Dean&Francis

ISSN 2959-6157

Improving methodology for CO_2 fixation in cyanobacteria

Jiaqian Tang

Guangdong Country Garden School, Foshan, China

howard73@nwfsc.edu

Abstract:

This paper explores recent advancements in the absorption of carbon dioxide by cyanobacteria, commonly known as blue-green algae. The escalating severity of climate change is primarily attributed to the rising carbon dioxide levels in the atmosphere. As organisms capable of photosynthesis, cyanobacteria can thrive in a wide range of habitats and engage in CO_2 fixation. In this review, we examine the ability of cyanobacteria to photosynthesize and their ecological adaptability, as well as explore ways to enhance their effectiveness in managing climate change by refining their carbon dioxide fixation processes. The aim is to find innovative approaches to mitigate climate change. We delve into the mechanisms and strategies that can optimize the efficiency of cyanobacteria in carbon sequestration, including discovering novel species, genetic engineering, and manipulating environmental conditions. By enhancing cyanobacteria's capacity for CO_2 fixation, this research seeks to contribute to global efforts in climate change mitigation and offer new directions for climate governance strategies.

Keywords: Cyanobacteria; photosynthesis; global warming; climate change; CO_2 fixation.

1. Introduction

Presently, global warming poses a major threat to the sustainability of the environment as well as to human communities. By 2024, the mean surface temperature of the Earth had escalated by about 1.2°C beyond its pre-industrial state, mainly due to human-made greenhouse gas emissions [1]. The primary cause of this temperature rise is the increased levels of carbon dioxide (CO_2) in the air, rising from roughly 280 ppm (parts per million) in the late 1700s to about 420 ppm today [2]. Addressing climate change concerns primarily involves two key tactics: mitigation and adaptation. Strategies for mitigation involve transitioning to renewable sources such as solar and wind energy, which decrease CO_2 emissions from fossil fuels. Enhancing energy efficiency in both buildings and transportation also lowers emissions. Additionally, the adoption of carbon capture and storage (CCS) technology effectively traps CO_2 from various industries and power stations, ensuring its safe underground storage. Strategies for adaptation encompass the investment in infrastructure resistant to climate change, aiding communities in coping with severe weather conditions, safeguarding natural habitats

such as wetlands and forests, and enhancing carbon capture and ecological advantages. Worldwide treaties, such as the Paris Agreement, strive to curb the increase in temperature. Similar to taxes or cap-and-trade systems, carbon pricing promotes reducing emissions by imposing a price on them. Even with significant progress in climate policy, numerous essential shortcomings continue to impede advancement. A significant number of existing policies and pledges fall short of international agreement objectives, underscoring a discrepancy between stated plans and their actual execution [3]. Additionally, the introduction of sophisticated technologies like Carbon Capture and Storage (CCS) is in its early stages, encountering significant financial and scalability hurdles. Likewise, the shift of entire economies toward renewable energy sources requires considerable investment and alterations in infrastructure. Furthermore, the successful oversight and implementation of climate strategies continue to face challenges, stemming from concerns about clarity, responsibility, and precision in measurements, all of which affect the success of climate initiatives. Consequently, addressing these shortcomings is vital for significant advancements in climate change mitigation and adaptation. Cyanobacteria, alternatively termed blue-green algae, represent a varied collection of photosynthetic microbes vital for water ecosystems and worldwide biogeochemical processes [4]. Cyanobacteria, known for their oxygenic photosynthesis capabilities, play a crucial role in the primary generation and nitrogen fixation processes in marine and freshwater habitats. The ability of these organisms to prosper in diverse environments, especially harsh ones, along with their metabolic adaptability, highlights their importance in ecology and evolution. Cyanobacteria, being extensively researched, provide a crucial understanding of microbial ecology, evolutionary biology, and possible biotechnological uses [5].

This paper aims to investigate ways to enhance the carbon dioxide fixation techniques in cyanobacteria. Cyanobacteria are crucial in both ecosystems and worldwide biogeochemical processes. Their ability to photosynthesize and adapt to environmental conditions renders them an essential subject for research on CO_2 fixation. By examining the traits and photosynthesis processes of cyanobacteria, we suggest techniques to enhance their carbon dioxide absorption, aiming to offer innovative concepts and technical solutions for managing global climate change.

2. Features and properties of cyanobacteria

2.1 Photosynthetic capabilities

In cyanobacteria, chlorophyll a, phycocyanin, and phycoerythrin serve as the pigments responsible for photosynthesis. Chlorophylls play a role in photosystem I, while phycocyanin pigments are linked with photosystem II. The organism photosynthesizes oxygen and possesses the capability to stabilize atmospheric nitrogen.

2.2 Cell Structure and Morphology

Cyanobacterial cells may exist as single cells or develop into colonies, filaments, or multicellular formations. Their cellular architecture is uncomplicated and devoid of membrane-bound organelles, yet they possess unique internal structures like thylakoids, which are the sites of photosynthesis. Certain species develop akinetes, resilient spores that aid in their survival in adverse environments.

2.3 Habitat versatility

Cyanobacteria exhibit remarkable adaptability, flourishing in diverse habitats such as freshwater, marine, and terrestrial environments. These species are recognized for inhabiting harsh environments such as hot springs, saltwater lakes, and deserts.

3. Photosynthetic metabolisms and CO_2 fixation

3.1 Photosynthesis metabolism

The process of photosynthesis in cyanobacteria, which are ancient and extensively dispersed photosynthetic bacteria, is crucial for terrestrial life. Cyanobacteria can transform light energy into chemical energy, a process that generates organic compounds and emits oxygen into the air. The process is divided into two primary phases: light-dependent reactions and the Calvin cycle, also known as light-independent reactions.

Light-based reactions take place in cyanobacteria's thylakoid membranes, arranged in layered formations known as granules in the cytoplasmic membrane or as distinct thylakoid formations. The thylakoids in question are composed of chlorophyll-a, a key pigment in harnessing light energy, along with supplementary pigments like phycobiliproteins, which are components of phycobilisomes. Phycobilisomes, intricate formations affixed to thylakoid membranes, are crucial in absorbing light energy and conveying it to chlorophyll-a. Upon photon absorption by chlorophyll-a, its electrons are stimulated and conveyed via a sequence of proteins integrated into the thylakoid membrane, called the electron transport chain (ETC) [6].

When stimulated electrons traverse the ETC, initiate photophosphorylation and lead to the creation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP)

ISSN 2959-6157

and inorganic phosphate (Pi) via chemiosmosis. Simultaneously, $NADP^+$ is reduced to NADPH. Water serves as the primary electron donor in this process, splitting into oxygen, protons, and electrons within the oxygen-evolving complex associated with Photosystem II. The splitting of water provides the necessary electrons for the ETC and releases molecular oxygen (O_2) as a byproduct, which is then released into the environment.

Subsequent to the reactions involving light, the thylakoid membranes' stroma undergoes the Calvin Cycle, alternatively termed light-independent reactions. The process involves converting carbon dioxide (CO_2) into a stable intermediate molecule. This process is facilitated by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which integrates CO_2 into the five-carbon sugar, ribulose-1,5-bisphosphate (RuBP). The produced compound, consisting of six carbon atoms, becomes unstable and promptly divides into a pair of three-carbon molecules. Subsequently, these molecules undergo reduction with ATP and NADPH generated in light reactions, resulting in the formation of glyceraldehyde-3-phosphate (G3P), a sugar with three carbon atoms. A portion of this G3P is utilized in the production of glucose and various carbohydrates, which serve as energy sources for cyanobacteria [7].

Cyanobacteria engage in cyclic photophosphorylation in addition to the non-cyclic electron transport route. During this process, electrons revert to Photosystem I, leading to additional ATP production without generating NADPH. The repetitive nature of this process helps maintain equilibrium between ATP and NADPH levels, which is essential for various biosynthetic pathways.

3.2 Carbon fixation via cyanobacteria

Engineered cyanobacteria are in the process of creating biofuels, including bioethanol and biodiesel. Studies are concentrated on refining metabolic routes to enhance biofuel production. In the realm of carbon capture technologies, cyanobacteria play a role in reducing CO_2 emissions resulting from industrial activities. These can be grown in photobioreactors, which capture CO_2 from exhaust gases, thereby lowering greenhouse gas levels and aiding in the mitigation of climate change. Cyanobacteria exhibit considerable promise in capturing CO_2. CO_2 absorption efficiency differs across various species and strains, shaped by environmental factors and metabolic abilities. Research indicates that cyanobacteria are capable of absorbing CO_2 at a rate varying between 0.1 and 5 grams per square meter daily, influenced by variables such as light intensity, nutrient presence, and temperature. The capacity of these organisms to stabilize nitrogen in the atmosphere and endure different pollutants enables them to enhance the quality of soil and water while simultaneously absorbing CO_2 [8].

4. Technologies to improve carbon fixation efficiency

4.1 Discovering novel species

4.1.1 High-quality screening

The process of high-quality screening involves meticulously choosing and assessing the strain's traits to ensure it possesses the necessary characteristics for industry relevance. Typically, this screening technique requires in-depth experiments and analyses to assess the strain's growth rate, adaptability, genetic actionability, and other aspects. High-quality screening aims to identify strains that yield biologically high outputs and are highly efficient, catering to the demands of industrial uses.

The appeal of cyanobacterial strains as biotechnological systems lies in their industry-specific characteristics, including adaptability to environmental shifts and the simplicity of genetic alteration. Nonetheless, the expansion pace of frequently utilized model strains is comparatively sluggish, and this reduced growth rate hampers their capacity to produce biomass in large quantities. Consequently, scientists are searching for superior photosynthetic substrates capable of swift growth by examining and discovering novel cyanobacterial strains suitable for use in biotechnological applications. Numerous research efforts have led to the discovery and characterization of novel cyanobacterial strains capable of swift growth in ideal environments, accompanied by the creation of various genetic tools for converting CO_2 into chemical compounds. Take, for instance, the rapidly proliferating cyanobacterium S. elongates UTEX 2973 (subsequently referred to as UTEX 2973), which multiplies in merely 1.5 hours under ideal cultivation settings. Conversely, S. elongates PCC 11801 (subsequently referred to as PCC 11801), sourced from India, demonstrates a remarkable replication duration of 0.29 hours under ideal conditions, marking the briefest duration ever recorded for a cyanobacterium in normal CO_2 environments. A different variant, S. elongates PCC 11802, which is closely related phylogenetically to PCC 11801 and shares a 97% genome identity, exhibiting a doubling duration of 2.8 hours under ideal growth environments. Furthermore, under ideal conditions, the Synechococcus species PCC 11901, sourced from Singapore, shows a biomass accumulation 1.7 to 3 times greater than that of typical model cyanobacteria and achieves the shortest doubling time of nearly 2 hours at 30°C and 5% CO_2 [9].

4.1.2 High-throughput screening

Conversely, high-throughput screening denotes the swift examination of numerous strains using automated and high-throughput methods to identify strains with distinct characteristics or roles. Commonly, this screening approach employs methods such as high-throughput culture, measurement, and analysis to concurrently process various samples and swiftly evaluate the strains' efficacy. The benefit of high-throughput screening lies in its ability to swiftly screen numerous strains, enhancing both the efficiency and speed of the process. Previous research employing single-cell resonance Raman (SCRR) spectroscopy revealed that embedding 13 CO_2 in carotenoids led to a redshift in the spectra of single-cell resonance Raman. The SCRR-SIP method is capable of directly examining CO_2-fixed cells, uncovering the ecophysiological aspects of microorganisms previously uncultured. The advancement in Raman spectroscopy enables the metabolic examination and categorization of algal cell species without the need for culturing. Lately, scientists have innovated droplet systems to analyze and screen genetically modified cyanobacterial strains, including those producing lactic acid and ethanol. Furthermore, the droplet system is applicable for extensive screening of single-celled cyanobacterial strains apt for salinity cultivation environments.

4.2 Genetically editing

4.2.1 Marker-Free Genome Editing

The process of genetic alteration in cyanobacteria is lengthy and becomes more complex due to the haploid or polyploid nature of certain cyanobacteria. The emergence of gene-editing technologies based on CRISPR has enhanced the efficiency of metabolic engineering in cyanobacteria, enabling editing without the need for markers [10]. A range of CRISPR systems, such as gene knockout, knock-in, and specific point mutations, has been investigated and utilized in gene editing. Recent research has shown that in Anabaena, the CRISPR-Cpfl method can eliminate extensive genomic areas ranging from 43 to 118 kb, suggesting that polyploidy doesn't impede the effective deletion of substantial DNA segments [11]. Furthermore, gene knock-ins have utilized CRISPR systems, such as the CRISPR-Cas9 system, to successfully insert a shortened thioesterase gene (tesA) into Escherichia coli, leading to a UTEX 2973 mutant with increased free fatty acid synthesis [44]. Utilizing the CRISPR/Cpfl system, every SNP in UTEX 2973 has been transformed into matching alleles in S. elongatus PCC 7942 (referred to as PCC 7942), given PCC 7942's status as a highly competitive strain in biofuel manufacturing [12].

4.2.1 Control of Gene Expression

A range of instruments and tactics, such as regulatory components, inducible mechanisms, and regulatory networks, has been devised to precisely regulate gene expression instead of modifying DNA sequences. CRISPR interference (CRISPRi) employs CRISPR-linked (Cas) proteins alongside a nuclease-lacking mutant (Cas proteins) to attach to specific sequences without causing double-strand breaks, thereby suppressing native gene expression and diminishing carbon flow in competitive metabolic routes. For example, the CRISPRi mechanism was employed to inhibit the psbD gene in the photosystem II reaction centre (D2 protein). There was a notable reduction of over 95% in psbD expression, which drastically hindered PSII function and the proliferation of PCC 6803 in photosynthetic growth environments. Utilizing the previously mentioned regulatory mechanisms, cyanobacteria have developed genetic circuits for intricate programmable control. CRISPRi exemplifies a versatile and adaptable genetic control mechanism that, when integrated with two physically and chemically inducible promoters, governs the activity of circadian rhythm genes in PCC 7942 cells [13]. Recently, PCC 7942 has been developed with a protein degradation system that can be induced using Escherichia coli's SsrA tag, an SspB adaptor, and the ClpXP protease [14].

4.3 Regulating cultivation

4.3.1 Environmental stresses

Environmental elements such as the intensity of light, pH levels, salinity, and the presence of nutrients affect the proliferation and biomass buildup of cyanobacterial cells. In numerous natural microbial environments, salinity plays a crucial role as a non-living element, and there has been notable advancement in understanding how salt stress affects the production of valuable chemicals in recent years. GG stands out as an important compatible solute in marine and moderately halotolerant cyanobacteria, recognized for its various advantageous uses in cosmetics, health foods, and as an enzyme stabilizer. Following partial sustained growth in saline conditions, the production of GG in PCC 6803 mutants, which had GG absorption mechanisms and ggps deactivation, escalated to as much as 982 mg/L [15]. Spirulina platensis, one of the rare cyanobacteria capable of generating GG, thrives in high-salinity outdoor environments, aiding in almost sterile farming conditions. In response to the detrimental impacts of solar UV radiation (UVR), certain cyanobacteria have devised efficient methods, including the creation of UV-absorbing agents such as mycosporine-like amino acids (MAAs). As an example, when exposed to UVA stress, Nostoc punctiforme's mycosporine constitutes roughly 1.3% of its total

ISSN 2959-6157

dry cell weight (DCW), with its production being negligible in standard white light conditions. Moreover, when subjected to UVB stress, Nostoc flagelliforme's MAAs can constitute as much as 3.2% of the DCW [16].

4.3.2 Nutrition limitations

Research indicates that variations in nutrient levels can modify the development and metabolic processes of microalgae and cyanobacteria. Glycogen, lipids, and PHB stand out as key bioenergy and biopolymer components in cyanobacteria, with their quantities influenced by the concentration of nutrients. Studies show that in photoautotrophic environments, PCC 6803 accumulates glycogen, lipids, and PHB at rates of 22.7%, 14.1%, and 2.4% of its dry cell weight (DCW) respectively. When deprived of nitrogen (-N), the levels of glycogen and PHB rise by 62% and 4.5 times, respectively, in contrast to their levels in phosphorus (-P), sulfur, iron, or calcium scarcity scenarios. While glycogen and PHB may build up in environments lacking nutrients, they also have the potential to hinder cellular growth and diminish the accumulation of biomass. A recent investigation aimed at attaining elevated concentrations of both elements explored how partial nitrogen or phosphorus availability impacts the biomass and production of these three biological products. Findings indicated that with less than 20% phosphorus availability, glycogen build-up constituted as much as 51.8% of DCW, while PHB levels accounted for up to 6.3% of DCW. With no phosphorus present, the lipid build-up amounted to 12.7% of DCW [17].

4.3.3 Chemical Interference

Latest studies indicate that certain substances, such as plant hormones, signalling molecules, amines, and various chemical groups, can be augmented by chemical regulators. Take, for instance, calliterpenone (CT), a diterpene substance extracted from the callus tissue of the Calli-carpa macrophylla plant, which is known to enhance plant and microorganism development. Initial research showed that with 0.01 mM CT, the dry biomass, lipid, and carbohydrate production of PCC 6803 increased by 316.1%, 130.76%, and 140.34%, respectively, in comparison to the control [18].

4.3.4 Co-culture system

Biological co-cultivation methods can significantly impact the process of carbon fixation in cyanobacteria, which is driven by light. As an illustration, cyanobacteria are capable of generating reactive oxygen species (ROS) in the process of photosynthesis, and the buildup of ROS in the culture medium may impede their proliferation. Li and others. Created a synthetic co-cultivation setup involving the sugar-generating PCC 7942 and the red yeast Rhodotorula glutinis. The discovery was made that Rhodotorula glutinis can efficiently eliminate ROS from the system, thereby mitigating the growth suppression caused by cyanobacteria. Furthermore, Ducat and colleagues combined the sugar-generating PCC 7942 with a mutant strain of Saccharomyces cerevisiae W303 that uses sucrose [19]. Synthetic co-cultures are capable of maintaining stability for durations ranging from weeks to months. Under alternating light and dark conditions, both species can continue to thrive despite certain disruptions. The inclusion of yeast aids in the efficient elimination of ROS from the culture medium, markedly enhancing the system's stability compared to that of the pure cultures of the strains.

5. Conclusion

Cyanobacteria have demonstrated significant capabilities in adapting to climate change, thanks to their distinct photosynthetic processes and adaptability to the environment. Despite the availability of various enhanced techniques, additional studies are required to refine their effectiveness and tackle the difficulties present in current methods. Upcoming research ought to concentrate on enhancing the CO_2 absorption ability of cyanine bacteria and evaluating their real-world effectiveness. This approach will enhance the real-world effectiveness of cyanobacteria in reducing climate change impacts and foster the creation and advancement of associated technologies.

References

[1] National Aeronautics and Space Administration. Global climate change: Vital signs of the planet. 2024. Retrieved on August 21, 2024, retrieved from https://climate.nasa.gov/.

[2] Intergovernmental Panel on Climate Change. Climate change 2023: The physical science basis. 2023. Retrieved on August 21, 2024, retrieved from https://www.ipcc.ch/report/ar6/wg1/.

[3] Intergovernmental Panel on Climate Change. Climate Change 2023: Synthesis Report. 2023. Retrieved on August 21, 2024, retrieved from https://www.ipcc.ch/report/ar6/syr/.

[4] Whitton, B. A., & Potts, M. The ecology of cyanobacteria: Their diversity in time and space. Springer, 2012.

[5] Singh, P., & Singh, J. Biotechnological potential of cyanobacteria: An overview. Journal of Applied Phycology, 2021, 33(3): 1193-1208.

[6] Hall, D. O., & Rao, K. K. Photosynthesis. Cambridge University Press, 1999.

[7] Berg, J. M., Tymoczko, J. L., & Gatto, G. J. Biochemistry. W.H. Freeman and Company, 2015.

[8] Capone, D. G., & Zehr, J. P. Nitrogen fixation in cyanobacteria: Ecological and biotechnological implications.

Frontiers in Microbiology, 2020, 11: 572845.
[9] Wang T., et al. Pooled CRISPR interference screening enables genome-scale functional genomics study in bacteria with superior performance. Nature Communications, 2018, 9: 04899.
[10] Wendt K.E, Ungerer J., Cobb R.E., et al. CRISPR/Cas9 mediated targeted mutagenesis of the fast growing cyanobacterium Synechococcus elongatus UTEX 2973. Microbial Cell Factories, 2016, 15: 115.
[11] Niu T.C., Lin G.M., Xie L.R., et al. Expanding the potential of CRISPR-Cpf1-based genome editing technology in the cyanobacterium Anabaena PCC 7120. ACS Synthetic Biology, 2019, 8: 170-180.
[12] Racharaks R., Arnold W., Peccia J. Development of CRISPR-Cas9 knock-in tools for free fatty acid production using the fast-growing cyanobacterial strain Synechococcus elongatus UTEX 2973. Journal of Microbiological Methods, 2021, 189:106315.
[13] Ungerer J., Wendt K.E., Hendry J.I., et al. Comparative genomics reveals the molecular determinants of rapid growth of the cyanobacterium Synechococcus elongatus UTEX 2973. Proceedings of the National Academy of Sciences USA, 2018, 115: 11761-11770.
[14] Lee M., Woo H.M. A logic NAND gate for controlling gene expression in a circadian rhythm in cyanobacteria. ACS Synthetic Biology, 2020, 9: 3210-3216.
[15] Tan, X., Du, W., Lu, X. Photosynthetic and extracellular production of glucosylglycerol by genetically engineered and gel-encapsulated cyanobacteria. Appl. Microbiol. Biotechnol., 2015, 99: 2147-2154.
[16] Ferroni, L., Klisch, M., Pancaldi, S., et al. Complementary UV-absorption of mycosporine-like amino acids and scytonemin is responsible for the UV-insensitivity of photosynthesis in Nostoc flagelliforme. Mar. Drugs, 2010, 8: 106-121.
[17] Singhon, P., Phoraksa, O., Incharoensakdi, A., et al. Increased bioproduction of glycogen, lipids, and poly(3-hydroxybutyrate) under partial supply of nitrogen and phosphorus by photoautotrophic cyanobacterium Synechocystis sp. PCC 6803. J. Appl. Phycol., 2021, 33: 2833-2843.
[18] Patel, V.K., Maji, D., Singh, A.K., et al. A natural plant growth promoter, calliterpenone, enhances growth and biomass, carbohydrate, and lipid production in cyanobacterium Synechocystis PCC 6803. J. Appl. Phycol., 2013, 26: 279-286.
[19] Hays, S.G., Yan, L.L.W., Silver, P.A., et al. Synthetic photosynthetic consortia define interactions leading to robustness and photoproduction. J. Biol. Eng., 2017, 11: 4.