Anabaena: A New Chassis for Space Exploration

Abstract

The establishment of sustainable human presence beyond low Earth orbit necessitates paradigm-shifting approaches to life-support systems that can operate independently from terrestrial supply chains. Among the biological candidates for enabling this autonomy, the filamentous, nitrogen-fixing cyanobacterium *Anabaena* has emerged as a remarkably versatile chassis for space bioengineering. This review critically examines *Anabaena*'s potential to serve as the foundational organism for bioregenerative life-support systems (BLSS) in extraterrestrial environments, particularly on Mars. We analyze the unique physiological attributes that position *Anabaena* as a biological interface between in situ resource utilization (ISRU) and closed-loop life support, including its capacity for atmospheric nitrogen fixation, photosynthetic oxygen production, and mineral bioleaching. Recent advances demonstrate that *Anabaena* sp. PCC 7938 can sustain robust growth under Martian atmospheric conditions at reduced pressures (100 hPa) while utilizing regolith simulants as nutrient sources[6][7][8]. We evaluate the molecular mechanisms underlying heterocyst differentiation and metabolic compartmentalization, assess current synthetic biology tools for genetic manipulation, and discuss engineering challenges including culture optimization, contamination control, and integration with downstream biological processes. Critical knowledge gaps are identified, particularly regarding long-term genetic stability under space conditions, optimization of photobioreactor designs for reduced gravity, and quantification of resource conversion efficiencies. Looking forward, we propose that *Anabaena*-based systems represent not merely an incremental improvement but a transformative platform that could anchor multi-trophic bioregenerative architectures, potentially reducing launch mass requirements by orders of magnitude and enabling true settlement-scale life support beyond Earth.

Introduction

The Challenge of Sustainable Space Exploration

Humanity stands at the threshold of becoming a multi-planetary species, with NASA's Artemis program targeting sustained lunar presence by 2030 and crewed Mars missions projected for the late 2030s[1][2]. However, the mass-logistics equation for these missions presents a fundamental constraint: current architectures require importing virtually all consumables from Earth, creating exponential cost scaling and severe mission duration limitations. For a crewed Mars mission lasting 500-900 days, life-support requirements for a crew of six include approximately 30 metric tons of oxygen, 18 metric tons of water, and 10 metric tons of food—masses that translate to launch costs exceeding $1 billion using current heavy-lift vehicles[3][4]. This paradigm is economically and logistically untenable for establishing permanent human presence beyond Earth.

The solution lies in transitioning from supply-based to production-based life support through two complementary strategies: in situ resource utilization (ISRU), which harvests and processes local materials, and bioregenerative life-support systems (BLSS), which employ living organisms to regenerate consumables through cyclic biological processes[1][4]. While ISRU technologies have focused primarily on abiotic chemical processes—such as extracting water from regolith or producing methane and oxygen via the Sabatier reaction—these approaches require substantial energy inputs and produce limited product diversity[2]. BLSS concepts, conversely, leverage the metabolic versatility of photosynthetic organisms to convert raw materials into complex biomolecules while simultaneously producing oxygen, but historically have been disconnected from local resource streams and dependent on Earth-supplied growth media[4][13].

Cyanobacteria as Biological ISRU Interfaces

The critical innovation enabling truly sustainable extraterrestrial life support is the integration of ISRU with BLSS through primary producers that can directly utilize planetary resources[4][7][28]. Cyanobacteria—ancient photosynthetic prokaryotes responsible for oxygenating Earth's atmosphere over 2.4 billion years ago—possess a unique combination of metabolic capabilities that make them ideal biological interfaces[3][9]. Unlike higher plants or eukaryotic algae, certain cyanobacterial lineages are lithotrophic, diazotrophic, and photosynthetic, meaning they can extract nutrients from rocks, fix atmospheric nitrogen gas, and convert carbon dioxide to biomass using only light energy[7][8][28]. This metabolic trifecta allows cyanobacteria to establish primary productivity using nothing but regolith minerals, atmospheric gases, water, and sunlight—resources demonstrably available on both the Moon and Mars[6][7][9].

Within the taxonomic diversity of cyanobacteria, heterocyst-forming filamentous genera, particularly *Anabaena* (also classified as *Nostoc* in some taxonomic schemes), exhibit specialized cellular differentiation that spatially separates the oxygen-sensitive process of nitrogen fixation from oxygenic photosynthesis[14][17][19]. This morphological and metabolic compartmentalization represents an evolutionary solution to the "oxygen problem" that has profound implications for bioengineering applications[11][17]. By differentiating specialized nitrogen-fixing cells (heterocysts) at regular intervals along photosynthetic vegetative cell chains, *Anabaena* creates microenvironments optimized for each metabolic function while maintaining filament-wide metabolic integration through intercellular nutrient exchange[14][17][27].

Rationale for *Anabaena* as Space Chassis

The selection of *Anabaena* sp. PCC 7938 as a model chassis for space biotechnology reflects a systematic evaluation process[8]. Key selection criteria included: (1) robust growth on Mars regolith simulants under diazotrophic conditions; (2) tolerance to low-pressure atmospheres compatible with engineering constraints; (3) efficient nitrogen fixation rates sufficient to support biomass accumulation; (4) suitability as feedstock for secondary heterotrophic organisms; (5) genetic tractability for synthetic biology applications; and (6) extensive physiological characterization in terrestrial laboratory settings[8][16][18]. *Anabaena* sp. PCC 7938 emerged as the leading candidate strain, demonstrating superior performance across these metrics compared to unicellular cyanobacteria such as *Synechococcus* or *Synechocystis* species[8].

This review synthesizes current understanding of *Anabaena* as a space biotechnology platform, critically evaluating physiological capabilities, molecular mechanisms, genetic

engineering tools, and system integration challenges. We identify critical knowledge gaps that must be addressed to transition *Anabaena* from a promising candidate to a flight-qualified biological system. Finally, we provide a forward-looking perspective on how *Anabaena*-based platforms could evolve over the next decade to enable not just survival, but thriving human communities beyond Earth.

Physiological Foundations for Space Applications

Growth Under Mars-like Atmospheric Conditions

One of the most significant recent breakthroughs in cyanobacterial space biotechnology was the demonstration that *Anabaena* sp. PCC 7938 can sustain photoautotrophic, diazotrophic growth under atmospheric conditions approximating the Martian surface[7][9]. Verseux and colleagues developed a specialized low-pressure photobioreactor (termed "Atmos") capable of maintaining precise atmospheric control across nine cultivation chambers, enabling systematic evaluation of growth parameters under reduced total pressure[7]. Their landmark study revealed that *Anabaena* could grow robustly in a 96% N_2, 4% CO_2 gas mixture at 100 hPa total pressure—conditions they designated as "Mars Design Atmosphere-1" (MDA-1)[7][15].

This finding carries profound engineering implications. The Martian atmospheric pressure ranges from 6-11 hPa at the surface, far too low to maintain liquid water[9][12]. Creating Earth-like atmospheric conditions (101.3 kPa) in habitation or cultivation facilities would require massive structural reinforcement to withstand the pressure differential, dramatically increasing habitat mass and construction complexity[7][9]. The demonstration that cyanobacteria can thrive at intermediate pressures (100 hPa) represents a critical compromise: pressures high enough to maintain liquid water and support robust biological metabolism, yet low enough to substantially reduce structural requirements and facilitate the use of Martian atmospheric gases with minimal processing[7][15].

Subsequent investigations extended these findings, demonstrating that lowering atmospheric pressure from 1000 hPa down to 80 hPa—while maintaining constant partial pressures of metabolizable gases (N_2 and CO_2)—did not reduce *Anabaena* growth rates[6]. These studies also derived mathematical relationships analogous to Monod kinetics describing the dependence of growth on partial pressures of nitrogen and carbon dioxide, providing quantitative frameworks for photobioreactor design optimization[6]. The gas requirements were found to be remarkably modest: nitrogen partial pressures as low as 20 hPa and CO_2 partial pressures of 0.4 hPa supported near-maximal growth rates[6][7]. Given that Mars's atmosphere contains approximately 2.7% N_2 (equivalent to 0.2-0.3 hPa partial pressure at the surface), enrichment by only 50-100 fold would suffice—an achievable target using membrane separation or cryogenic distillation[7][9].

Regolith Bioleaching and Nutrient Acquisition

The capacity to extract bioavailable nutrients from planetary regolith represents the second pillar of *Anabaena*'s ISRU capabilities[8][18]. Martian regolith, analyzed extensively by rover missions including Curiosity's investigation of the Rocknest aeolian deposit at Gale Crater, contains substantial concentrations of essential mineral nutrients: silicon (18-21% SiO_2), iron (17-21% total Fe), calcium (5-7% CaO), magnesium (7-9% MgO), potassium (0.4-0.6% K_2O), and phosphorus (0.8-1.0% P_2O_5)[8][18]. However, these nutrients are

primarily locked in crystalline mineral phases including olivine, pyroxene, plagioclase feldspar, and magnetite—forms not directly bioavailable[8].

Verseux et al. demonstrated that *Anabaena* sp. PCC 7938 could grow in media containing Mars Global Simulant-1 (MGS-1), a high-fidelity analog of Rocknest regolith, as the sole nutrient source under diazotrophic, photoautotrophic conditions[8][15][18]. Growth dynamics studies revealed that phosphorus was the primary limiting nutrient in MGS-1, with growth rates and carrying capacities directly correlated with phosphate bioavailability[18]. Critically, when cyanobacterial cells and regolith particles were physically separated using dialysis membranes (15 kDa molecular weight cutoff), growth was severely impaired, indicating that direct cell-mineral contact is required for efficient nutrient mobilization[18]. This suggests that *Anabaena* actively promotes mineral dissolution through bioweathering mechanisms, likely involving the secretion of organic acids, siderophores, and other chelating agents[8][13][18].

The requirement for cell-regolith contact has important implications for bioreactor design: systems must enable intimate mixing while also facilitating downstream biomass-regolith separation for purification[18]. The growth kinetics in regolith-supplemented media showed typical bacterial patterns, with specific growth rates of 0.15-0.25 d^{-1} and doubling times of 3-5 days when phosphorus was not limiting[18]. These rates, while slower than terrestrial agricultural crops, are entirely compatible with Mars mission timelines where biological systems can be initiated months before crew arrival and operated continuously throughout surface operations[4][8].

Nitrogen Fixation and Heterocyst Function

The ability to fix atmospheric nitrogen represents perhaps *Anabaena*'s most critical capability for space applications, as fixed nitrogen (ammonia, amino acids, proteins) is essential for all life and is energetically expensive to import from Earth[7][8][14]. *Anabaena* accomplishes nitrogen fixation through the enzyme complex nitrogenase, localized within specialized cells called heterocysts that differentiate at semi-regular intervals (approximately every 10-15 vegetative cells) along the filament[14][17][25][27].

Heterocyst differentiation is triggered by nitrogen starvation and orchestrated by a complex genetic regulatory cascade[25][27][30]. Within 18-24 hours of nitrogen deprivation, pre-determined vegetative cells commit to the heterocyst developmental program, undergoing profound metabolic and structural remodeling[14][27]. The developing heterocyst down-regulates photosystem II (PSII), eliminating oxygen-generating photosynthesis while maintaining photosystem I (PSI) for ATP generation via cyclic photophosphorylation[17][27]. Simultaneously, the cell synthesizes a multi-layered envelope consisting of an inner glycolipid layer and an outer polysaccharide layer, creating a diffusion barrier that maintains internal microaerobic conditions despite the oxygen-rich environment of adjacent photosynthesizing vegetative cells[17][27][30].

Nitrogenase, the enzyme catalyzing the reduction of N_2 to NH_3, is irreversibly inactivated by oxygen exposure[14]. The heterocyst's elaborate structural and metabolic specializations—PSII elimination, envelope formation, enhanced respiration—collectively create an intracellular environment with oxygen concentrations approximately 1000-fold lower than in vegetative cells, protecting nitrogenase from inactivation[17]. Recent nanoscale elemental imaging using synchrotron X-ray fluorescence revealed elevated concentrations of iron, calcium, and potassium in heterocysts compared to vegetative

cells, consistent with the high metal cofactor demands of nitrogenase (which contains 16 Fe atoms and 2 Mo atoms per complex) and elevated metabolic activity[11].

The spatial separation of nitrogen fixation (heterocysts) and photosynthetic carbon fixation (vegetative cells) necessitates extensive intercellular metabolite exchange[14][17]. Isotopic labeling studies using ^{13}C-bicarbonate and $^{15}N_2$, coupled with nanoscale secondary ion mass spectrometry (NanoSIMS), revealed the dynamic flux patterns: fixed carbon (primarily as sucrose) flows from vegetative cells to heterocysts to fuel the energetically expensive nitrogen fixation process (8 ATP per N_2 reduced), while fixed nitrogen (as amino acids, particularly glutamine) flows from heterocysts to vegetative cells for assimilation into proteins and nucleotides[14][17]. This bidirectional nutrient exchange occurs through septal junctions—proteinaceous channels connecting adjacent cells, composed of proteins including FraCD[14].

Photosynthetic Efficiency and Biomass Production

Photosynthetic oxygen production is central to *Anabaena*'s potential role in life support[3][7][13][31]. Cyanobacterial photosynthesis employs the same oxygenic pathway as higher plants: capturing photons via phycobiliproteins and chlorophyll a, transferring excitation energy to photosystems II and I, splitting water to extract electrons, and using the resulting reducing power to fix CO_2 via the Calvin-Benson-Bassham cycle[13]. The theoretical maximum photosynthetic efficiency (light energy converted to chemical energy) is approximately 10-12%, though realized efficiencies in cultivation systems typically range from 3-8% depending on light intensity, wavelength, temperature, and nutrient availability[13][31].

Under optimized laboratory conditions with continuous illumination and nutrient sufficiency, *Anabaena* cultures can achieve optical densities (OD_{750}) of 2-4 within 7-14 days, corresponding to dry biomass concentrations of 1-2 g/L[7][8][18]. Photosynthetic oxygen production rates scale with biomass, typically yielding 1-3 mg O_2 per gram dry weight per hour under moderate light intensities (50-100 µmol photons m^{-2} s^{-1})[3][13][31]. For a photobioreactor with 100 L working volume at 1.5 g/L biomass density, this translates to 3.6-10.8 kg O_2 per day—sufficient to support the respiratory demands of approximately one to three adult humans[3][13].

However, translating these laboratory productivity metrics to space operational contexts requires addressing multiple challenges[4][13][31]. Microgravity effects on bubble formation and gas-liquid mass transfer may reduce oxygen removal efficiency, potentially leading to oxygen inhibition of photosynthesis[31]. Variable lighting conditions (e.g., Martian dust storms reducing insolation) necessitate energy storage mechanisms or artificial lighting backup systems[4][7]. Temperature fluctuations on planetary surfaces require thermal regulation of cultivation systems[4][8]. Recent ground-based studies preparing for space flight experiments with the related species *Limnospira indica* identified insufficient gas transfer as a critical bottleneck, leading to oxygen inhibition and growth limitation[31]. These practical engineering challenges underscore that transitioning *Anabaena* from a functional laboratory model to a reliable operational system requires intensive technology development.

Capability	Requirement	Performance	Reference
Low-pressure growth	80-100 hPa	Growth rate maintained	[6][7]
N_2 partial pressure	>20 hPa	Near-maximal growth	[6][7]
CO_2 partial pressure	>0.4 hPa	Supports photosynthesis	[6][7]
Regolith utilization	MGS-1 simulant	Growth as sole nutrient source	[8][18]
N_2 fixation rate	0.5-2 nmol N_2 min^{-1} mg^{-1}	Sufficient for biomass	[14][17]
Doubling time	3-5 days (regolith)	Compatible with missions	[8][18]
O_2 production	1-3 mg g^{-1} h^{-1}	Life-support relevant	[3][13][31]
Biomass yield	1-2 g L^{-1}	Scalable production	[7][8]

Table 1: Summary of *Anabaena* sp. PCC 7938 physiological performance parameters relevant to space life-support applications, demonstrating capabilities that meet or exceed minimum requirements for bioregenerative systems.

Molecular Mechanisms and Genetic Regulation

Heterocyst Differentiation Regulatory Networks

The differentiation of nitrogen-fixing heterocysts from photosynthetic vegetative cells represents one of the most sophisticated examples of prokaryotic cellular differentiation, involving coordinated transcriptional cascades, cell-cell signaling, and pattern formation mechanisms[25][27][30][33]. Understanding these regulatory networks is essential for synthetic biology approaches aimed at optimizing *Anabaena* performance or engineering novel capabilities[20][26][32].

The master regulator of heterocyst differentiation is HetR, a serine-type protease that functions as both a DNA-binding transcriptional regulator and a direct sensor of developmental signals[25][27]. Upon nitrogen depletion, the global nitrogen regulator NtcA activates *hetR* transcription, initiating the differentiation cascade[27][33]. HetR then auto-activates its own expression and directly or indirectly activates numerous downstream genes required for heterocyst maturation, including those encoding envelope components (*hepA*, *hepB*, *hepC*, *hglD*, *hglE*), nitrogen fixation machinery (*nifB*, *nifH*, *nifD*, *nifK*), and DNA rearrangement systems (*xisA*, *xisC*)[27][30][33].

A critical aspect of heterocyst biology is pattern formation—the establishment of heterocysts at regular intervals rather than random or clustered distributions[27]. This patterning is achieved through lateral inhibition mediated by two diffusible peptide signals, PatS and HetN, which act as inhibitors of heterocyst differentiation[27][30]. Genetic and molecular evidence indicates that both PatS and HetN function by binding to HetR,

preventing its DNA-binding activity and thereby blocking differentiation in cells exposed to these signals[27]. The resulting model posits that cells committed to heterocyst fate produce and export PatS and HetN, creating inhibitory gradients that prevent immediate neighbors from also differentiating, thereby establishing the characteristic spacing pattern[27][30].

Recent studies have identified mutations in *hetR* (specifically, the R223W substitution) that render the protein insensitive to PatS and HetN inhibition, resulting in essentially random heterocyst distribution and, at high expression levels, complete differentiation of all cells in the filament—a lethal phenotype[27]. This demonstrates that HetR serves as the central integration point for both positive differentiation signals (nitrogen starvation via NtcA) and negative patterning signals (PatS and HetN), making it a key target for genetic engineering approaches aimed at modulating heterocyst frequency or distribution[20][27][32].

Gene Expression Control in *Anabaena*

Beyond heterocyst differentiation, comprehensive genetic engineering of *Anabaena* requires robust tools for controlling gene expression with temporal precision and appropriate dynamic range[20][26][32]. The heterologous protein expression toolkit for *Anabaena* sp. PCC 7120 (the most extensively characterized strain genetically) has expanded significantly in recent years but remains less developed than tools available for model heterotrophs like *Escherichia coli* or *Saccharomyces cerevisiae*[20][26][32].

Svoboda et al. systematically evaluated a library of promoter-riboswitch constructs for inducible protein expression in *Anabaena* sp. PCC 7120[20][26]. Their approach combined six cyanobacterial promoters spanning a range of constitutive strengths (weak, medium, strong) with two theophylline-responsive riboswitches (designated E and F), which undergo conformational changes upon ligand binding to modulate translation initiation[20][26]. Quantification of chloramphenicol acetyltransferase (CAT) activity as a reporter revealed several important findings: (1) theophylline addition increased protein production for nearly all promoter-riboswitch combinations, demonstrating functional induction; (2) riboswitch F generally exhibited lower basal expression than riboswitch E, providing better off-state repression; (3) strong promoters yielded high baseline expression that was not substantially increased by induction, indicating that promoter strength and riboswitch dynamic range must be matched; and (4) medium-strength promoters paired with riboswitch F provided the best combination of low basal expression and high induction fold-change[20][26].

These findings have practical implications for metabolic engineering and synthetic biology applications[20][26][29][32]. For applications requiring tight transcriptional control—such as conditionally expressing toxic genes, controlling metabolic flux, or creating feedback regulatory circuits—medium-strength promoters with low-leakage riboswitches offer optimal performance[20][26]. For applications requiring maximal expression levels—such as producing heterologous proteins or overexpressing rate-limiting enzymes—strong constitutive promoters may be preferred despite limited dynamic range[26][29].

Genome Editing Technologies

The application of programmable nucleases, particularly CRISPR-Cas systems, has revolutionized cyanobacterial genetic engineering over the past decade[29][32]. While *Synechococcus* elongatus PCC 7942 and *Synechocystis* sp. PCC 6803 were the first cyanobacteria to be successfully edited using CRISPR-Cas9, *Anabaena* sp. PCC 7120 initially proved more recalcitrant, possibly due to high levels of Cas9 toxicity[26][29][32].

The introduction of CRISPR-Cas12a (also known as Cpf1) systems provided a breakthrough for *Anabaena* genome editing[29][32]. Cas12a offers several advantages over Cas9: (1) recognition of T-rich PAM sequences (TTTV) rather than G-rich PAMs (NGG), expanding target site options in AT-rich genomes; (2) generation of staggered DNA ends rather than blunt ends, potentially facilitating homology-directed repair; (3) processing of its own CRISPR array, enabling multiplex editing from a single transcript; and (4) apparently lower toxicity in cyanobacteria[29][32]. CRISPR-Cas12a has been successfully applied in *Anabaena* sp. PCC 7120 for both targeted gene disruption and markerless gene insertion[29][32].

A particularly innovative advancement is the adaptation of CRISPR-associated transposase (CAST) systems for *Anabaena*[29]. Unlike standard CRISPR editing, which relies on host recombination machinery for inserting donor DNA (often inefficient in cyanobacteria), CAST systems use a transposase enzyme fused to the Cas protein, enabling RNA-guided transposition that inserts genetic cargo 63 bp downstream of the PAM sequence with high precision and without activating endogenous transposons[29]. This technology has been successfully demonstrated in *Anabaena* sp. PCC 7120, opening possibilities for high-efficiency, predictable genomic insertions[29].

Despite these advances, cyanobacterial genome editing remains challenged by relatively low transformation efficiencies, long generation times (compared to bacteria like *E. coli*), and incomplete understanding of DNA repair pathways[26][29][32]. Most *Anabaena* genetic modifications still require conjugative transfer from *E. coli* using mobilizable plasmids, a time-consuming process typically requiring 2-4 weeks to obtain edited strains[21][26]. The development of neutral chromosomal integration sites—genomic loci where insertions do not disrupt essential functions—has facilitated the construction of engineered strains carrying multiple heterologous pathways, as demonstrated by successful expression of natural product biosynthetic gene clusters in *Anabaena*[29][32].

Tool Type	Specific Tool	Application/Notes	Reference
Promoters	Strong, medium, weak	Constitutive expression with varying strength; strong promoters for maximal expression	[20][26]
Riboswitches	Theophylline-responsive E and F	Inducible expression; F has lower basal activity; best with medium promoters	[20][26] [32]
Genome editing	CRISPR-Cas12a	Markerless editing, lower toxicity than Cas9, multiplex capability	[29][32]
Genome editing	CRISPR-associated transposase (CAST)	Precise insertion without relying on recombination; 63 bp downstream of PAM	[29]
DNA transfer	Conjugation with *E. coli*	Primary transformation method; requires 2-4 weeks	[21][26]
Selection markers	Antibiotic resistance (chloramphenicol, spectinomycin, kanamycin)	Required for most transformations; markerless systems in development	[21][26] [29]
Integration sites	Neutral chromosomal sites (NS1, NS2, NS3)	Stable multi-copy or multi-pathway expression without disrupting essential genes	[29][32]

Table 2: Synthetic biology toolkit available for genetic engineering of *Anabaena* species, highlighting recent advances that enable more sophisticated metabolic engineering and chassis optimization for space applications.

System Integration and Engineering Challenges

Photobioreactor Design for Reduced Pressure and Microgravity

Translating *Anabaena* cultivation from laboratory flasks to space-qualified bioreactor systems requires addressing multiple engineering constraints simultaneously[4][6][7][31]. The development of the Atmos photobioreactor by Verseux et al. represented a crucial proof-of-concept for low-pressure cultivation, but operational systems must additionally contend with microgravity, limited power availability, mass and volume constraints, and long-term sterility maintenance[4][7][31].

Pressure containment and atmospheric composition control are paramount. Operating at 100 hPa rather than 1013 hPa (Earth sea level) reduces structural stress by approximately 90%, but the bioreactor must still maintain a stable pressure differential relative to the external environment (near-vacuum in space, 6-11 hPa on Mars)[7]. The atmosphere must be actively managed to prevent depletion of CO_2 (consumed in photosynthesis) and accumulation of O_2 (produced by photosynthesis), requiring gas separation, recycling, and replenishment systems[6][7]. The modest partial pressure requirements identified by recent studies—20 hPa N_2 and 0.4 hPa CO_2—suggest that gas processing systems can be relatively compact, but long-term reliability remains undemonstrated[6][7].

Microgravity introduces particularly complex challenges for gas-liquid systems[4][31]. On Earth, buoyancy drives bubble rise, facilitating gas-liquid separation and mass transfer. In microgravity, bubbles do not rise, instead accumulating and coalescing into large gas pockets that can impede circulation and reduce gas exchange efficiency[4][31]. Studies with *Limnospira indica* photobioreactors identified insufficient gas transfer as a critical performance limitation, leading to oxygen accumulation and photosynthetic inhibition[31]. Potential solutions include membrane gas exchange systems (analogous to hollow-fiber bioreactors), active mixing to disperse bubbles, or counter-current flow designs, but each adds complexity, mass, and power requirements[4][31].

Light delivery presents another design constraint. While Mars receives sufficient solar radiation (approximately 590 W/m^2 peak, compared to 1000 W/m^2 on Earth) to drive photosynthesis, dust storms can reduce surface insolation by 90% or more for weeks to months[4][7]. Photobioreactors must therefore incorporate light distribution systems—either fiber optic light guides concentrating external sunlight or LED arrays powered by nuclear or solar sources—capable of delivering 50-200 µmol photons m^{-2} s^{-1} uniformly throughout the culture volume[4][7][13]. LED technology offers spectral tuning advantages, allowing optimization of wavelengths matching phycobiliprotein and chlorophyll absorption peaks (approximately 440 nm, 620-650 nm, and 680 nm)[13].

Integration with Secondary Biological Processes

A critical advantage of *Anabaena*-based systems is their capacity to serve as primary producers feeding downstream secondary biological processes, potentially supporting multi-trophic food webs that dramatically expand the functional capabilities of bioregenerative life support[7][8][10][13][28]. Demonstration of this principle is essential for validating the "CyBLiSS" (cyanobacterium-based BLSS) concept[28].

Verseux et al. demonstrated that *Anabaena* biomass grown under MDA-1 conditions on MGS-1 regolith could successfully support the growth of *Escherichia coli*, a representative heterotrophic bacterium[7]. While *E. coli* itself is not proposed as a space organism, this

experiment validated the proof-of-concept that cyanobacterial biomass provides bioavailable carbon, nitrogen, and cofactors for downstream heterotrophs[7][10]. Extensions of this work should evaluate more application-relevant secondary organisms, including edible mushroom-forming fungi (e.g., *Pleurotus* species producing oyster mushrooms), oleaginous yeasts capable of lipid accumulation for biofuel production, and potentially higher plants that could provide nutritional diversity[4][10][13].

The European Space Agency's MELiSSA (Micro-Ecological Life Support System Alternative) project represents the most comprehensive effort to develop closed-loop bioregenerative architectures[10][13]. MELiSSA employs a four-compartment design: (1) liquefaction of solid waste by thermophilic anaerobes; (2) photoheterotrophic conversion to organic acids and ammonia; (3) nitrification by autotrophic bacteria converting ammonia to nitrate; and (4) photosynthetic production of food and oxygen using microalgae or higher plants[10][13]. Integration of *Anabaena* into MELiSSA-type architectures could potentially replace or augment compartments 3 and 4 while adding the crucial ISRU interface capability—utilizing regolith minerals and atmospheric gases to supply nutrients that would otherwise need to be recycled or imported[10][13][28].

Recent theoretical analyses suggest that optimized multi-organism BLSS incorporating cyanobacteria as primary producers could achieve closure levels exceeding 90% for oxygen, 80% for water, and 60-70% for food, dramatically reducing resupply requirements compared to purely physicochemical life support systems (typically <50% closure)[4][13]. However, these projections rely on numerous assumptions requiring experimental validation, including nutrient transfer efficiencies between trophic levels, genetic and community stability over multi-year timescales, and resilience to perturbations or component failures[4][10][13].

Contamination Control and Planetary Protection

The utilization of living organisms for life support in extraterrestrial environments raises critical planetary protection concerns[10]. International agreements embodied in the Outer Space Treaty (1967) and subsequent Committee on Space Research (COSPAR) policies mandate that space-faring nations prevent both forward contamination (Earth microbes transported to other worlds that could compromise scientific investigations or harm potential indigenous life) and backward contamination (extraterrestrial materials returned to Earth that could threaten terrestrial ecosystems)[10].

For Mars, classified as a Category IV target under COSPAR policy due to the possibility of extant or extinct life, planetary protection requirements are stringent[10]. Biological systems intended for use on Mars must be completely contained, with multiple redundant barriers preventing release to the Martian environment[10]. This necessitates photobioreactor designs incorporating fail-safe containment—potentially including physical barriers, HEPA filtration of gas streams, and sterilization of waste outputs[10]. Verseux noted in interviews that the risk of *Anabaena* contaminating Mars can be reduced to extremely low levels through appropriate engineering controls, but acknowledged that absolute guarantees are impossible[10].

Conversely, *Anabaena* cultures must be protected from potential Martian contaminants, particularly oxidizing compounds such as perchlorates and hydrogen peroxide detected in Martian regolith[18]. Studies have evaluated perchlorate tolerance in *Anabaena* sp. PCC 7938, finding that concentrations up to 1% (mass/mass) in growth media did not prevent growth, though higher concentrations showed inhibitory effects[18]. Martian regolith

contains perchlorates at approximately 0.5-1% levels, suggesting that with appropriate bioreactor design (e.g., pre-washing regolith or selective extraction of nutrients), perchlorate toxicity should be manageable[18].

An additional consideration is genetic containment—preventing horizontal gene transfer from engineered *Anabaena* strains to any hypothetical indigenous Martian microorganisms[10]. Strategies could include auxotrophies (engineering dependency on compounds not available in the Martian environment), genetic instability circuits (kill switches activated by environmental signals), or physical containment rendering escape impossible[10]. The development and validation of these safeguards should proceed in parallel with technological development to ensure responsible implementation[10].

Critical Analysis: Controversies and Knowledge Gaps

Scalability and Resource Conversion Efficiency

While laboratory demonstrations of *Anabaena* growth under Mars-relevant conditions are encouraging, critical questions remain regarding scalability to mission-relevant production levels[4][8][18]. A human requires approximately 0.8-1.0 kg oxygen per day, 2-3 kg water per day (for drinking and hygiene), and 1-2 kg dry food per day[3][4]. For a six-person crew, this translates to approximately 5-6 kg O_2, 12-18 kg water, and 7-12 kg food daily. Meeting these demands through cyanobacterial cultivation requires either extremely large photobioreactor volumes or exceptionally high productivity per unit volume[3][4][13].

Consider oxygen production as a benchmark. Laboratory cultures achieving 1.5 g/L biomass density with photosynthetic rates of 2 mg O_2 g^{-1} h^{-1} produce 3 mg O_2 L^{-1} h^{-1}, equivalent to 72 mg L^{-1} day^{-1} or 0.072 g L^{-1} day^{-1}[3][13]. To generate 5 kg O_2 per day would require approximately 70,000 L of culture—impractical for launch mass constraints[3][4]. Achieving practical scales demands either increasing biomass density 5-10 fold (to 7-15 g/L) or increasing specific photosynthetic rates by similar factors[4][13]. High-density cultivation approaches, such as membrane-based perfusion systems or immobilized-cell configurations, may offer paths to these productivity targets but remain largely unexplored for *Anabaena*[4][13].

An equally critical but less frequently addressed question concerns the overall resource conversion efficiency: how much regolith must be processed, how much water consumed, and how much light energy input to generate each kilogram of usable biomass?[8][18] The phosphorus limitation observed in MGS-1 cultivation suggests that Martian regolith contains sufficient minerals for substantial biomass production, but the ratio of regolith mass to biomass yield has not been systematically quantified[18]. If, hypothetically, 100 kg of regolith must be processed (dissolved, filtered, disposed) to generate 1 kg biomass, the logistical burden may outweigh the benefits compared to importing nutrients from Earth[8][18]. Comprehensive mass and energy balance analyses are urgently needed to evaluate whether *Anabaena*-based ISRU offers genuine advantages over alternative strategies[4][8].

Genetic Stability and Evolutionary Dynamics

Long-duration space missions—18 months for a Mars surface mission, years or decades for permanent settlements—raise concerns about the genetic and phenotypic stability of *Anabaena* cultures[5]. Cyanobacteria are capable of rapid adaptive evolution, with mutation rates of approximately 10^{-9} to 10^{-8} per base pair per generation[5]. Over hundreds or thousands of generations (achievable within months given 3-5 day doubling times), spontaneous mutations, gene duplications, deletions, and horizontal gene transfer events can significantly alter strain characteristics[5][19].

Studies of bacterial adaptation to the International Space Station environment revealed multiple genomic changes, including alterations in mechanosensitive channel proteins (potentially adapting to hypoosmotic stress associated with microgravity), increased DNA repair capacity, and modifications to biofilm formation genes[5]. While these specific adaptations may not apply to *Anabaena* in photobioreactors, they illustrate that space-associated selective pressures can drive evolutionary change. For *Anabaena*, potential evolutionary trajectories of concern include: (1) loss of nitrogen fixation capability (energetically expensive and potentially selected against if fixed nitrogen becomes available from dead cells); (2) reduced heterocyst frequency (if nitrogen fixation load can be shared among fewer heterocysts); (3) loss of regolith utilization capacity (if bioavailable nutrients accumulate from incomplete recycling); or (4) emergence of faster-growing variants that outcompete the original strain but have reduced functionality[8][19].

Mitigation strategies include periodic re-inoculation with frozen reference cultures, genetic engineering of stabilization systems (e.g., toxin-antitoxin modules penalizing loss of key functions), and continuous monitoring of culture performance with early detection of productivity decline[5]. However, these approaches add complexity and may not be foolproof. More fundamentally, rigorous long-term evolution experiments—maintaining *Anabaena* under Mars-simulated conditions for hundreds of generations while tracking genetic and phenotypic changes—are needed to empirically assess stability risks[5][8].

Physiological Stress and Multi-Stressor Interactions

Laboratory studies of *Anabaena* under Mars conditions typically evaluate one or two stressors at a time (e.g., low pressure plus limited nutrients, or low pressure plus perchlorate exposure)[6][7][18]. Real Martian environments, however, present multiple simultaneous stressors whose interactions may be non-additive[4]. Temperature fluctuations on Mars range from -120°C to +20°C diurnally near the equator, requiring active thermal control of bioreactors but also imposing thermal cycling on any external components[4]. Ionizing radiation—cosmic rays and solar energetic particles—reaches the Martian surface at approximately 200 mSv/year, roughly 100 times higher than Earth's surface, due to the lack of a protective magnetosphere and thin atmosphere[4]. While photobioreactors would provide some shielding, and cyanobacteria have demonstrated radiation resistance in low Earth orbit exposure experiments, chronic radiation effects on growth, mutation rates, and long-term viability require systematic investigation[3][4].

An additional poorly characterized stressor is nutrient imbalance. Martian regolith has nutrient ratios (e.g., N:P:K) that differ substantially from terrestrial soils or optimized growth media[8][18]. While phosphorus has been identified as the primary limiting nutrient in MGS-1, other elements—including trace metals (Mn, Zn, Cu, Mo, Ni) essential for metalloenzymes like nitrogenase—may become limiting under different conditions or with different regolith compositions[8][11][18]. Regional variations in Martian geology could

significantly impact bioleaching efficiency and nutrient profiles, introducing geographic constraints on where *Anabaena*-based systems could be deployed[8].

The lack of multi-stressor interaction studies represents a significant knowledge gap. Synergistic effects—where the combination of stressors is more detrimental than the sum of individual effects—are common in microbial stress biology[4][5]. For example, radiation damage to DNA repair systems could reduce tolerance to chemical stressors, or temperature stress could exacerbate oxygen inhibition of nitrogen fixation. Comprehensive experimental designs evaluating *Anabaena* performance under realistic multi-stressor Mars simulation conditions are essential for risk assessment and technology maturation[4][8].

Alternative Organisms and Comparative Chassis Selection

While this review focuses on *Anabaena*, it is crucial to acknowledge that alternative cyanobacterial species and even non-cyanobacterial organisms offer competing or complementary capabilities[8][13]. Unicellular cyanobacteria such as *Synechococcus* sp. PCC 7002 (a coastal marine strain) exhibit faster growth rates, higher genetic tractability, and superior tolerance to high light intensities compared to *Anabaena*[9][13]. However, *Synechococcus* 7002 lacks nitrogen fixation capability, requiring an external nitrogen source—potentially sourced from nitrate minerals in regolith or from atmospheric nitrogen processed through abiotic Haber-Bosch reactors[9][13].

The siderophilic (iron-loving) cyanobacterium *Leptolyngbya* sp. JSC-1, isolated from extreme environments, demonstrates exceptionally high rates of bioleaching, particularly for iron-rich minerals, and exhibits elevated photosystem I:II ratios suggesting superior performance under iron-replete conditions like Mars[13]. Another candidate, *Arthrospira* (spirulina), while not a nitrogen fixer, produces high-quality biomass rich in protein (60-70% dry weight), essential amino acids, vitamins, and bioactive compounds including phycocyanins with antioxidant and anti-inflammatory properties, making it particularly attractive for nutritional applications[13][31].

The question of chassis selection—whether to deploy a single optimized *Anabaena* strain, multiple complementary cyanobacterial species, or complex consortia combining cyanobacteria with other microorganisms—remains unresolved[8][13]. Single-organism systems offer simplicity, predictability, and ease of genetic engineering but limited functional capacity[8]. Multi-organism consortia offer functional diversity and potentially greater stability through ecological interactions but at the cost of complexity, unpredictability, and difficulty in maintaining desired community composition[10][13]. This design tension mirrors debates in terrestrial biotechnology between pure culture and mixed culture systems, without clear resolution[10][13]. Mission-specific requirements—particularly the balance between closure level targets, acceptable complexity, and risk tolerance—will likely dictate optimal strategies[4][10].

Future Perspectives: 5-10 Year Horizon

Synthetic Biology-Driven Chassis Optimization

The convergence of systems biology, synthetic biology, and space biotechnology over the next decade will likely drive transformation of *Anabaena* from a naturally occurring organism to an extensively engineered chassis optimized for extraterrestrial deployment[20][26][29][32][34]. Key engineering targets include:

Enhanced photosynthetic efficiency: Natural photosynthesis captures only 3-8% of incident light energy, leaving substantial room for improvement[13]. Strategies include optimizing light-harvesting antenna size to reduce self-shading and energy dissipation, introducing carbon-concentrating mechanisms (already present in cyanobacteria but potentially improvable), expressing algal or C4 plant enzymes to augment CO_2 fixation rates, and engineering photosystem stoichiometry to match Mars light spectra[13][32]. Computational models based on genome-scale metabolic reconstructions could guide rational design, prioritizing modifications with maximal impact on productivity[29][32].

Expanded nutrient mobilization: Engineering secretion of siderophores (iron chelators), organic acids (phosphate solubilizers), and other bioweathering compounds could enhance regolith utilization[8][13]. Expression of heterologous phosphatases capable of liberating phosphate from organic or mineral sources could alleviate the phosphorus limitation observed in MGS-1 cultivation[18]. Alternatively, introducing biosynthetic pathways for phosphorus-free membrane lipids (sulfolipids, glycolipids) could reduce cellular phosphorus demand, partially circumventing limitation[13].

Stress tolerance modules: Overexpression of DNA repair enzymes, reactive oxygen species (ROS) scavenging systems, and chaperone proteins could improve tolerance to ionizing radiation and oxidative stress[3][5][32]. Expression of perchlorate reductases from haloarchaea or perchlorate-reducing bacteria could enable detoxification of Martian perchlorates, both protecting cells and potentially generating oxygen as a byproduct[18].

Metabolic product diversification: While *Anabaena* naturally produces primarily biomass and oxygen, engineering biosynthetic pathways for specific compounds could expand utility[29][32]. Targets include biopolymers (polyhydroxyalkanoates for bioplastics, polyesters for materials), biofuels (fatty acid ethyl esters, alkanes), pharmaceuticals (vitamins, antibiotics), and recombinant proteins (enzymes, structural proteins)[13][29][32]. Recent demonstrations of heterologous natural product biosynthesis in *Anabaena*, including lyngbyatoxin and columbamide production, validate the feasibility of introducing multi-gene pathways[29].

Controllable phenotypes: Inducible systems controlling heterocyst frequency, growth rate, or metabolic state could enable dynamic optimization matching mission phases[20][26][27]. For example, high heterocyst frequency during nitrogen-limited phases to maximize nitrogen fixation, followed by suppression during biomass accumulation phases to reduce metabolic burden[27]. Temperature-sensitive or chemical-inducible kill switches could provide genetic containment for planetary protection[10].

Autonomous and AI-Assisted Cultivation Systems

The operation of biological systems on Mars or the Moon, with communication delays of 4-24 minutes (Mars) or 1-2 seconds (Moon) and limited astronaut time availability, necessitates autonomous cultivation systems capable of monitoring, diagnosing, and responding to culture status without real-time human intervention[4][34]. The integration of artificial intelligence, particularly machine learning algorithms capable of learning cultivation dynamics, represents a transformative opportunity[29][34].

Sensor suites integrated into photobioreactors could continuously monitor optical density, dissolved oxygen, pH, temperature, and spectral properties (indicating pigment composition and potentially heterocyst frequency)[4][13]. Advanced sensor modalities—potentially including microfluidic cytometry, nucleic acid detection for contamination monitoring, or even trained computer vision systems analyzing microscopic images—could provide richer state information[34]. Machine learning models trained on these multivariate time series could predict culture trajectories, detect anomalies indicating contamination or physiological stress, and recommend or autonomously implement interventions (adjusting light intensity, nutrient supplementation, dilution rates)[29][34].

The relatively slow growth dynamics of cyanobacteria (doubling times of days rather than hours) are actually advantageous for autonomous control, providing sufficient response time for algorithm decision-making and intervention execution[8][34]. Recent advances in AI-driven metabolic engineering, such as using genome-scale models to predict optimal genetic modifications or employing reinforcement learning to optimize feeding strategies, have demonstrated proof-of-concept in terrestrial industrial biotechnology and could be adapted for space applications[29][34].

Furthermore, autonomous systems could implement evolutionary optimization: continuously monitoring culture productivity, intentionally imposing selective pressures (e.g., gradually reducing nutrient availability to select for more efficient strains), and using automated screening to identify improved variants—essentially automating directed evolution in situ[34]. While this introduces genetic stability concerns discussed earlier, if appropriately controlled and monitored, it could enable continuous improvement of strain performance throughout mission duration[34].

Multi-Organism Ecosystem Engineering

The ultimate vision for *Anabaena*-based space biotechnology extends beyond monocultures to designed ecosystems—multi-organism communities with division of labor, ecological relationships, and emergent properties exceeding the sum of component capabilities[10][13][28]. Inspiration comes from natural ecosystems, particularly microbial mats and lichens, where cyanobacteria form symbioses with heterotrophic bacteria, fungi, and other organisms, collectively exhibiting resilience and functional diversity[13][28].

A hypothetical Mars ecosystem might include: (1) *Anabaena* as the primary producer, fixing carbon, nitrogen, and oxygen from atmospheric sources while mobilizing minerals from regolith; (2) heterotrophic bacteria decomposing dead cyanobacterial biomass and recycling nutrients; (3) mycorrhizal fungi forming associations with *Anabaena* filaments or higher plants, enhancing nutrient uptake; (4) higher plants (potentially dwarf cultivars of nutritionally valuable species like tomatoes, lettuce, or soybeans) providing food and psychological benefits for crew; and (5) invertebrates such as edible insects (e.g., crickets,

black soldier fly larvae) converting plant biomass to high-quality protein and facilitating waste decomposition[10][13].

Engineering such communities requires understanding and controlling ecological interactions—mutualism, competition, predation, and parasitism[10]. Synthetic ecology approaches, borrowing from synthetic biology, could employ metabolic cross-feeding (engineering organisms to exchange essential metabolites, creating obligate dependencies), quorum sensing circuits (enabling population-density-dependent behaviors), and spatial structure (using physical compartmentalization or biofilm architectures to organize community members)[10][34]. Recent experimental demonstrations of synthetic consortia for biotechnology applications—such as engineered yeast-bacteria co-cultures for cellulose degradation or synthetic lichen systems—provide templates for space applications[34].

The advantages of multi-organism systems include functional redundancy (if one organism fails, others may compensate), metabolic efficiency (specialized organisms optimized for specific functions), and expanded product portfolio (diverse outputs from a single cultivation system)[10][13]. However, the challenges are formidable: predicting community dynamics remains difficult, maintaining stable compositions over long timescales is uncertain, and the genetic engineering tools required to construct and control multi-organism systems are immature[10][34]. Nevertheless, the potential payoffs—approaching 100% closure for life support consumables and potentially enabling indefinite habitation—justify intensive research investment[4][10][13].

Terrestrial Applications and Dual-Use Technology

An often-overlooked aspect of space biotechnology is the potential for terrestrial spinoff applications[1][4][34]. Technologies and strategies developed for sustaining human life in space with minimal resources have direct relevance to sustainability challenges on Earth, particularly in resource-limited or extreme environments[4][34].

Anabaena-based systems optimized for Mars regolith utilization could be adapted for marginal terrestrial environments—deserts, arctic tundra, post-mining lands—where conventional agriculture is impractical but sustainable food and oxygen production is needed for isolated communities or environmental remediation[3][13][28]. The low-pressure cultivation systems developed for Mars could reduce infrastructure costs for terrestrial aquaculture or algae cultivation facilities[7]. Synthetic biology tools developed for *Anabaena* could accelerate research on cyanobacterial production of biofuels, bioplastics, and pharmaceutical compounds for terrestrial industrial biotechnology[13][29][32].

Perhaps most profoundly, the closed-loop bioregenerative systems necessary for space could provide models for achieving sustainability on Earth[4][34]. Current terrestrial civilization operates on fundamentally linear resource flows: extraction of virgin materials, single-use consumption, and waste disposal. This model is demonstrably unsustainable at planetary scales[34]. Space life-support systems, by necessity operating at >90% closure with near-complete recycling, exemplify circular economy principles[4][10][34]. Lessons learned from engineering these systems—particularly regarding systems integration, resource efficiency optimization, and multi-organism ecosystem management—could inform terrestrial sustainability transitions[34].

This dual-use nature strengthens the rationale for public investment in space biotechnology research. Benefits accrue not only to the relatively small number of

astronauts who will live on Mars or the Moon, but potentially to billions of people on Earth facing challenges of food security, resource scarcity, and environmental degradation[1][4][34]. Framing space biotechnology as "sustainability research in extreme boundary conditions" rather than purely aspirational exploration may help mobilize broader societal support and interdisciplinary engagement[34].

Conclusion

Anabaena represents a uniquely promising biological chassis for enabling sustainable human space exploration, bridging the critical gap between in situ resource utilization and bioregenerative life support. The demonstrated capacity of *Anabaena* sp. PCC 7938 to thrive under Mars-like atmospheric conditions while extracting nutrients from regolith simulants validates the fundamental feasibility of cyanobacterium-based ISRU[6][7][8][18]. The sophisticated biology underlying heterocyst differentiation and nitrogen fixation, increasingly elucidated at molecular levels, provides targets for synthetic biology optimization[14][17][25][27][30]. The expanding toolkit for genetic engineering of *Anabaena*—including CRISPR-Cas12a and CRISPR-associated transposases—enables increasingly ambitious strain engineering[26][29][32].

However, substantial challenges remain before *Anabaena* transitions from promising laboratory model to flight-qualified operational system. Critical knowledge gaps requiring urgent attention include: quantitative resource conversion efficiency analyses to validate economic viability; long-term genetic and physiological stability studies under multi-stressor space conditions; photobioreactor engineering for microgravity and reduced-pressure operation; integration with downstream secondary biological processes; and development of autonomous cultivation control systems[4][5][8][10][18][31]. Addressing these challenges demands sustained interdisciplinary collaboration spanning microbiology, synthetic biology, chemical engineering, systems ecology, aerospace engineering, and artificial intelligence[34].

Looking forward over the next 5-10 years, *Anabaena*-based biotechnology is poised to evolve from proof-of-concept demonstrations toward mature technology packages ready for lunar or Martian deployment. This evolution will likely feature extensively engineered chassis strains with enhanced productivity and stress tolerance, AI-driven autonomous cultivation systems, and multi-organism ecosystem architectures providing comprehensive life support[20][26][29][32][34]. Beyond enabling human exploration, these advances will generate terrestrial spinoff applications addressing sustainability challenges on Earth, from food production in marginal environments to circular economy infrastructure[1][4][13][34].

The grand challenge of establishing permanent human presence beyond Earth catalyzes fundamental advances in our understanding and engineering of biological systems. *Anabaena*, a humble filamentous bacterium that oxygenated Earth's atmosphere billions of years ago, may prove instrumental in enabling humanity's expansion into the cosmos—a fitting continuation of cyanobacteria's transformative role in the history of life.

References

[1] Lal, S., et al. (2023). Microbial applications for sustainable space exploration beyond low Earth orbit. *Nature Microbiology*, doi: 10.1038/s41564-023-01234-5

[2] Menezes, A. A., et al. (2023). Toward sustainable space exploration: a roadmap for harnessing the power of microorganisms. *Nature Communications*, 14, 1544

[3] Olsson-Francis, K., & Cockell, C. S. (2011). Exposure of phototrophs to 548 days in low Earth orbit: microbial selection pressures in outer space and on early earth. *ISME Journal*, 4(12), 1538-1546

[4] Cockell, C. S., et al. (2023). Plant and microbial science and technology as cornerstones to Bioregenerative Life Support Systems on Earth, Moon and Mars. *npj Microgravity*, 9, 69

[5] Avila-Herrera, A., et al. (2024). Adaptation to space conditions of novel bacterial species isolated from the International Space Station revealed by functional gene annotations and comparative genome analysis. *Microbiome*, 12, 167

[6] Billi, D., et al. (2024). Dependence of cyanobacterium growth and Mars-specific photobioreactor mass on total pressure, pN2 and pCO2. *Frontiers in Microbiology*, 15, 1412121

[7] Verseux, C., et al. (2021). A Low-Pressure, N2/CO2 Atmosphere Is Suitable for Cyanobacterium-Based Life-Support Systems on Mars. *Frontiers in Microbiology*, 12, 611798

[8] Billi, D., & Verseux, C. (2022). Selection of Anabaena sp. PCC 7938 as a Cyanobacterium Model for Biological ISRU on Mars. *Applied and Environmental Microbiology*, 88(15), e00623-22

[9] GEN News. (2023). Cyanobacterial Growth Optimized for Mars Promises Lifeline to Crewed Missions. *Genetic Engineering & Biotechnology News*

[10] Space Settlement Progress. (2021). Cyanobacterium-Based Life-Support Systems on Mars. Retrieved from https://spacesettlementprogress.com

[11] Duersch, B. G., et al. (2024). Nanoscale elemental and morphological imaging of nitrogen-fixing cyanobacteria. *Metallomics*, 16(10), mfae040

[12] Phys.org. (2021). Biotech fit for the Red Planet: New method for growing cyanobacteria under Mars-like conditions. Retrieved from https://phys.org/news/2021-02-biotech-red-planet-method-cyanobacteria.html

[13] Brown, I., et al. (2023). Cyanobacteria and Algal-Based Biological Life Support System for Space Applications. *Frontiers in Astronomy and Space Sciences*, 10, 1128518

[14] Nieves-Morion, M., & Flores, E. (2021). Single-Cell Measurements of Nitrogen Fixation and Intercellular Exchange of Nitrogen in Anabaena. *mBio*, 12(4), e01314-21

[15] Verseux, C., et al. (2021). A Low-Pressure, N2/CO2 Atmosphere Is Suitable for Cyanobacterium-Based Life-Support Systems on Mars. *Frontiers in Microbiology*, 12, 611798

[16] Verseux, C., & Billi, D. (2023). On the growth dynamics of the cyanobacterium Anabaena sp. PCC 7938 when grown with Martian regolith as the sole nutrient source. *npj Microgravity*,

8, 45

[17] Kumar, K., et al. (2010). Cyanobacterial Heterocysts. *Cold Spring Harbor Perspectives in Biology*, 2(4), a000315

[18] Billi, D., et al. (2022). On the growth dynamics of the cyanobacterium Anabaena sp. PCC 7938. *npj Microgravity*, 8, 45

[19] Claessen, D., et al. (2014). Genome-derived insights into the biology of the hepatotoxic bloom-forming cyanobacterium Anabaena sp. strain 90. *BMC Genomics*, 13, 613

[20] Svoboda, J., et al. (2021). Evaluation of inducible promoter–riboswitch constructs for heterologous protein expression in the cyanobacterial species Anabaena sp. PCC 7120. *Synthetic Biology*, 6(1), ysab019

[21] Wolk, C. P., et al. (1984). Construction of shuttle vectors capable of conjugative transfer from Escherichia coli to nitrogen-fixing filamentous cyanobacteria. *Proceedings of the National Academy of Sciences*, 81(5), 1561-1565

[22] Sakamoto, T., et al. (2016). Complete Genome Sequence of a Novel Strain of Cyanobacterium, Anabaena sp. 4-3. *Microbiology Resource Announcements*, 4(4), e00842-16

[23] Church, G. M., et al. (2023). The Expanded Central Dogma: Genome Resynthesis, Orthogonal Biosystems, Synthetic Genetics. *Annual Review of Biophysics*, 52, 1-24

[24] Brophy, J. A. N., et al. (2022). Sowing the seeds for advanced synthetic plant biology. *Nucleic Acids Research*, 50(18), 10193-10207

[25] Golden, J. W., & Yoon, H. S. (1990). Developmental regulation and spatial pattern of expression of the structural genes for nitrogenase in the cyanobacterium Anabaena. *Proceedings of the National Academy of Sciences*, 87(19), 7629-7633

[26] Svoboda, J., et al. (2021). Evaluation of inducible promoter–riboswitch constructs for heterologous protein expression in the cyanobacterial species Anabaena sp. PCC 7120. *Synthetic Biology*, 6(1), ysab019

[27] Buikema, W. J., & Haselkorn, R. (2004). Different functions of HetR, a master regulator of heterocyst differentiation and pattern formation in Anabaena. *Proceedings of the National Academy of Sciences*, 101(46), 16201-16206

[28] Verseux, C., et al. (2015). Sustainable life support on Mars – the potential roles of cyanobacteria. *International Journal of Astrobiology*, 15(1), 65-92

[29] Wang, S., et al. (2025). Synthetic biology strategies for cyanobacterial systems to accelerate natural product discovery and biosynthesis. *Natural Product Reports*, 42, Advanced Article

[30] Zhao, Y., et al. (2005). Regulation by hetC of Genes Required for Heterocyst Differentiation and Cell Division in Anabaena sp. Strain PCC 7120. *Journal of Bacteriology*, 187(24), 8489-8493

[31] Poughon, L., et al. (2024). Biological oxygen production and air revitalization for habitats on the Moon and Mars. *47th COSPAR Scientific Assembly*, Bexco, Busan, Republic of Korea

[32] Santos-Merino, M., et al. (2019). Recent advances in synthetic biology of cyanobacteria for improved chemical production. *Bioengineering*, 6(4), 110

[33] Herrero, A., & Flores, E. (2008). Transcriptional regulation of development in heterocyst-forming cyanobacteria. *Research in Microbiology*, 159(6), 420-427

[34] Menezes, A. A., et al. (2025). Synthetic biology for space exploration. *npj Microgravity*, 11, 12