a controlled photobioreactor (growth chamber) conditions: continuous illumination at 15–22 µmol photons/m^2/s (Power Glo fluorescent bulb, 30W, Hagen, QC, Canada) at 29 °C ± 1 °C under sterile conditions for 21 days. The photobiocatalytic reaction was initiated by adding 15 µL (16.6 mg) of epoxymethyl dimethyl phosphonate to each 100 mL cyanobacterial biomass solution, followed by gentle mixing to achieve a uniform substrate concentration of 1 mM. The cultures were then maintained under consistent photobioreactor conditions for 1 to 7 days to optimize conversion rates. Each experiment was conducted in triplicate. After biotransformation, the reaction mixture from each strain was centrifuged at 4000 rpm for 10 min, and the supernatant was collected. The supernatant was subjected to extraction twice using 50 mL of ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ for 24 h, and then filtered. The ethyl acetate was evaporated under reduced pressure using a rotary evaporator at 60 °C. The resulting oil residue (product) was purified using High Pressure Preparative Liquid Chromatography System (Teledyne ISCO ACCQPrep HP125, Lincoln, NE, USA) with reversed phase column RediSep Prep C18, 5 µm, 250 mm (Teledyne ISCO, Lincoln, NE, USA). General procedure of purification using HPLC: 10 min of isocratic flow of 10% of acetonitrile in water, 5 min of 15% of acetonitrile in water, 5 min from 15 to 30% of acetonitrile in water, 10 min of isocratic flow of 30% of acetonitrile in water; flow 5 mL/min, Rf1 = 23 min. The obtained products were analyzed by Mass Spectroscopy (MS), Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (NMR)—MS (TOF MS ES+) Calcd for $C_5H_{11}O_4P$ [M + H]+ 167.0473; found: 167.0481; IR: 3362 (OH), 1638 (CH=CH) cm^{-1}; ^{31}P NMR ($CDCl_3$, δ, ppm): 22.64; ^1H NMR ($CDCl_3$, δ, ppm): 3.69 (d, J = 11 Hz, 6H, 2×P(O)OC$\underline{H}_3$), 4.26–4.31 (m, 2H, C$\underline{H}_2$OH), 5.96 (ddt, J = 2, 17, 21 Hz, 1H, P-C$\underline{H}$=CH), 6.86 (ddt, J = 3, 17, 23 Hz, 1H, P-CH=C$\underline{H}$); ^{13}C NMR ($CDCl_3$, δ, ppm): 52.58 (d, J = 6 Hz, 1C, PO$\underline{C}H_3$), 62.36 (d, J = 22 Hz, 1C, HO$\underline{C}H_2$CH=CH), 113.22 (d, J = 191 Hz, 1C, P-$\underline{C}$H=CH), 153.69 (d, J = 5 Hz, 1C, P-CH=$\underline{C}$H) [64].

The assignment of signals on ^1H and ^{13}C NMR were further confirmed by $^{1\text{-}1}H$ COSY, ^1H-^{13}C HMQC, and ^1H-^{13}C HMBC spectra. The product was also synthesized by a chemical method [63]: sodium methoxide in methanol (0.1 equiv) was added dropwise to epoxymethyl dimethyl phosphonate (3.01 mmol; 500 mg) at 0 °C under stirring. After the addition, the reaction was stirred for 1 h at room temperature. Then, 2.00 g of Dowex 50W-X8 (H^+) resin (200–400 mesh) (Sigma-Aldrich, St. Louis, MO, USA), previously washed with methanol, was added and the mixture was stirred for 15 min. Afterwards, the resin was filtered off, washed with methanol, and the combined filtrate was evaporated to dryness to give the crude product (490 mg). After synthesis, the products were purified using Flash Chromatography System: Combi Flash® Rf 150 (Teledyne ISCO, Lincoln, NE, USA) and reversed phase column PuriFlash C18-HP, 15 µm, 25 g (Teledyne ISCO, Lincoln, NE, USA). The general procedure of purification included isocratic flow of pure water, flow 10 mL/min, Rf = 2.5 min. The product was analyzed by NMR, the assignment of signals on ^1H and ^{13}C NMR were further confirmed by $^{1\text{-}1}H$ COSY, ^1H-^{13}C HMQC, and ^1H-^{13}C HMBC spectra. The spectroscopic characterization was in agreement with the data reported earlier in the literature [64]. This synthetic approach allowed the obtainment of the desired product in 81.4% yield with 85% purity.

3. Results

Bioconversion results were thoroughly analyzed using Mass Spectroscopy (MS); ^1H, ^{13}C, ^{31}P and 2D Nuclear Magnetic Resonance (NMR); and Infrared Spectroscopy (IR). Figure 2 highlights key findings from the ^{31}P NMR and IR analyses.

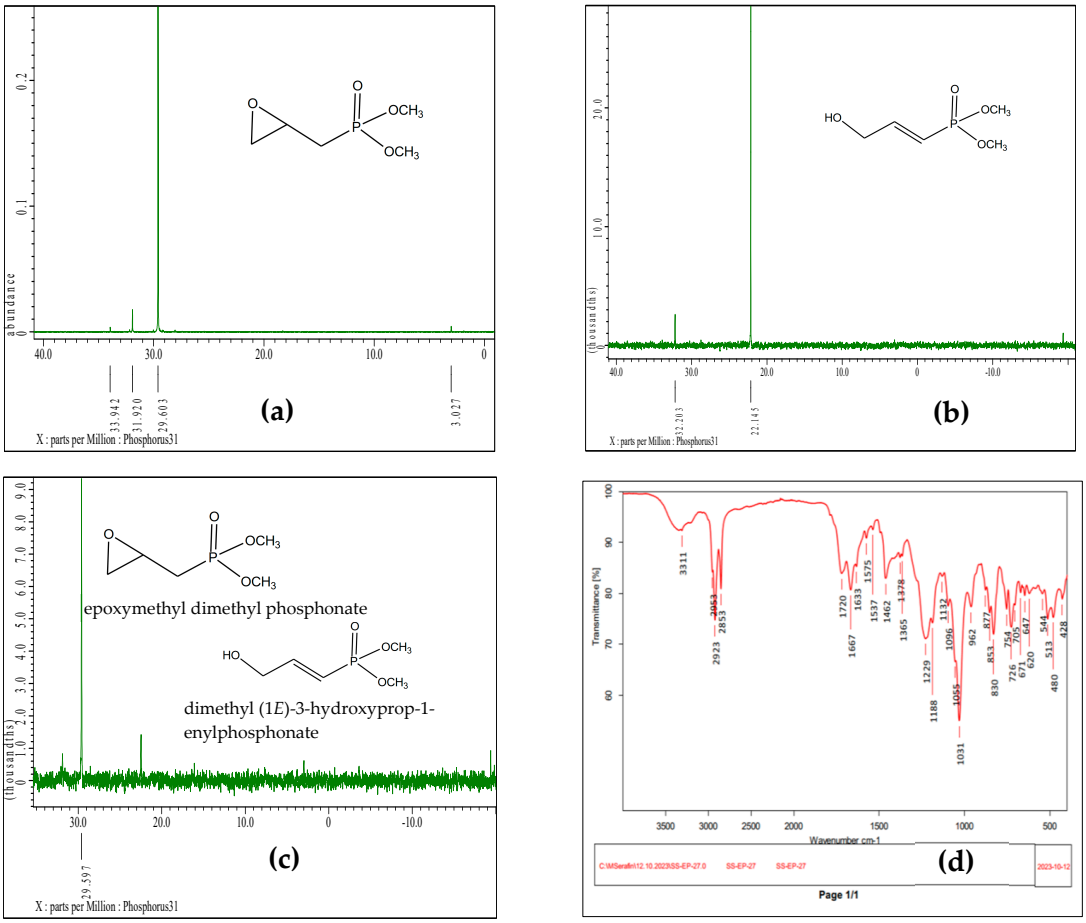


Figure 2. Spectral analysis of the bioconversion process: (**a**) ^{31}P NMR spectrum of the substrate, epoxymethyl dimethyl phosphonate; (**b**) ^{31}P NMR spectrum of the fully converted product, dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate; (**c**) ^{31}P NMR spectrum depicting partial substrate conversion; (**d**) IR spectrum of the fully converted bioconversion product.

3.1. Comparative Bioconversion Rates of Cyanobacterial Strains

Figure 3 shows the comparative bioconversion efficiency of six cyanobacterial strains—*Synechococcus bigranulatus*, *Nostoc cf-muscorum*, *Limnospira indica*, *Limnospira maxima*, *Leptolyngbya foveolarum*, and *Nodularia moravica*—in the conversion of epoxymethyl dimethyl phosphonate (EMDP) into dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate. The figure reveals a time-dependent increase in bioconversion across all strains, with conversion degrees evaluated at intervals up to 7 days. The data indicate that *Limnospira* strains (*Limnospira indica* and *Limnospira maxima*) and *Synechococcus bigranulatus* all achieved the highest conversion efficiency, 99% after 3 days, and maintained this efficiency through subsequent days. However, *Limnospira* strains demonstrated rapid bioconversion rates, achieving 99% after 24 h and sustaining these levels. The high efficiency of these strains suggests a robust capability for photobiocatalysis, likely facilitated by their optimized photosynthetic pathways and compatibility with the experimental light conditions used.

In contrast, *Leptolyngbya foveolarum* and *Nodularia moravica* exhibited slower conversion rates, achieving 96% and 75% bioconversion, respectively, by day 7. *Nostoc cf-muscorum* showed the least conversion, with only 14% bioconversion by day 7, which may be attributed to inherent metabolic or structural limitations within this strain that affect substrate uptake or enzyme activity relevant to the transformation of EMDP. This variance among strains underscores the importance of strain-specific metabolic features in determining bioconversion efficiency.

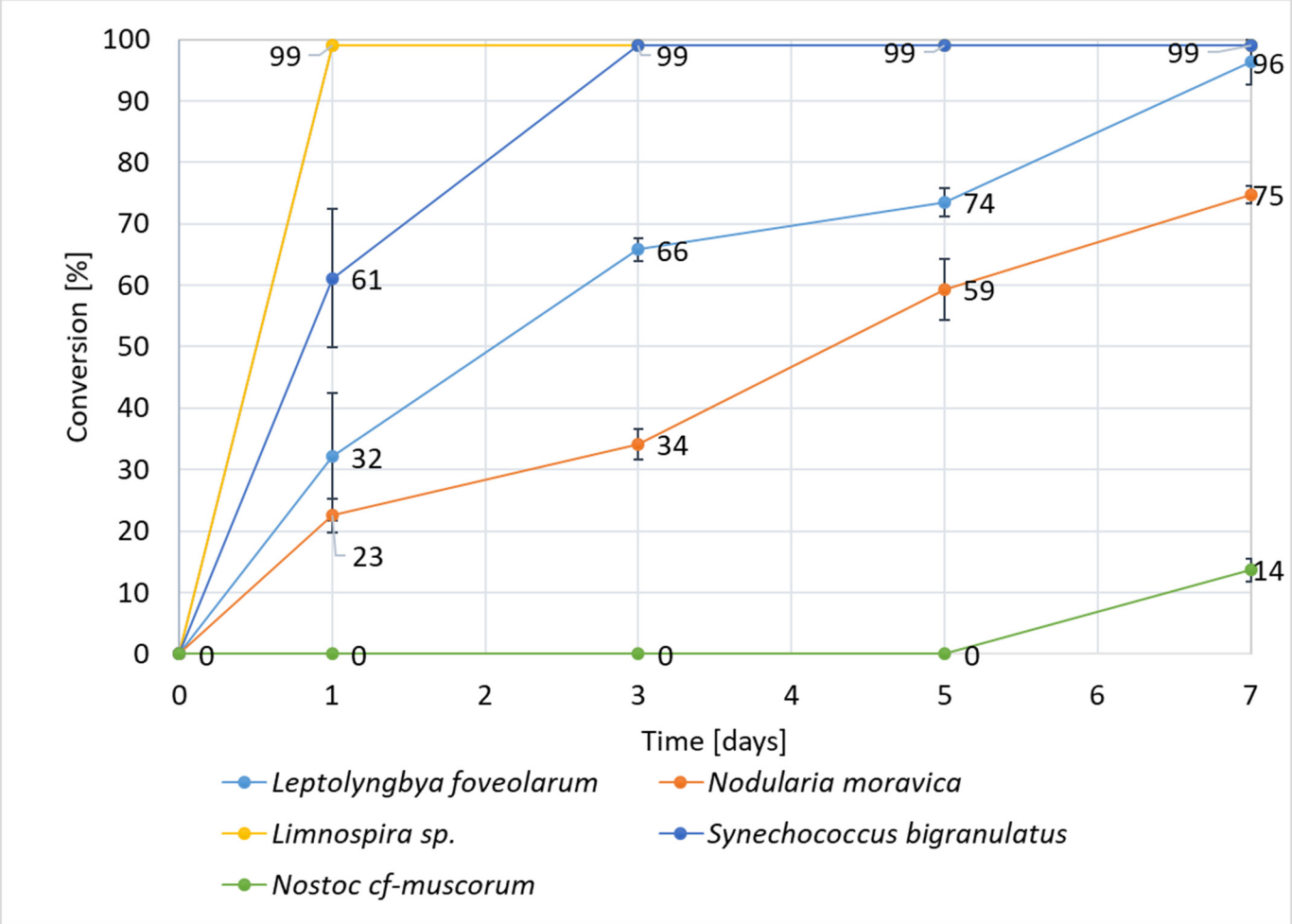


Figure 3. Comparative analysis of the bioconversion degrees by cyanobacterial biocatalysts.

3.2. Spectral Characterization of Biotransformation

Thorough spectral characterization of the bioconversion process, using both ^{31}P NMR and IR spectroscopy is presented in Figure 2. The spectral data provides insight into the structural changes occurring as EMDP is transformed into dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate. Figure 2a presents the ^{31}P NMR spectrum of the initial substrate, EMDP, with typical peaks that align with the characteristic phosphonate spectral signatures. Figure 2b displays the ^{31}P NMR spectrum following complete bioconversion to the target product. Here, notable shifts in the ^{31}P signal indicate successful transformation, corresponding to structural alterations involving the formation of an unsaturated C=C bond and the introduction of a hydroxyl group. Figure 2c shows the spectrum of a partially converted sample, where residual substrate peaks are visible alongside product signals, providing an intermediate perspective on the bioconversion process. This spectrum illustrates the sequential conversion stages and is useful in understanding the kinetics of the reaction. Figure 2d presents the IR spectrum of the fully converted product, indicating key vibrational modes that confirm the presence of both a hydroxyl group and an unsaturated C=C bond in the final product structure. The presence of these functional groups aligns with the proposed structure of dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate and corroborates findings from the NMR spectra [65–67]. Therefore, the data presented in Figure 2 confirms the structural integrity and reliability of the bioconversion process, with the spectral signatures validating the transition from EMDP to the targeted high-value-added phosphonate product. These results provide a fundamental understanding of how cyanobacterial

systems can be harnessed for efficient biocatalytic transformation of organophosphorus compounds, with each spectral shift marking a critical step in the pathway.

4. Discussion

The research's findings highlight the effectiveness of cyanobacterial strains as biocatalysts in transforming epoxymethyl dimethyl phosphonate into dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate (Figure 2a–d). Among the six strains tested, *Synechococcus bigranulatus* and *Limnospira* strains achieved an exceptional conversion rate (Figure 3). This exceptional performance emphasizes the metabolic adaptability and photosynthetic competence of these strains, predominantly their capability to harvest light energy and optimize energy transfer for biotransformation processes. The performance of *Synechococcus* aligns with previous research which demonstrated that *Synechococcus* photoautotrophic abilities, coupled with genetic engineering, improve the synthesis of high-value-added biochemicals under controlled light conditions [68]. For example, engineered *Synechococcus elongatus* strains have been advanced for the effective generation of high-value compounds like succinic acid, highlighting the potential of *Synechococcus* in scalable chemical production [68,69]. *Limnospira* strains which were formerly classified under *Spirulina* have shown promise in renewable energy applications, including their contribution to bioethanol and biodiesel production [70–72]. The strong photosynthetic machinery and adaptability of *Limnospira* strains to diverse light intensities make them highly suitable for biocatalytic applications [73]. In addition, the high biomass productivity of *Limnospira* sp makes them cost-effective options for industrial-scale production and their applicability in photobiocatalysis [25].

Other strains such as *Leptolyngbya foveolarum* and *Nodularia moravica* exhibited moderate conversion efficiencies while *Nostoc cf-muscorum* exhibited the least conversion efficiency. Although previous investigation demonstrated that these strains show potential in enantioselective synthesis of chiral phosphonates [25,61,74]; however, their performance in this study was limited, possibly due to their slower growth rates and metabolic path toward nitrogen fixation. *Nostoc* species, for example, direct significant metabolic resources towards nitrogen fixation, that may constrain high-rate biocatalysis for phosphonates [25]. Previous studies have also highlighted *Nostoc's* applications in nutrient-deficient environments where nitrogen fixation is prioritized over other metabolic functions [75,76]. Hence, while these strains may be less efficient for EMDP conversion, they could serve specialized roles in environments that require multifunctional biocatalysis or bioremediation [76–78]. Cyanobacterial species, like *Nostoc* and *Nodularia*, can synthesize chiral hydroxyphosphonates via enantioselective bioreduction, suggesting further investigation of these strains for diverse chiral synthesis applications [49].

Prospective research efforts could focus on advancing genetic engineering techniques to improve biocatalytic efficacy across various cyanobacterial strains. For instance, offering metabolic pathways for advanced phosphonate synthesis in strains such as *Leptolyngbya* and *Nodularia* could increase their applicability. In addressing challenges faced by conventional chemical synthesis of phosphonates in terms of selectivity and environmental impact, incorporating these cyanobacterial systems with green chemistry practices, like enzyme immobilization or continuous flow reactors, could significantly boost process scalability and efficiency in eco-friendly production of high-value-added phosphonates [79–82].

5. Conclusions

This research presents a pioneering exploration of cyanobacteria as biocatalysts for the eco-efficient transformation of epoxymethyl dimethyl phosphonate into the valuable product dimethyl (1*E*)-3-hydroxyprop-1-enylphosphonate. Notably, *Synechococcus bigranulatus* and *Limnospira* strains emerged as highly effective agents, demonstrating the potential of cyanobacteria to revolutionize biocatalysis through natural photosynthetic mechanisms. These findings highlight a feasible approach to chemical production, with significant implications for the pharmaceutical and agricultural industries. The scalability and cost-

effectiveness of cyanobacteria underscore their promise for industrial applications, offering an environmentally friendly alternative to traditional synthetic methods.

Future research should prioritize advanced genetic and metabolic engineering strategies to further enhance biocatalytic efficiency and expand the utility of cyanobacteria in complex synthetic pathways, including chiral phosphonate production. This innovative pathway could establish a greener, more resourceful framework for the synthesis of valuable compounds, positioning cyanobacteria as integral to the future of feasible biochemical products.

Author Contributions: E.Ż.-D., S.O.S. and M.S.-L. conceptualized the idea and methodology; E.Ż.-D. and M.S.-L. supervised the article; S.O.S., E.Ż.-D., M.S.-L., M.B.-R. and M.K.-O. wrote the original drafted manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Mcpherson Iii, J.C.; Runner, R.; Buxton, T.B.; Hartmann, J.F.; Farcasiu, D.; Bereczki, I.; Tollas, S.; Ostorházi, E.; Rozgonyi, F.; Herczegh, P. Synthesis of osteotropic hydroxybisphosphonate derivatives of fluoroquinolone antibacterials. *Eur. J. Med. Chem.* **2011**, *47*, 615–618. [CrossRef] [PubMed]
2. Mikołajczyk, M. Phosphonate reagents and building blocks in the synthesis of bioactive compounds, natural products and medicines. *Pure Appl. Chem.* **2019**, *91*, 811–838. [CrossRef]
3. Krečmerová, M.; Majer, P.; Rais, R.; Slusher, B.S. Phosphonates and Phosphonate Prodrugs in Medicinal Chemistry: Past Successes and Future Prospects. *Front. Chem.* **2022**, *10*, 889737. [CrossRef] [PubMed]
4. Sevrain, C.M.; Berchel, M.; Couthon, H.; Jaffrès, P.-A. Phosphonic acid: Preparation and applications. *Beilstein J. Org. Chem* **2017**, *13*, 2186–2213. [CrossRef]
5. Kaboudin, B.; Daliri, P.; Faghih, S.; Esfandiari, H. Hydroxy- and Amino-Phosphonates and -Bisphosphonates: Synthetic Methods and Their Biological Applications. *Front. Chem.* **2022**, *10*, 890696. [CrossRef]
6. Perlmutter, P. Alkenoic acids and their derivatives. In *Conjugate Addition Reactions in Organic Synthesis*; Tetrahedron Organic Chemistry Series; Pergamon Press: Oxford, UK, 1992; Volume 9, pp. 199–282.
7. Turhanen, P.A.; Demadis, K.D.; Kafarski, P. Editorial: Phosphonate Chemistry in Drug Design and Development. *Front. Chem.* **2021**, *9*, 695128. [CrossRef]
8. Manghi, M.C.; Masiol, M.; Calzavara, R.; Graziano, P.L.; Peruzzi, E.; Pavoni, B. The use of phosphonates in agriculture. Chemical, biological properties and legislative issues. *Chemosphere* **2021**, *283*, 131187. [CrossRef]
9. Xuan, C.; Zhu, Z.; Li, Z.; Shu, C. Recent developments in the synthesis of pharmacological alkyl phosphonates. *Adv. Agrochem*, 2024; *in press*. [CrossRef]
10. Kaur, B.; Singh, P. Epoxides: Developability as active pharmaceutical ingredients and biochemical probes. *Bioorg. Chem.* **2022**, *125*, 105862. [CrossRef]
11. Montchamp, J.L. Challenges and solutions in phosphinate chemistry. *Pure Appl. Chem.* **2019**, *91*, 113–120. [CrossRef]
12. Cybulska, P.; Legrand, Y.M.; Babst-Kostecka, A.; Diliberto, S.; Leśniewicz, A.; Oliviero, E.; Bert, V.; Boulanger, C.; Grison, C.; Olszewski, T.K. Green and Effective Preparation of α-Hydroxyphosphonates by Ecocatalysis. *Molecules* **2022**, *27*, 3075. [CrossRef] [PubMed]
13. Serafin-Lewańczuk, M.; Brzezińska-Rodak, M.; Lubiak-Kozłowska, K.; Majewska, P.; Klimek-Ochab, M.; Olszewski, T.K.; Żymańczyk-Duda, E. Phosphonates enantiomers receiving with fungal enzymatic systems. *Microb. Cell Fact.* **2021**, *20*, 81. [CrossRef] [PubMed]
14. Kolodiazhna, A.O.; Kolodiazhnyi, O.I. Catalytic Asymmetric Synthesis of C-Chiral Phosphonates. *Symmetry* **2022**, *14*, 1758. [CrossRef]
15. Sobkowski, M.; Kraszewski, A.; Stawinski, J. Recent Advances in H-Phosphonate Chemistry. Part 2. Synthesis of C-Phosphonate Derivatives. *Top. Curr. Chem.* **2014**, *361*, 179–216. [CrossRef]
16. Van der Jeught, S.; Masschelein KG, R.; Stevens, C.V. Synthesis of Phosphonobenzocarbacephems by Intramolecular Radical Cyclization of Haloaryl-Substituted β-Lactams. *Eur. J. Org. Chem.* **2010**, *2010*, 1333–1338. [CrossRef]
17. Schrewe, M.; Julsing, M.K.; Bühler, B.; Schmid, A. Whole-cell biocatalysis for selective and productive C–O functional group introduction and modification. *Chem. Soc. Rev.* **2013**, *42*, 6346–6377. [CrossRef]
18. Ran, N.; Zhao, L.; Chen, Z.; Tao, J. Recent applications of biocatalysis in developing green chemistry for chemical synthesis at the industrial scale. *Green Chem.* **2008**, *10*, 361–372. [CrossRef]
19. Groaz, E.; De Jonghe, S. Overview of Biologically Active Nucleoside Phosphonates. *Front. Chem.* **2021**, *8*, 616863. [CrossRef]

20. Hilderbrand, R.L.; Henderson, T.O. Phosphonic acids in nature. In *The Role of Phosphonates in Living Systems*; CRC Press: Boca Raton, FL, USA, 2018; pp. 5–30. [CrossRef]
21. Hanson, J.E.; Kaplan, A.P.; Bartlett, P.A. Phosphonate analogues of carboxypeptidase A substrates are potent transition-state analogue inhibitors. *Biochemistry* **1989**, *28*, 6294–6305. [CrossRef]
22. Ruffolo, F.; Dinhof, T.; Murray, L.; Zangelmi, E.; Chin, J.P.; Pallitsch, K.; Peracchi, A. The Microbial Degradation of Natural and Anthropogenic Phosphonates. *Molecules* **2023**, *28*, 6863. [CrossRef]
23. Harsági, N.; Keglevich, G. The Hydrolysis of Phosphinates and Phosphonates: A Review. *Molecules* **2021**, *26*, 2840. [CrossRef] [PubMed]
24. Horsman, G.P.; Zechel, D.L. Phosphonate Biochemistry. *Chem. Rev.* **2017**, *117*, 5704–5783. [CrossRef] [PubMed]
25. Górak, M.; Zymańczyk-Duda, E. Application of cyanobacteria for chiral phosphonate synthesis. *Green Chem.* **2015**, *17*, 4570–4578. [CrossRef]
26. Tüllinghoff, A.; Djaya-Mbissam, H.; Toepel, J.; Bühler, B. Light-driven redox biocatalysis on gram-scale in Synechocystis sp. PCC 6803 via an in vivo cascade. *Plant Biotechnol. J.* **2023**, *21*, 2074. [CrossRef]
27. Włodarczyk, A.; Selão, T.T.; Norling, B.; Nixon, P.J. Newly discovered Synechococcus sp. PCC 11901 is a robust cyanobacterial strain for high biomass production. *Commun. Biol.* **2020**, *3*, 215. [CrossRef]
28. Leu, S.; Boussiba, S. Advances in the Production of High-Value Products by Microalgae. *Ind. Biotechnol.* **2014**, *10*, 169–183. [CrossRef]
29. Vijay, D.; Akhtar, M.K.; Hess, W.R. Genetic and metabolic advances in the engineering of cyanobacteria. *Curr. Opin. Biotechnol.* **2019**, *59*, 150–156. [CrossRef]
30. El-fayoumy, E.A.; Shanab, S.M.; Hassan, O.M.A.; Shalaby, E.A. Enhancement of active ingredients and biological activities of Nostoc linckia biomass cultivated under modified BG-110 medium composition. *Biomass Convers. Biorefinery* **2023**, *13*, 6049. [CrossRef]
31. Flores, E.; López-Lozano, A.; Herrero, A. Nitrogen fixation in the oxygenic phototrophic prokaryotes (cyanobacteria): The fight against oxygen. *Biol. Nitrogen Fixat.* **2015**, *2*, 879–890. [CrossRef]
32. Kulasooriya, S.A.; Magana-Arachchi, D.N. Nitrogen fixing cyanobacteria: Their diversity, ecology and utilization with special reference to rice cultivation. *J. Natl. Sci. Found. Sri Lanka* **2016**, *44*, 111–128. [CrossRef]
33. Pineda-Rodríguez, Y.Y.; Herazo-Cárdenas, D.S.; Vallejo-Isaza, A.; Pompelli, M.F.; Jarma-Orozco, A.; Jaraba-Navas, J.d.D.; Cordero-Ocampo, J.D.; González-Berrio, M.; Arrieta, D.V.; Pico-González, A.; et al. Optimal Laboratory Cultivation Conditions of Limnospira maxima for Large-Scale Production. *Biology* **2023**, *12*, 1462. [CrossRef] [PubMed]
34. Segers, C.; Mysara, M.; Coolkens, A.; Wouters, S.; Baatout, S.; Leys, N.; Lebeer, S.; Verslegers, M.; Mastroleo, F. Limnospira indica PCC 8005 Supplementation Prevents Pelvic Irradiation-Induced Dysbiosis but Not Acute Inflammation in Mice. *Antioxidants* **2023**, *12*, 572. [CrossRef] [PubMed]
35. Poughon, L.; Laroche, C.; Creuly, C.; Dussap, C.-G.; Paille, C.; Lasseur, C.; Monsieurs, P.; Heylen, W.; Coninx, I.; Mastroleo, F. limnospira indica pcc8005 growth in photobioreactor: Model and simulation of the iss and ground experiments. *Life Sci. Sp. Res.* **2020**, *25*, 53–65. [CrossRef] [PubMed]
36. López-Rodríguez, A.; Mayorga, J.; Flaig, D.; Fuentes, G.; Hernández, V.; Gómez, P.I. Genetic characterization and assessment of the biotechnological potential of strains belonging to the genus Arthrospira/Limnospira (Cyanophyceae) deposited in different culture collections. *Algal Res.* **2023**, *73*, 103164. [CrossRef]
37. Ali, S.S.; El-Sheekh, M.; Manni, A.; Ruiz, H.A.; Elsamahy, T.; Sun, J.; Schagerl, M. Microalgae-mediated wastewater treatment for biofuels production: A comprehensive review. *Microbiol. Res.* **2022**, *265*, 127187. [CrossRef]
38. Keshari, N.; Zhao, Y.; Das, S.K.; Zhu, T.; Lu, X. Cyanobacterial Community Structure and Isolates From Representative Hot Springs of Yunnan Province, China Using an Integrative Approach. *Front. Microbiol.* **2022**, *13*, 872598. [CrossRef]
39. Negi, R.; Nigam, M.; Singh, R.K. Harnessing Leptolyngbya for antiproliferative and antimicrobial metabolites through lens of modern techniques: A review. *Algal Res.* **2024**, *83*, 103702. [CrossRef]
40. Ramesh Kumar, V.; Narendrakumar, G.; Thyagarajan, R.; Melchias, G. A comparative analysis of biodiesel production and its properties from Leptolyngbya sp. BI-107 and Chlorella vulgaris under heat shock stress. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 502–506. [CrossRef]
41. Cui, J.; Morita, M.; Ohno, O.; Kimura, T.; Teruya, T.; Watanabe, T.; Suenaga, K.; Shibasaki, M. Leptolyngbyolides, Cytotoxic Macrolides from the Marine Cyanobacterium Leptolyngbya sp.: Isolation, Biological Activity, and Catalytic Asymmetric Total Synthesis. *Chem. A Eur. J.* **2017**, *23*, 8500–8509. [CrossRef]
42. Żymańczyk-Duda, E.; Głąb, A.; Górak, M.; Klimek-Ochab, M.; Brzezińska-Rodak, M.; Strub, D.; Śliżewska, A. Reductive capabilities of different cyanobacterial strains towards acetophenone as a model substrate—Prospect of applications for chiral building blocks synthesis. *Bioorg. Chem.* **2019**, *93*, 102810. [CrossRef]
43. Jodlbauer, J.; Rohr, T.; Spadiut, O.; Mihovilovic, M.D.; Rudroff, F. Biocatalysis in Green and Blue: Cyanobacteria. *Trends Biotechnol.* **2021**, *39*, 875–889. [CrossRef] [PubMed]
44. Cui, J.; Sun, H.; Chen, R.; Sun, J.; Mo, G.; Luan, G.; Lu, X. Multiple routes toward engineering efficient cyanobacterial photosynthetic biomanufacturing technologies. *Green Carbon* **2023**, *1*, 210–226. [CrossRef]
45. Baunach, M.; Guljamow, A.; Miguel-Gordo, M.; Dittmann, E. Harnessing the potential: Advances in cyanobacterial natural product research and biotechnology. *Nat. Prod. Rep.* 2023. [CrossRef] [PubMed]

46. Prasanna, R.; Saxena, A.K.; Jaiswal, P.; Nayak, S. Development of alternative support system for viable count of cyanobacteria by most probable number method. *Folia Microbiol.* **2006**, *51*, 455–458. [CrossRef]
47. Parte, S.; Sirisha, V.L.; D'Souza, J.S. Biotechnological Applications of Marine Enzymes From Algae, Bacteria, Fungi, and Sponges. *Adv. Food Nutr. Res.* **2017**, *80*, 75–106. [CrossRef]
48. Knoop, H.; Gründel, M.; Zilliges, Y.; Lehmann, R.; Hoffmann, S.; Lockau, W.; Steuer, R. Flux Balance Analysis of Cyanobacterial Metabolism: The Metabolic Network of Synechocystis sp. PCC 6803. *PLOS Comput. Biol.* **2013**, *9*, e1003081. [CrossRef]
49. Zhao, L.; Lin, L.Z.; Zeng, Y.; Teng, W.K.; Chen, M.Y.; Brand, J.J.; Zheng, L.L.; Gan, N.Q.; Gong, Y.H.; Li, X.Y.; et al. The facilitating role of phycospheric heterotrophic bacteria in cyanobacterial phosphonate availability and Microcystis bloom maintenance. *Microbiome* **2023**, *11*, 142. [CrossRef]
50. González-Morales, S.I.; Pacheco-Gutiérrez, N.B.; Ramírez-Rodríguez, C.A.; Brito-Bello, A.A.; Estrella-Hernández, P.; Herrera-Estrella, L.; López-Arredondo, D.L. Metabolic engineering of phosphite metabolism in Synechococcus elongatus PCC 7942 as an effective measure to control biological contaminants in outdoor raceway ponds. *Biotechnol. Biofuels* **2020**, *13*, 119. [CrossRef]
51. Dexter, J.; Fu, P. Metabolic engineering of cyanobacteria for ethanol production. *Energy Environ. Sci.* **2009**, *2*, 857–864. [CrossRef]
52. Pattharaprachayakul, N.; Choi, J.L.; Incharoensakdi, A.; Woo, H.M. Metabolic Engineering and Synthetic Biology of Cyanobacteria for Carbon Capture and Utilization. *Biotechnol. Bioprocess Eng.* **2020**, *25*, 829–847. [CrossRef]
53. Gomaa, M.A.; Al-Haj, L.; Abed, R.M.M. Metabolic engineering of Cyanobacteria and microalgae for enhanced production of biofuels and high-value products. *J. Appl. Microbiol.* **2016**, *121*, 919–931. [CrossRef] [PubMed]
54. Bhati, R.; Mallick, N. Production and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) co-polymer by a N 2-fixing cyanobacterium, Nostoc muscorum Agardh. *J. Chem. Technol. Biotechnol.* **2012**, *87*, 505–512. [CrossRef]
55. Dodds, W.K.; Gudder, D.A.; Mollenhauer, D. THE ECOLOGY OF NOSTOC. *J. Phycol.* **1995**, *31*, 2–18. [CrossRef]
56. Nostoc Muscorum—Microbewiki. Available online: https://microbewiki.kenyon.edu/index.php/Nostoc_muscorum (accessed on 16 September 2024).
57. Wang, Y.; Tao, F.; Ni, J.; Li, C.; Xu, P. Production of C3 platform chemicals from CO2 by genetically engineered cyanobacteria. *Green Chem.* **2015**, *17*, 3100–3110. [CrossRef]
58. Pembroke, J.T.; Ryan, M.P. *Cyanobacterial Biofuel Production: Current Development, Challenges and Future Needs*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 35–62. [CrossRef]
59. Gu, F.; Li, C.; Zheng, H.; Ni, J. Engineering cyanobacteria for the production of aromatic natural products. *Blue Biotechnol.* **2024**, *1*, 2. [CrossRef]
60. Wang, Y.; Lv, X.; Ji, X.-J.; Ji, B.; Satta, A.; Esquirol, L.; Ebert, B.E. Current Metabolic Engineering Strategies for Photosynthetic Bioproduction in Cyanobacteria. *Microorganisms* **2023**, *11*, 455. [CrossRef]
61. Lai, M.C.; Lan, E.I. Advances in Metabolic Engineering of Cyanobacteria for Photosynthetic Biochemical Production. *Metabolites* **2015**, *5*, 636–658. [CrossRef]
62. Salehi, S.; Abdollahi, K.; Panahi, R.; Rahmanian, N.; Shakeri, M.; Mokhtarani, B. Applications of Biocatalysts for Sustainable Oxidation of Phenolic Pollutants: A Review. *Sustainability* **2021**, *13*, 8620. [CrossRef]
63. Rippka, E.; Deruelles, J.; Waterbury, N.B. Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. *Microbiology* **1979**, *111*, 1–61. [CrossRef]
64. Just, G.; Potvin, P.; Hakimelahi, G.H. 3-Diethylphosphonoacrolein diethylthioacetal anion (6*), a reagent for the conversion of aldehydes to u,p-unsaturated ketene dithioacetals and three-carbon homologated u,p-unsaturated aldehydes. *Can. J. Chem.* **1980**, *58*, 2780–2783.
65. Galezowska, J.; Gumienna-Kontecka, E. Phosphonates, their complexes and bio-applications: A spectrum of surprising diversity. *Coord. Chem. Rev.* **2012**, *256*, 105–124. [CrossRef]
66. Thornburg, C.C.; Cowley, E.S.; Sikorska, J.; Shaala, L.A.; Ishmael, J.E.; Youssef, D.T.A.; McPhail, K.L. Apratoxin H and Apratoxin A Sulfoxide from the Red Sea Cyanobacterium Moorea producens. *J. Nat. Prod.* **2013**, *76*, 1781. [CrossRef] [PubMed]
67. Carey, F.A.; Giuliano, R.M. *Organic Chemistry*, 9th ed.; McGraw-Hill Education: New York, NY, USA, 2014; Available online: https://www.columbia.edu/itc/chemistry/ARCHIVE/old_orgo/text_chapters/ (accessed on 14 December 2024).
68. Sengupta, S.; Jaiswal, D.; Sengupta, A.; Shah, S.; Gadagkar, S.; Wangikar, P.P. Metabolic engineering of a fast-growing cyanobacterium Synechococcus elongatus PCC 11801 for photoautotrophic production of succinic acid. *Biotechnol. Biofuels* **2020**, *13*, 89. [CrossRef]
69. Zhang, S.; Sun, J.; Feng, D.; Sun, H.; Cui, J.; Zeng, X.; Wu, Y.; Luan, G.; Lu, X. Unlocking the potentials of cyanobacterial photosynthesis for directly converting carbon dioxide into glucose. *Nat. Commun.* **2023**, *14*, 3425. [CrossRef]
70. Sachdeva, N.; Poughon, L.; Gerbi, O.; Dussap, C.G.; Lasseur, C.; Leroy, B.; Wattiez, R. Ground Demonstration of the Use of Limnospira indica for Air Revitalization in a Bioregenerative Life-Support System Setup: Effect of Non-Nitrified Urine–Derived Nitrogen Sources. *Front. Astron. Sp. Sci.* **2021**, *8*, 700270. [CrossRef]
71. Rahman, M.A.; Aziz, M.A.; Al-khulaidi, R.A.; Sakib, N.; Islam, M. Biodiesel production from microalgae Spirulina maxima by two step process: Optimization of process variable. *J. Radiat. Res. Appl. Sci.* **2017**, *10*, 140–147. [CrossRef]
72. Spínola, M.P.; Mendes, A.R.; Prates, J.A.M. Chemical Composition, Bioactivities, and Applications of Spirulina (*Limnospira platensis*) in Food, Feed, and Medicine. *Foods* **2024**, *13*, 3656. [CrossRef]
73. Poughon, L.; Creuly, C.; Godia, F.; Leys, N.; Dussap, C.G. Photobioreactor Limnospira indica Growth Model: Application From the MELiSSA Plant Pilot Scale to ISS Flight Experiment. *Front. Astron. Sp. Sci.* **2021**, *8*, 700277. [CrossRef]

74. Ciebiada, M.; Kubiak, K.; Daroch, M. Modifying the Cyanobacterial Metabolism as a Key to Efficient Biopolymer Production in Photosynthetic Microorganisms. *Int. J. Mol. Sci.* **2020**, *21*, 7204. [CrossRef]
75. Mouga, T.; Pereira, J.; Moreira, V.; Afonso, C. Unveiling the Cultivation of Nostoc sp. under Controlled Laboratory Conditions. *Biology* **2024**, *13*, 306. [CrossRef]
76. Brenes-Álvarez, M.; Olmedo-Verd, E.; Vioque, A.; Muro-Pastor, A.M. A nitrogen stress-inducible small RNA regulates CO_2 fixation in Nostoc. *Plant Physiol.* **2021**, *187*, 787–798. [CrossRef] [PubMed]
77. Ciani, M.; Adessi, A. Cyanoremediation and phyconanotechnology: Cyanobacteria for metal biosorption toward a circular economy. *Front. Microbiol.* **2023**, *14*, 1166612. [CrossRef] [PubMed]
78. Cepoi, L.; Zinicovscaia, I.; Valuta, A.; Codreanu, L.; Rudi, L.; Chiriac, T.; Yushin, N.; Grozdov, D.; Peshkova, A. Bioremediation Capacity of Edaphic Cyanobacteria Nostoc linckia for Chromium in Association with Other Heavy-Metals-Contaminated Soils. *Environments* **2021**, *9*, 1. [CrossRef]
79. Lourembam, J.; Haobam, B.; Singh, K.B.; Verma, S.; Rajan, J.P. The molecular insights of cyanobacterial bioremediations of heavy metals: The current and the future challenges. *Front. Microbiol.* **2024**, *15*, 1450992. [CrossRef]
80. Zhu, Y.; Chen, Q.; Shao, L.; Jia, Y.; Zhang, X. Microfluidic immobilized enzyme reactors for continuous biocatalysis. *React. Chem. Eng.* **2019**, *5*, 9–32. [CrossRef]
81. Yamaguchi, H.; Miyazaki, M. Bioremediation of Hazardous Pollutants Using Enzyme-Immobilized Reactors. *Molecules* **2024**, *29*, 2021. [CrossRef]
82. Sheldon, R.A.; Basso, A.; Brady, D. New frontiers in enzyme immobilisation: Robust biocatalysts for a circular bio-based economy. *Chem. Soc. Rev.* **2021**, *50*, 5850–5862. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.