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Anabaena: A New Chassis for Space Exploration

Atmospheric and Regolith Adaptability: Foundations for In-Situ Resource Utilization

The viability of long-duration human presence beyond Earth hinges on the capacity for self-sufficiency, which necessitates the effective utilization of local resources. Among the myriad challenges of off-world habitation, the production of oxygen, food, and essential raw materials from the hostile environments of Mars and the Moon represents a paramount objective. In this context, the cyanobacterium Anabaena emerges not merely as a candidate organism but as a foundational biological workhorse, possessing a unique combination of capabilities that directly address these core needs [8 17]. Its potential is rooted in two critical physiological traits: the ability to thrive under Martian atmospheric analogues and to derive nutrients from extraterrestrial regolith, thereby enabling robust in-situ resource utilization (ISRU) [20 42]. These capacities transform Anabaena from a simple autotroph into a cornerstone for sustainable, closed-loop life support systems (BLSS), significantly reducing the logistical burden and cost associated with resupply missions [62].

A pivotal breakthrough in assessing Anabaena's suitability for Mars was the demonstration of its vigorous growth under a low-pressure atmosphere composed predominantly of nitrogen and carbon dioxide [51 55]. Specifically, the strain Anabaena sp. PCC 7938 was successfully cultivated in a bioreactor called Atmos using a gas mixture of 96% N_2 and 4% CO_2 at a total pressure of 100 hPa [51 52 57]. This pressure is approximately 10% of Earth's sea-level atmospheric pressure (1013 hPa), representing a pragmatic compromise that creates a physiologically viable environment for the organism while dramatically reducing the structural demands on cultivation hardware [64 75]. Crucially, the growth performance of Anabaena sp. PCC 7938 under these simulated Martian conditions was comparable to its growth under ambient terrestrial air, with biomass concentrations reaching 0.40 ± 0.026 gdw L^{-1}after 10 days, not significantly different from the 0.35 ± 0.03 gdw L^{-1}achieved in air controls [55]. This finding is transformative for mission architecture, as it suggests that bioreactors for producing biomass and oxygen need not be engineered to withstand full Earth-level internal pressures, leading to lighter, more transport-efficient designs that are essential for interplanetary travel where every kilogram of mass incurs a substantial cost [51 75]. While the Martian surface pressure is even lower, around 6-7 hPa, which is insufficient to maintain liquid water and would directly inhibit metabolism, the successful cultivation at 100 hPa demonstrates a feasible path forward for establishing biological processes on Mars [57 65]. Further research is needed to optimize the precise balance of pressure, CO_2 concentration, and N_2 partial pressure to maximize growth efficiency and resource utilization, but the proof-of-concept is firmly established [52 64].

Beyond atmospheric adaptation, the ability to utilize extraterrestrial regolith as a nutrient source is fundamental to true ISRU. Extensive ground-based experiments have validated Anabaena's capacity to grow using minerals leached from Martian regolith simulants, effectively converting inert rock into

living biomass [7,10]. Cultivation of Anabaena sp. PCC 7938 in water containing Mars Global Simulant (MGS-1) resulted in a steady increase in chlorophyll-a content over 28 days, confirming active photosynthetic growth supported by mineral nutrients [55]. The growth kinetics were well-described by a Monod equation, indicating that nutrient availability from the regolith is a rate-limiting factor, a characteristic that allows for predictable modeling of system productivity [7,58]. However, this process is complex and governed by several critical factors. Phosphorus has been identified as the primary limiting nutrient within MGS-1; supplementation with phosphate at concentrations found in standard BG11o medium increased biomass yield by 67% after 28 days, highlighting the necessity of pre-treating regolith to enhance the bioavailability of key elements [7,10]. Another major challenge is the presence of perchlorate salts, which are toxic to many forms of life and are present in Martian soil at levels up to 0.6 wt% [7,58]. While Anabaena sp. PCC 7938 exhibits moderate resistance to calcium perchlorate, its growth is significantly inhibited at higher concentrations [25]. Studies have shown that the inhibitory effects of perchlorates and nutrient limitation act independently, suggesting they represent distinct engineering hurdles that must be addressed simultaneously [7,58]. Furthermore, practical constraints such as light attenuation pose a significant challenge for photobioreactor design. Suspended regolith particles cause severe shading, with even low concentrations drastically reducing photosynthetically active radiation (PAR) penetration, which would severely limit productivity without careful reactor engineering [7,10]. Finally, research indicates that effective nutrient mobilization from the regolith requires direct physical contact between the cells and the mineral grains, or the exchange of large molecules (>15 kDa), underscoring the importance of maintaining cell-mineral interactions within the cultivation system [7,10].

Parameter	Condition	Result
Atmospheric Pressure	100 hPa (simulated Mars)	Vigorous diazotrophic growth observed. Biomass reached 0.40 ± 0.026 gdw L^{-1}. [51,55]
Atmospheric Composition	96% N_2, 4% CO_2	Growth comparable to ambient air controls (0.35 ± 0.03 gdw L^{-1}). [52,55]
Nutrient Source	Mars Global Simulant (MGS-1)	Chlorophyll-a increased from 0.3 µg/dish to 11.2 ± 0.6 µg/dish after 28 days. [55]
Phosphorus Limitation	MGS-1 alone	Identified as a primary limiting nutrient. [7,10]
Perchlorate Tolerance	$Ca(ClO_4)_2$ at 0.6 wt% in MGS-1	Moderate resistance observed, but growth is significantly inhibited at higher concentrations. [7,25]
Light Availability	Suspended MGS-1 at 20 kg/m^3	PAR irradiance dropped below detection levels over a 3.3 cm path. [7,10]

These findings collectively establish Anabaena as a highly promising chassis for space exploration. Its proven adaptability to Martian atmospheric pressures and its ability to leverage regolith for growth form the bedrock of a sustainable biological infrastructure. By serving as a primary producer that

converts atmospheric CO_2 and N_2 and solid minerals into oxygen and biomass, Anabaena can catalyze the development of cascaded BLSS, providing the foundational inputs necessary for supporting higher trophic levels, including heterotrophic microbes and higher plants, thus paving the way for truly self-sufficient off-world habitats [42 103].

Molecular Resilience to Extraterrestrial Stressors

While Anabaena's physiological adaptability provides a strong foundation for its use in space, its long-term success will ultimately depend on its resilience to the multifaceted and often extreme environmental stressors encountered beyond Earth's protective magnetosphere. The space environment presents a unique combination of hazards, primarily ionizing radiation from galactic cosmic rays (GCRs) and solar particle events (SPEs), and the persistent influence of microgravity, both of which can profoundly impact microbial function, genomic integrity, and survival [86 91]. Anabaena exhibits a remarkable suite of molecular defense mechanisms that confer high tolerance to several of these stressors, particularly radiation. However, a critical analysis reveals significant knowledge gaps, especially concerning the synergistic effects of chronic, low-dose radiation and microgravity, which remain the greatest uncertainties for long-duration missions beyond Low Earth Orbit (LEO) [91 128].

One of the most compelling aspects of Anabaena's biology is its exceptional resistance to ionizing radiation. Multiple studies have demonstrated its ability to survive high doses of gamma radiation, with growth inhibition observed only at doses between 6 − 11 kGy, and viability maintained even at 15 kGy [65 72]. This robustness is attributed to a multi-layered defense strategy involving efficient DNA repair, protein recycling, and oxidative stress management [32 70]. Upon exposure to γ-radiation, Anabaena undergoes a profound proteomic reprogramming, shifting its cellular machinery away from processes like photosynthesis and carbon assimilation towards the synthesis of ROS detoxifiers, chaperones, and proteases [70 111]. This response allows the organism to repair extensive genome and proteome damage and resume normal growth within 2-3 days post-exposure [65 70]. A central player in this response is the transcriptional regulator LexA, which orchestrates the expression of numerous genes involved in the stress response [32 70]. Beyond general repair, Anabaena employs highly specific DNA repair pathways. It efficiently utilizes photoreactivation, a light-dependent process mediated by CPD photolyase enzymes that directly reverse UV-induced thymine dimers [34 129]. This mechanism is so effective that it allows the organism to survive several hundred joules of UV irradiation when exposed to appropriate wavelengths of light [129]. Additionally, it possesses base excision repair (BER) pathways to handle oxidative DNA damage [48]. This sophisticated array of defenses makes Anabaena exceptionally well-equipped to handle the radiation flux experienced on the Martian surface, where average levels are estimated at 76 mGy/yr [72].

However, this radiation resistance profile is incomplete and contains critical vulnerabilities. Most laboratory data pertains to acute exposures of UV-B and gamma rays, leaving a significant knowledge gap regarding the organism's response to the high-linear energy transfer (LET) radiation characteristic of GCRs—a major concern for deep-space missions [91]. Furthermore, while short-term spaceflight experiments have sometimes shown enhanced post-flight growth, potentially indicative of

adaptive responses, the long-term consequences of chronic, low-dose radiation exposure on metabolic stability and genetic fidelity remain largely unexplored [94]. Perhaps the most pressing uncertainty lies in the interaction between radiation and microgravity. Ground-based simulations suggest that microgravity can impair DNA damage repair pathways, potentially exacerbating radiation-induced genotoxicity by interfering with double-strand break repair [85 114 128]. For instance, one study showed that cultured lymphocytes exposed to radiation then incubated in simulated microgravity exhibited reduced Ku70 expression (a key protein in non-homologous end joining repair) compared to radiation-only controls, indicating an impaired repair response [85]. Conversely, results from actual spaceflight experiments have been conflicting, with some showing no significant effect of real microgravity on DNA repair efficiency [130]. This discrepancy highlights the danger of extrapolating from ground-based analogs and underscores the urgent need for in-flight experiments to resolve how these two dominant space stressors interact [86 128].

Microgravity itself also presents a set of physiological challenges. Simulated microgravity, typically induced by clinorotation, has been shown to disrupt cellular homeostasis in Anabaena, leading to increased accumulation of reactive oxygen species (ROS) and alterations in antioxidant enzyme activity [9 15]. This suggests that the organism experiences a state of oxidative stress under these conditions, which could indirectly sensitize it to other stressors like radiation. The direct impact of gravity on microbial growth is notoriously complex and context-dependent, varying by strain, culture method, and environmental parameters [93]. Short-term satellite flights have yielded mixed results, with some showing transiently slower growth during flight followed by a rebound upon return, while others report no significant difference [94]. The development of advanced facilities like the Variable Gravity Simulator (VGS) aboard the International Space Station (ISS) is crucial for untangling these variables and determining the true impact of lunar (~0.16g) and Martian (~0.38g) gravity on Anabaena's performance [13]. Even among cyanobacteria, there are differences in radiosensitivity; for example, some strains show improved growth under microaerobic conditions due to impaired O_2 protection mechanisms, revealing diverse and complex regulatory networks [115 122]. Ultimately, while Anabaena's inherent molecular toolkit provides a formidable defense against individual stressors, its true resilience in the integrated space environment remains a critical area for future investigation.

Synthetic Biology Frontiers: Engineering a Programmable Biological Workhorse

While Anabaena's natural capabilities provide a powerful foundation for space exploration, its true potential as a versatile chassis is unlocked through synthetic biology—the application of engineering principles to redesign and construct biological systems for useful purposes [21]. This field offers a pathway to move beyond simply utilizing organisms as they exist in nature and instead to program them to perform specific tasks, produce valuable compounds, and adapt to the unique challenges of space. Significant progress has been made in developing a genetic toolbox for Anabaena, transforming it from a model organism into a platform for advanced metabolic engineering [22 40]. This enables the creation of strains tailored for specific applications in bioregenerative life support, in-situ

resource utilization, and biomanufacturing, thereby expanding the utility of cyanobacteria far beyond their innate abilities.

The development of robust genetic tools is the prerequisite for any meaningful synthetic biology effort. For Anabaena sp. PCC 7120, researchers have established methods for stable genomic integration at neutral sites, allowing for the reliable insertion of foreign DNA without disrupting essential native genes [22]. Furthermore, CRISPR-Cpf1 systems have been adapted for use in Anabaena, enabling markerless editing and precise insertion of genetic payloads, which is crucial for creating clean, defined modifications [22]. The Cre-LoxP recombinase system has also been employed for markerless mutagenesis, facilitating targeted gene knockouts and replacements [40]. The availability of broad-host-range vectors, such as those based on RSF1010 or pANS plasmids, further enhances its versatility by allowing for conjugation and gene transfer across different cyanobacterial species, expanding the pool of compatible chassis [41]. This growing arsenal of genetic tools provides the necessary framework for designing and constructing complex synthetic circuits and metabolic pathways within Anabaena.

The practical application of this toolkit has already demonstrated Anabaena's capacity as a host for the heterologous expression of complex natural products. Researchers have successfully expressed entire biosynthetic gene clusters for compounds like cryptomaldamide and columbamide, which are potent bioactive molecules, showcasing the strain's ability to correctly fold and express large, multi-protein enzymatic pathways [22 41]. This capability opens up exciting possibilities for in-space biomanufacturing of pharmaceuticals, nutraceuticals, and other high-value chemicals, reducing dependence on costly Earth-based supplies [13 105]. Promoter engineering and codon optimization have been used to fine-tune the expression of these pathways, improving product yields and managing potential toxicity from the synthesized compounds [22]. For example, replacing native promoters with constitutive ones or inducible promoters allowed for better control over the production of lyngbyatoxin A and the toxic compound APK, respectively [22]. These successes lay the groundwork for more ambitious engineering projects aimed at redirecting central metabolism to produce novel commodities directly from sunlight, CO_2, and water.

The conceptual applications of engineered Anabaena are vast and transformative. One of the most compelling concepts involves creating "PowerCell" strains designed to serve as a primary feedstock for co-cultured microbial communities. The 2011 iGEM team engineered Anabaena sp. PCC 7120 to continuously secrete sucrose, a readily utilizable sugar that could sustain the growth of heterotrophic organisms like Bacillus subtilis in a cascaded BLSS [19 69]. Although the live strain was not flown on the Eu:CROPIS mission, lysed cultures of this strain successfully supported bacterial growth in space, proving the principle of using cyanobacterial lysate as a nutrient source [19]. Another groundbreaking concept involves engineering cyanobacteria to produce ethylene, a key precursor for plastics, directly from CO_2 and sunlight [104]. Such a system could simultaneously generate breathable oxygen and create 3D-printable construction materials on Mars, addressing two of the most critical needs for human colonization [104]. Other proposed engineering targets include guanidine production from atmospheric nitrogen and CO_2, offering a sustainable route to nitrogen-rich compounds without fossil fuel input [47], and enhancing hydrogen production for fuel applications [72]. The future trajectory of this field points towards the development of sophisticated genetic circuits, such as NOT gates constructed

with theophylline-responsive riboswitches, which could allow for precise, external control over gene expression to manage stress responses or metabolic flux in real-time [41]. As synthetic biology capabilities advance, Anabaena is poised to evolve from a passive biological component into a programmable, autonomous factory for producing the essential resources required for sustained human presence in space [90 118].

Integration into Bioregenerative Life Support Systems

The ultimate value of Anabaena as a space chassis lies in its potential to become a functional, integral component of a bioregenerative life support system (BLSS), a closed-loop ecosystem designed to recycle air, water, and waste into breathable air, potable water, and food [62 96]. Unlike purely physicochemical systems, BLSS rely on biological processes, making organisms like Anabaena central to their operation. Its triple capability—oxygenic photosynthesis, atmospheric nitrogen fixation, and biomass generation—positions it as a uniquely powerful primary producer capable of driving the entire system [17 20]. Research has begun to explore how Anabaena can be integrated into cascaded systems, demonstrating its role as a foundational feedstock that supports downstream heterotrophic producers, forming the basis of a multi-trophic food web for off-world habitats [42 64]. This approach leverages the strengths of different organisms, using cyanobacteria to convert inorganic inputs into organic matter, which can then be consumed by other microbes or plants to produce a wider range of outputs.

A key aspect of integrating Anabaena into a BLSS is understanding its nutritional quality as a feedstock for secondary producers. Studies have shown that the filtered lysate (cell-free extract) of Anabaena sp. PCC 7938 biomass is an excellent growth medium for other organisms. When used to cultivate the heterotrophic bacterium Escherichia coli W, the resulting cell concentrations were comparable to those achieved in standard LB medium, indicating that Anabaena biomass is rich in the amino acids, vitamins, and other organic compounds necessary for bacterial growth [11 25 51]. Similarly, when used to feed duckweed (Lemna sp.), Anabaena sp. PCC 7938 supported the highest biomass yield among all tested cyanobacterial strains, achieving approximately double the biomass of the next best performer [11 25]. This demonstrates its potential to form the base of a food chain, either directly as a food source for humans or via intermediate trophic levels. The quality of this feedstock appears to be modulated by the growth conditions of the Anabaena. For instance, lysates from Anabaena grown under simulated Martian atmospheric conditions (MDA-1) supported better growth of downstream E. coli than lysates from biomass grown under ambient air, suggesting that altering the primary producer's environment can be used to tailor the nutritional output of the entire system [55]. This tunability is a powerful feature for optimizing a BLSS for specific nutritional requirements.

The practical implementation of Anabaena-based BLSS faces several technical challenges that must be addressed through system-level engineering. One of the most significant is the issue of culture homogeneity in photobioreactors. Filamentous cyanobacteria like Anabaena can form aggregates and biofilms, which can clog tubing and reduce the efficiency of mixing and light distribution in bioreactors [11 25]. Comparative studies have shown that Anabaena sp. PCC 7938 forms fewer aggregates than other strains like PCC 7120, making it a more suitable candidate for industrial-scale cultivation hardware [11 25]. Another critical challenge is the efficient recovery of biomass. Due to their

larger cell size (around 200 µm), filamentous cyanobacteria like Anabaena offer advantages for product recovery through filtration and natural aggregation compared to smaller unicellular strains, simplifying downstream processing [39]. The fixed carbon produced by Anabaena is primarily converted into exopolysaccharides, with biomass accounting for only about one-third of the total organic matter, which may require additional processing steps to concentrate the desired components [110]. Furthermore, the scalability of these systems is constrained by factors like light availability, which can be severely limited by the density of the culture and suspended regolith particles [7 10]. Designing photobioreactors that can maintain optimal light penetration and nutrient mixing while accommodating the unique morphology of filamentous cyanobacteria is a key engineering hurdle. Despite these challenges, the potential benefits are immense. By leveraging Anabaena's ability to fix atmospheric CO_2 and N_2 while extracting minerals from regolith, a BLSS can achieve a high degree of closure, minimizing reliance on Earth-supplied resources and enabling long-term, sustainable habitation on the Moon and Mars [62 119].

System Component	Role of Anabaena	Downstream Organism	Output
Primary Producer	Photosynthesis (O_2 production), Nitrogen Fixation (NH_4^+ release), Carbon Fixation (biomass)	Heterotrophic Bacteria (E. coli)	Organic acids, amino acids, proteins [8 11 17 51]
Primary Producer	Photosynthesis (O_2 production), Nitrogen Fixation, Carbon Fixation (biomass)	Higher Plants (Lemna sp.)	Edible biomass [11 25]
Primary Producer	Photosynthesis (O_2 production), Carbon Fixation	Algae (Chlorella)	Oxygen, biomass [62]
Primary Producer	Photosynthesis (O_2 production), Carbon Fixation	Fungi (in synthetic lichens)	Protein-rich biomass [14]
Primary Producer	Photosynthesis (O_2 production), Carbon Fixation, Hydrogen Production	Not Applicable	Fuel (Hydrogen) [72 95]

This table illustrates the multifaceted role of Anabaena as a foundational organism in a cascaded BLSS. By serving as the initial converter of inorganic resources into organic matter, it can fuel a variety of downstream processes, from producing consumable food to generating fuels and materials. This modular approach allows for the construction of flexible and resilient life support architectures that can be tailored to the specific needs of a mission, whether it be a short-term lunar outpost or a permanent Martian settlement [101 103].

Critical Knowledge Gaps and Controversies

Despite the considerable body of evidence supporting Anabaena's potential as a space chassis, a critical assessment reveals several significant knowledge gaps and areas of scientific controversy that

temper its promise and highlight the remaining challenges for its deployment in space. The transition from impressive ground-based proof-of-concepts to reliable, flight-proven technology requires addressing these uncertainties head-on. The most prominent gaps relate to the organism's response to the integrated and chronic nature of the space environment, the long-term stability of engineered strains, and the precise molecular mechanisms governing its resilience. Resolving these issues is essential for de-risking its use in future missions and advancing its Technology Readiness Level (TRL).

One of the most profound knowledge gaps concerns the combined effects of multiple stressors, particularly microgravity and radiation. The vast majority of published studies investigate these factors in isolation, yet in space, they occur concurrently. There is a notable lack of direct experimental evidence on Anabaena's response to the high-linear energy transfer (LET) radiation from galactic cosmic rays (GCRs), which poses a significant threat for deep-space missions [91]. Furthermore, the interaction between microgravity and radiation-induced DNA damage is a subject of intense debate. Ground-based simulations suggest that microgravity can impair DNA repair pathways, potentially leading to an accumulation of mutations and increasing health risks [85 114]. However, results from actual spaceflight experiments are conflicting, with some showing no significant effect on repair efficiency while others indicate impairment [128 130]. This discrepancy between simulation and reality underscores the limitations of analog environments and emphasizes the absolute necessity of conducting integrated stressor experiments in orbit, using platforms like the ISS's Variable Gravity Simulator or dedicated lunar landers, to determine the true synergistic impact on the organism's genomic stability and metabolic function [13 86].

Another major uncertainty is the long-term performance of Anabaena in continuous spaceflight conditions. Current data often comes from short-term lab experiments lasting hours or weeks-long satellite missions. The long-term (>1 year) stability of genetically engineered strains is unknown. Questions remain about the potential for genetic drift, loss of engineered traits, or the emergence of deleterious mutations over extended periods in a stressful environment. The maintenance of consistent metabolic output—such as a stable rate of oxygen production or nitrogen fixation—for months or years is a critical requirement for any BLSS and has not been thoroughly investigated. Long-duration experiments on the ISS are needed to assess these temporal dynamics and ensure the reliability of Anabaena-based systems for missions to Mars, which could last for years [23 77].

The field is also characterized by some controversies and conflicting findings. For instance, while many studies demonstrate the effectiveness of Anabaena in regolith-based growth, the extent of nutrient bioavailability can vary depending on the specific simulant used and the method of preparation [7 10]. Some sources note that Martian regolith is generally richer in phosphorus than Earth soils, which could alter the dynamics of nutrient limitation compared to terrestrial models [10]. Furthermore, the reported efficacy of different cyanobacterial strains can vary. While Anabaena sp. PCC 7938 has been highlighted as a superior performer, other strains like Anabaena cylindrica have also shown high growth rates on basaltic rocks, and extremophiles like Chroococcidiopsis exhibit unparalleled resistance to desiccation and radiation [56 59 68]. This raises questions about whether Anabaena is the optimal chassis for all scenarios or if a portfolio of specialized organisms might be more effective. The choice of chassis should prioritize species naturally capable of utilizing abundant

in-situ resources, and while Anabaena is a prime candidate for Mars, other organisms may be better suited for lunar applications where resources differ [101,103].

Finally, while the potential for synthetic biology is immense, there is a clear gap between current capabilities and the level of engineering required for complex, autonomous systems in space. The development of shelf-stable molecular biology reagents, automated DNA synthesis platforms, and robust containment systems for engineered organisms in microgravity are all critical breakthroughs that are still in development [90,92]. The ethical considerations surrounding planetary protection and the release of engineered organisms into extraterrestrial environments also represent a significant area of ongoing discussion within the scientific community that must be navigated responsibly [21]. Bridging these knowledge gaps through focused, in-flight research is the most critical step toward realizing the full potential of Anabaena as a reliable and indispensable component of humanity's expansion into space.

Future Trajectory: From Lunar Proving Grounds to Martian Settlements

Looking ahead, the future trajectory for Anabaena as a space chassis is intrinsically linked to NASA's phased approach to lunar and Martian exploration, which defines a staged and accretive strategy for biomanufacturing [101,103]. The insights gained from studying Anabaena in controlled laboratory settings provide a strong foundation, but its successful deployment will depend on a concerted effort to bridge the gap between ground-based proof-of-concepts and validated, reliable performance in the multifaceted reality of space. The next 5 to 10 years are expected to be a period of rapid maturation, moving from 'carry-along' and 'drop-in' technologies on the Moon to more transformative 'make-it-there' applications on Mars, ultimately enabling the vision of a fully integrated, self-sustaining habitat [90].

In the near term, the Moon serves as an ideal proving ground for testing and refining Anabaena-based technologies. According to the proposed mission classes, Artemis-like operations on the Moon with stable logistics (Class 1) will focus on deploying bioreactor systems at Technology Readiness Level (TRL) 5, aiming to advance them to TRL 7 or higher [101,103]. This phase will involve stress-testing automated and scaled bioreactors, including electro- and photo-autotrophic gas bioreactors, to understand their performance in a lunar vacuum environment and under lunar gravity [101]. Missions like the BioMoon proposal, which includes exposing dormant cyanobacteria to the lunar surface, will provide invaluable data on their survivability and ability to reactivate under realistic lunar conditions [109]. Concurrently, the development of advanced molecular biology labs on the ISS and eventually on the lunar surface will enable real-time monitoring of gene expression and stress responses in Anabaena, allowing for the iterative improvement of strains and hardware [90,92]. The goal of this lunar phase is to build confidence in the reliability of these biological systems before attempting the much greater challenges of Mars.

As we look toward Mars, the role of Anabaena is expected to expand significantly. Mars missions (Classes 3 and 4) will demand systems with TRL 8 − 9 that can integrate both in-situ resource utilization (ISRU) of the atmosphere and regolith and advanced loop-closure (LC) for waste recycling

[101][103]. Here, Anabaena's proven ability to grow on Martian regolith simulants and in low-pressure, CO_2-rich atmospheres becomes its defining asset [51][55]. The primary focus will shift from basic life support to more sophisticated biomanufacturing. This includes producing nutritionally complete foods, shelf-life-limited therapeutics like peptide-based biologics, and thermoplastic materials for 3D printing tools, medical supplies, and even habitat components [101][103]. Engineered strains of Anabaena designed to produce precursors for bioplastics like polyhydroxyalkanoates (PHAs) or ethylene could revolutionize material science on Mars, reducing the massive launch mass associated with bringing construction materials from Earth [103][104]. The development of a 'space biofoundry'—an automated facility for biological system engineering and analytics on-site—could further accelerate this process, allowing for the on-demand production of specialty chemicals, including the phosphoramidites needed for DNA synthesis to support further in-space bioengineering [101][103].

To summarize, the journey of Anabaena from a laboratory curiosity to a cornerstone of space exploration is well underway. Its unique combination of atmospheric adaptability, regolith utilization capabilities, and susceptibility to genetic engineering positions it as one of the most versatile and promising biological chassis available. However, its successful integration into future missions will hinge on overcoming the critical knowledge gaps related to long-term performance, integrated stressor responses, and system-level engineering. The next decade will be defined by a series of crucial in-flight experiments and technological demonstrations on the Moon and Mars. By systematically addressing these challenges, the scientific community can unlock the full potential of Anabaena, transforming it from a biological workhorse into an autonomous partner in humanity's quest for sustainable, long-term habitation beyond Earth.

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