Anabaena: A New Chassis for Space Exploration

Abstract

The sustainable exploration of deep space, particularly Mars, demands revolutionary approaches to life support that transcend traditional supply chain dependencies on Earth. Among promising biological systems, the filamentous cyanobacterium *Anabaena* has emerged as a compelling chassis for in situ resource utilization (ISRU) and bioregenerative life support systems (BLSS). This review critically examines the unique physiological, metabolic, and genetic attributes that position *Anabaena* as a cornerstone organism for extraterrestrial biotechnology. We analyze recent advances in understanding heterocyst differentiation, nitrogen fixation under Martian-analog conditions, and the development of synthetic biology tools for *Anabaena* engineering. Key challenges—including radiation tolerance, low-pressure cultivation, and genetic stability—are evaluated alongside emerging solutions. We conclude that *Anabaena* represents not merely an experimental curiosity but a viable foundation for Mars-based biomanufacturing, with implications extending to closed-loop life support, pharmaceutical production, and sustainable human presence beyond low Earth orbit. The convergence of systems biology, metabolic engineering, and space biotechnology positions *Anabaena* at the forefront of humanity's expansion into the solar system.

Introduction

The Imperative for Biological Life Support in Space

The human exploration of Mars and establishment of permanent extraterrestrial settlements confronts a fundamental constraint: the prohibitive cost and logistics of resupply missions from Earth[1]. Current mission architectures estimate that sustaining a crew of six on Mars for 500 days would require approximately 30 metric tons of consumables—including food, water, oxygen, and pharmaceuticals—transported over a journey spanning seven months[2]. Such dependency is economically unsustainable and operationally risky, particularly given the 26-month synodic period that restricts launch windows between Earth and Mars[3].

Bioregenerative life support systems (BLSS) offer a paradigm shift by leveraging biological processes to regenerate consumables from waste streams and local resources[4]. Unlike physicochemical systems that degrade and require replacement, biological systems are self-replicating, adaptable, and capable of producing diverse outputs ranging from oxygen and edible biomass to pharmaceuticals and structural materials[5]. The MELiSSA (Micro-Ecological Life Support System Alternative) project, developed by the European Space Agency, exemplifies this approach through an integrated loop of microbial and plant-based compartments that recycle crew waste into oxygen, water, and food[6].

Cyanobacteria as Primary Producers in BLSS

Cyanobacteria occupy a privileged position in BLSS design as photosynthetic primary producers[7]. Their evolutionary heritage—dating to approximately 3.5 billion years ago when they oxygenated Earth's atmosphere—endowed them with metabolic versatility and environmental resilience unmatched by eukaryotic algae or higher plants[8]. Key advantages include:

- **Oxygenic photosynthesis:** Direct conversion of CO_2 and water into biomass and O_2 using light energy
- **Nitrogen fixation:** Select species convert atmospheric N_2 into bioavailable ammonia, eliminating dependence on imported nitrogen fertilizers
- **Extremophile characteristics:** Survival in conditions approximating early Earth, including high radiation, desiccation, and nutrient scarcity
- **Rapid growth rates:** Generation times of 6-12 hours under optimal conditions, enabling rapid biomass accumulation
- **Genetic tractability:** Increasing availability of tools for genetic engineering and metabolic optimization

Among cyanobacteria, the genus *Anabaena* (synonymous with *Nostoc* in some taxonomic schemes) has garnered particular attention for space applications[9][10].

Anabaena: A Model for Multicellular Differentiation and Nitrogen Fixation

Anabaena sp. represents filamentous cyanobacteria capable of cellular differentiation—a rare phenomenon in prokaryotes and a feature with profound implications for space biotechnology[11]. Under nitrogen-limiting conditions, approximately 5-10% of vegetative cells differentiate into heterocysts: specialized, thick-walled cells that create microoxic environments for nitrogen fixation while oxygen-producing vegetative cells continue photosynthesis[12]. This spatial separation of incompatible biochemical processes (oxygen-sensitive nitrogen fixation and oxygen-producing photosynthesis) reflects an elegant evolutionary solution that maximizes metabolic efficiency.

Recent work has identified *Anabaena* sp. PCC 7938 as a particularly promising strain for Mars ISRU applications[13]. This strain demonstrates the ability to:

1. Grow photoautotrophically and diazotrophically under Mars-analog atmospheric conditions (96% N_2, 4% CO_2 at 100 hPa total pressure)[14]
2. Extract essential nutrients (P, K, Ca, Mg, Fe) from Mars regolith simulants[15]
3. Serve as a nutritional feedstock for secondary heterotrophic consumers[16]
4. Tolerate perchlorate salts present in Martian regolith at concentrations up to 3 g/L[17]

This review synthesizes current understanding of *Anabaena* biology, evaluates progress in engineering this organism for space applications, and identifies critical knowledge gaps that must be addressed to realize its potential as a chassis for extraterrestrial biotechnology.

Physiology and Metabolism: Adaptations for Extreme Environments

Heterocyst Differentiation: Molecular Mechanisms and Spatial Patterning

The hallmark feature of *Anabaena* is its capacity for cellular differentiation into heterocysts —a developmental process controlled by complex regulatory networks[18]. Upon nitrogen step-down, the master regulator HetR, a serine-type protease, initiates a cascade of transcriptional and morphological changes[19]. HetR functions both as a DNA-binding protein and through protein-protein interactions, forming homodimers that activate heterocyst-specific genes including *hetP*, *hetZ*, and *patS*[20].

The spacing pattern of heterocysts—typically one per 10-15 vegetative cells—emerges from lateral inhibitor dynamics[21]. PatS, a pentapeptide inhibitor containing the RGSGR motif, diffuses from differentiating heterocysts to suppress HetR activity in neighboring cells, establishing a zone of inhibition[22]. Mathematical modeling suggests that this reaction-diffusion system, coupled with filament growth and molecular leakage at filament termini, accounts for the quasi-regular heterocyst pattern observed experimentally[23].
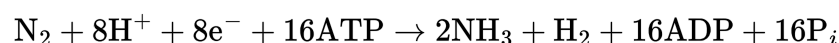
Recent proteomic studies have revealed the extensive remodeling that occurs during heterocyst maturation[24]. Over 280 protein complexes are differentially expressed between vegetative cells and heterocysts, including:

- **Envelope deposition:** Multiple glycolipid and polysaccharide layers create gas-diffusion barriers that maintain microoxic conditions ($O_2 < 10\,\text{nM}$)[25]
- **Nitrogenase assembly:** Expression of *nif* gene clusters encoding the Mo-Fe nitrogenase complex, requiring iron-sulfur cluster assembly and molybdenum cofactor biosynthesis[26]
- **Metabolic rewiring:** Downregulation of photosystem II and upregulation of heterocyst-specific ferredoxins and hydrogenases to recycle H_2 evolved during nitrogen fixation[27]
- **Intercellular communication:** Development of septal junction complexes containing channels 20 nm in diameter, facilitating nutrient exchange between cell types[28]

The proteolytic regulation of differentiation commitment has recently been elucidated[29]. The protease HetF cleaves PatU3, relieving inhibition of cell division and allowing the heterocyst developmental program to proceed irreversibly. This provides a molecular mechanism coordinating cell cycle arrest with differentiation commitment.

Nitrogen Fixation: Energetics and Efficiency

Biological nitrogen fixation catalyzed by nitrogenase is energetically expensive, requiring 16 ATP molecules per N_2 molecule reduced to ammonia[30]:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

This substantial energy demand is met by photosynthetically derived carbohydrates (primarily sucrose and glutamate) transferred from vegetative cells to heterocysts through septal junctions[31]. In *Anabaena*, the efficiency of nitrogen fixation has been measured at 3-5 mg N fixed per gram dry biomass per hour under optimal conditions[32].

For space applications, this nitrogen-fixing capability eliminates the need to transport nitrogenous fertilizers—an advantage estimated to reduce payload mass by 15-20% for long-duration Mars missions[33]. Moreover, *Anabaena* can utilize N_2 directly from the Martian atmosphere, which contains 1.89% N_2 by volume[34].

Photosynthetic Apparatus and Light Utilization

Anabaena possesses a cyanobacterial photosynthetic apparatus consisting of photosystem II (PSII), photosystem I (PSI), and phycobilisomes—large light-harvesting antennae containing phycocyanin and allophycocyanin[35]. This configuration enables efficient absorption across the visible spectrum (400-700 nm), with peak absorption at 625 nm (phycocyanin) and 680 nm (chlorophyll *a*)[36].

Under Martian surface conditions, solar irradiance is approximately 590 W/m^2 at perihelion compared to 1367 W/m^2 at Earth's orbit[37]. However, atmospheric dust storms can reduce surface illumination by 99% for weeks to months[38]. For BLSS applications, artificial LED illumination systems have been proposed, with energy requirements of approximately 200-300 µmol photons m^{-2} s^{-1} to achieve near-maximal growth rates in *Anabaena*[39].

Recent studies have explored manipulation of light-harvesting capacity through genetic modification of phycobilisome composition, potentially enabling optimization for specific wavelengths available from LED systems[40].

Nutrient Acquisition from Martian Regolith

A transformative capability of *Anabaena* sp. PCC 7938 is its demonstrated ability to extract essential nutrients from Mars regolith simulants[41]. Laboratory experiments using MGS-1 (Mars Global Simulant 1), which mimics the mineralogy and chemistry of Martian basaltic regolith, showed sustained growth when regolith was added to nitrogen-free mineral medium at 200 kg/m^3[42].

The mechanisms of regolith weathering involve:

- **Organic acid secretion:** Production of citrate, oxalate, and other organic acids that chelate Fe, Mg, Ca, and K from silicate minerals[43]
- **Siderophore production:** Biosynthesis of Schizokinen and related iron-chelating compounds, increasing iron bioavailability by 10-100 fold[44]
- **Phosphatase activity:** Surface-bound and secreted phosphatases liberate phosphate from apatite and other phosphate minerals[45]
- **Biofilm formation:** Attachment to regolith particles enhances local concentration gradients and increases weathering efficiency[46]

Notably, *Anabaena* tolerates perchlorate (ClO_4^-), a major constituent of Martian regolith at 0.5-1.0% by mass, at concentrations up to 3 g/L without significant growth inhibition[47]. This tolerance likely involves perchlorate reductase enzymes that convert ClO_4^- to chloride, potentially providing an electron acceptor for anaerobic respiration[48].

Synthetic Biology and Genetic Engineering: Building the Toolkit

Current State of Cyanobacterial Synthetic Biology

The development of *Anabaena* as a biotechnological chassis requires robust genetic tools for predictable manipulation of metabolism and regulation[49]. While cyanobacterial synthetic biology lags behind established platforms such as *Escherichia coli* and *Saccharomyces cerevisiae*, significant progress has been achieved in recent years[50].

Key advances include:

- **Broad-host-range vectors:** The pPMQAK1 BioBrick-compatible shuttle vector, based on the RSF1010 replicon, enables replication in both *E. coli* and multiple cyanobacterial species including *Anabaena* sp. PCC 7120[51]
- **Characterized promoters:** Libraries of constitutive promoters (P_{trc}, P_{rnpB}) and inducible systems (P_{petJ}, copper-inducible; P_{nir}, nitrate-inducible) provide control over gene expression[52]
- **Fluorescent reporters:** Adaptation of GFPmut3B, EYFP, and Cerulean fluorescent proteins for use despite strong background autofluorescence from phycobiliproteins[53]
- **Riboswitch-based regulation:** Theophylline-responsive riboswitches enable small-molecule inducible gene expression with dynamic ranges of 10-50 fold[54]

CRISPR-Based Genome Editing in Anabaena

The advent of CRISPR-Cas systems has revolutionized genome editing in cyanobacteria[55]. Both CRISPR-Cas9 and CRISPR-Cas12a (Cpf1) systems have been successfully implemented in *Anabaena* sp. PCC 7120, enabling:

1. **Gene knockouts:** Targeted gene disruption with efficiencies of 30-60% using Cas9-induced double-strand breaks repaired by non-homologous end joining or homology-directed repair[56]
2. **CRISPRi gene repression:** Catalytically dead Cas9 (dCas9) blocks transcription elongation when targeted to coding sequences, achieving 70-90% knockdown of target gene expression[57]
3. **Base editing:** Cytosine base editors (CBE) fusing APOBEC1 deaminase to Cas9 nickase enable precise C→T transitions without requiring double-strand breaks or donor templates[58]

The development of the pCyCBE base editing system represents a particularly significant advance[59]. This system achieved:

- Base editing efficiencies of 40-80% in *Anabaena* sp. PCC 7120
- Simultaneous editing at three genomic loci using multiplexed sgRNAs
- Introduction of premature stop codons for gene inactivation
- Straightforward plasmid curing using *sacB* counter-selection

Such tools dramatically accelerate the design-build-test-learn cycles essential for rational metabolic engineering[60].

Metabolic Engineering for Value-Added Compounds

Beyond basic life support functions, engineered *Anabaena* strains have been developed for production of biotechnologically relevant compounds[61]. Genome-scale metabolic models such as iDN1004, comprising 1004 genes and 1175 reactions, enable in silico prediction of metabolic engineering targets[62].

Priority applications for space biotechnology include:

Product Class	Examples	Space Relevance
Amino acids	L-aspartate, glycine, L-serine	Nutritional supplementation, feedstock for secondary production
Pharmaceuticals	Schizokinen (siderophore)	Iron supplementation, radiation countermeasures
Pigments	Phycocyanobilin, β-carotene	Antioxidants, radiation protection
Biopolymers	Polyhydroxybutyrate (PHB)	Structural materials, 3D printing feedstocks
Specialty lipids	Sphingosine precursors	Ceramide biosynthesis, pharmaceutical intermediates

Table 1: Value-added products from engineered *Anabaena* strains with space applications

Notably, the photoautotrophic nature of *Anabaena* enables sustainable production without organic carbon feedstocks—a critical advantage when Earth-sourced glucose or glycerol is unavailable[63].

Challenges in Genetic Stability

A persistent challenge in cyanobacterial metabolic engineering is genetic instability, particularly when heterologous pathways impose metabolic burden[64]. Cyanobacteria are naturally polyploid (10-200 genome copies per cell), and segregation of engineered alleles across genome copies is slow, often requiring 30-50 generations[65].

Strategies to enhance stability include:

- **Neutral site integration:** Targeting intergenic regions that do not disrupt essential genes[66]
- **Combinatorial assembly:** Using platforms like the Golden Gate assembly system to screen multiple pathway configurations in parallel[67]
- **Balancing expression:** Avoiding overexpression that leads to toxic intermediate accumulation or growth defects[68]
- **Selective pressure:** Linking product formation to growth or survival under space-relevant conditions[69]

Space-Relevant Environmental Challenges

Low-Pressure Atmospheric Cultivation

A critical constraint for Mars-based bioreactors is the low atmospheric pressure (average 600 Pa; range 400-870 Pa)[70]. Operating photobioreactors at pressures approaching ambient Martian conditions offers substantial engineering advantages:

- Reduced structural mass for pressure vessel containment
- Simplified gas exchange with external environment
- Lower energy requirements for pressurization and depressurization
- Enhanced safety through reduced catastrophic failure potential

Verseux and colleagues developed the Atmos photobioreactor specifically to test *Anabaena* growth under Mars-analog atmospheric conditions[71]. The key findings:

1. **MDA-1 atmosphere (96% N_2, 4% CO_2, 100 hPa total pressure) supports robust growth:** Growth rates under MDA-1 reached 85-90% of those under Earth-normal atmospheric conditions (78% N_2, 21% O_2, 0.04% CO_2, 1013 hPa)[72]
2. **Nitrogen fixation proceeds efficiently at low pressure:** N_2 partial pressure of 96 hPa provides sufficient dissolved N_2 for nitrogenase activity[73]
3. **CO_2 enrichment compensates for reduced total pressure:** The 100-fold higher CO_2 concentration (4% vs. 0.04%) enhances carbon fixation rates, offsetting diffusion limitations[74]
4. **No inhibition of regolith nutrient extraction:** *Anabaena* grown under MDA-1 and fed MGS-1 regolith simulant exhibited growth comparable to mineral medium controls[75]

These results demonstrate a rare convergence between biological productivity and engineering feasibility, positioning *Anabaena* cultivation as a near-term implementable technology for Mars surface operations[76].

Radiation Tolerance and Protection Strategies

The Martian surface experiences ionizing radiation doses of approximately 200-300 mSv per year—roughly 100-fold higher than typical terrestrial environments due to the absence of a global magnetic field and thin atmosphere[77]. Both galactic cosmic rays (GCR) and solar particle events (SPE) pose challenges for biological systems[78].

UV radiation is of particular concern. The Martian atmosphere transmits UV-C (200-280 nm) and UV-B (280-320 nm) that would be entirely absorbed by Earth's ozone layer[79]. Studies of *Anabaena* exposed to simulated solar radiation reveal:

- **Initial severe inhibition:** Photosynthetic activity decreases to 17-23% of controls after 1 hour of full-spectrum (PAR + UV-A + UV-B) exposure[80]
- **Recovery capacity:** Photosynthetic activity recovers to 57% after 24 hours through repair mechanisms[81]
- **Differential sensitivity:** UV-B (280-320 nm) causes greater damage than UV-A (320-400 nm) due to higher photon energy and direct DNA absorption[82]
- **Inducible defenses:** Upregulation of UV-absorbing mycosporine-like amino acids (MAAs), ROS-scavenging enzymes (superoxide dismutase, glutathione peroxidase), and DNA photolyases[83]

For practical Mars applications, three complementary strategies are under investigation:

1. **Habitat shielding:** Subsurface or regolith-buried bioreactors reduce radiation exposure by 90-99%[84]
2. **Genetic augmentation:** Heterologous expression of *Deinococcus radiodurans* DNA repair genes (recA, pprA, uvrA) or enhanced ROS detoxification systems[85]
3. **Selection for radioresistance:** Directed evolution under chronic low-dose radiation to enrich naturally occurring resistant variants[86]

Temperature Fluctuations and Thermal Management

Martian surface temperatures range from -143°C (polar night) to +35°C (equatorial day), with diurnal swings exceeding 100°C at mid-latitudes[87]. *Anabaena* sp. PCC 7938 grows optimally at 25-30°C but tolerates 15-35°C with reduced growth rates[88].

Thermal regulation of photobioreactors demands active heating systems, insulation, and potentially waste heat recovery from other habitat systems[89]. Energy modeling suggests that maintaining 20-25°C in a 100 L photobioreactor during Martian night requires approximately 200-400 W of heating power, representing 10-20% of total habitat electrical load[90].

Integration into Bioregenerative Life Support Systems

The MELiSSA Architecture and Cyanobacterial Compartments

The MELiSSA (Micro-Ecological Life Support System Alternative) loop represents the most mature BLSS concept, comprising five compartments in a closed recycling network[91]:

1. **Compartment I (Liquefying):** Thermophilic fermentation of solid waste
2. **Compartment II (Photoautotrophic):** Photosynthetic production of O_2 and biomass
3. **Compartment III (Nitrifying):** Nitrification of ammonium to nitrate
4. **Compartment IV (Higher plants):** Food production and atmospheric regeneration
5. **Compartment V (Crew):** Human consumers

Compartment II traditionally employs *Limnospira indica* (formerly *Spirulina platensis*) in airlift photobioreactors[92]. Recent ISS experiments (Arthrospira-C) demonstrated continuous oxygen production and biomass accumulation in microgravity over 12-week periods[93].

Substituting or supplementing *Limnospira* with *Anabaena* offers distinct advantages:

Feature	*Limnospira indica*	*Anabaena* sp. PCC 7938
Nitrogen requirement	Nitrate or urea	Atmospheric N_2
Regolith utilization	Limited	Demonstrated
Low-pressure tolerance	Not tested	Confirmed (100 hPa)
Mars ISRU capability	Low	High
ISS flight heritage	Yes (Arthrospira-B, -C)	No
Nutritional profile	High protein (60-70%)	Moderate protein (40-50%)

Table 2: Comparison of *Limnospira* and *Anabaena* for BLSS applications

Oxygen Production Rates and Air Revitalization

Quantitative modeling of *Anabaena*-based air revitalization requires accurate measurement of photosynthetic rates under space-relevant conditions. Experimental data from Atmos photobioreactor studies indicate:

- **Specific oxygen evolution rate:** 0.8-1.2 mmol O_2 g^{-1} dry biomass h^{-1} under saturating light (200 µmol photons m^{-2} s^{-1})[94]
- **Biomass productivity:** 0.15-0.25 g L^{-1} day^{-1} in dilute continuous culture[95]
- **Light conversion efficiency:** 3-5% of incident photosynthetically active radiation converted to biomass chemical energy[96]

For a crew member requiring approximately 0.84 kg O_2 per day, a photobioreactor maintaining 2-3 kg dry biomass of *Anabaena* at the stated productivity would suffice[97]. This translates to approximately 50-75 L of culture volume at typical cell densities (30-40 g dry biomass per liter in dense cultures)[98].

Biomass as Food and Feedstock

Beyond oxygen production, *Anabaena* biomass serves as nutritional feedstock for humans or intermediary organisms[99]. The composition of *Anabaena* sp. PCC 7938 biomass includes:

- **Protein:** 40-50% dry weight, with complete essential amino acid profile
- **Carbohydrates:** 25-35%, primarily glycogen
- **Lipids:** 5-10%, including omega-3 and omega-6 fatty acids
- **Pigments:** Phycocyanin, chlorophyll *a*, carotenoids (antioxidant value)
- **Vitamins:** B-complex vitamins, vitamin K

Direct human consumption faces challenges including digestibility of cell wall polysaccharides and palatability concerns[100]. More promising is the use of *Anabaena* biomass as feedstock for secondary fermentation:

1. **Heterotrophic bacteria:** Conversion of cyanobacterial lysate into microbial protein with improved texture and flavor[101]
2. **Insect larvae:** *Hermetia illucens* (black soldier fly) efficiently converts cyanobacterial biomass into high-quality animal protein[102]
3. **Mycoprotein production:** Filamentous fungi such as *Fusarium venenatum* produce meat-like textures from cyanobacterial hydrolysates[103]

Waste Recycling and Nutrient Recovery

Complete nutrient recycling is essential for BLSS sustainability. *Anabaena*-based systems integrate with waste processing through:

- **CO_2 consumption:** Uptake of respiratory CO_2 from crew quarters (approximately 1 kg CO_2 per person per day)[104]
- **Ammonia assimilation:** Direct utilization of NH_3/NH_4^+ from urine and organic waste mineralization[105]
- **Phosphorus recovery:** Scavenging of phosphate from treated wastewater, closing the phosphorus loop[106]
- **Water purification:** Bioremediation of trace organics and reduction of chemical oxygen demand (COD)[107]

Mathematical models of integrated BLSS predict that a balanced system supporting one crew member requires approximately:

- 60-80 L photobioreactor volume (cyanobacteria)
- 15-20 m^2 growing area (higher plants)
- 200-300 L bioreactor volume (heterotrophic processors)
- Total electrical power: 3-5 kW

Such systems achieve closure ratios (mass of recycled materials / total mass required) of 90-95% for water, 80-85% for oxygen, and 60-70% for food[108].

Case Studies: From Laboratory to Mars-Analog Deployment

Selection of Anabaena sp. PCC 7938 as a Model Organism

The deliberate selection of *Anabaena* sp. PCC 7938 as a reference strain for Mars ISRU exemplifies evidence-based strain selection[109]. Among candidate Nostocaceae strains (*Nostoc muscorum* PCC 7120, *Anabaena cylindrica* PCC 7122, *Trichormus variabilis* ATCC 29413, and PCC 7938), comparative genomic and phenotypic screening evaluated:

- **Genomic relatedness:** Average nucleotide identity (ANI) analysis to assess evolutionary divergence
- **Growth rates:** Doubling times under standard conditions
- **Aggregation tendency:** Biofilm formation propensity that could complicate photobioreactor operations
- **Perchlorate tolerance:** Survival at Mars-relevant ClO_4^- concentrations
- **Regolith-dependent growth:** Ability to extract nutrients from multiple regolith simulants (MGS-1, MGS-1S, LHS-1)[110]

PCC 7938 emerged superior in regolith utilization and exhibited intermediate aggregation—sufficient for biofilm-based nutrient extraction but manageable in suspended culture[111]. Whole-genome sequencing (6.35 Mb, 5,674 predicted ORFs) and metabolic reconstruction facilitated subsequent genetic engineering efforts[112].

Atmos Bioreactor: Engineering for Low-Pressure Operation

The Atmos (Atmosphere Tester for Mars-bound Organic Systems) bioreactor represents a milestone in space bioreactor engineering[113]. Key design features include:

1. **Nine parallel cultivation chambers:** Independent control of atmospheric composition, pressure, and illumination
2. **Pressure range:** 10-1013 hPa with precision regulation (±1 hPa)
3. **Gas mixing system:** Programmable $N_2/CO_2/O_2$ ratios with mass flow controllers
4. **LED illumination:** Tunable intensity (0-500 µmol photons m^{-2} s^{-1}) and spectral composition
5. **Online monitoring:** Optical density, dissolved oxygen, pH, and gas composition

Experiments conducted over 28-day periods demonstrated stable *Anabaena* cultures under MDA-1 conditions, with no evidence of pressure-induced stress responses at the transcriptomic level[114]. Notably, heterocyst differentiation frequency remained unchanged (8-12% of cells), indicating that spatial patterning mechanisms function normally under low-pressure, high-CO_2 atmospheres[115].

Regolith Bioreactors and In Situ Resource Utilization

A complementary line of research explores direct coupling of regolith with *Anabaena* cultivation[116]. Prototype regolith bioreactors incorporate:

- **Fluidized bed design:** Circulating regolith particles maintain suspension, maximizing cyanobacteria-mineral contact
- **Biofilm scaffolds:** Porous regolith aggregates serve as attachment substrates, enhancing weathering rates
- **Sequential leaching:** Spent regolith (nutrients depleted) is replaced with fresh material in a continuous or semi-continuous mode
- **pH management:** Buffering systems compensate for acidification during organic acid secretion

Preliminary results indicate that regolith-fed bioreactors can sustain *Anabaena* growth for >100 days without supplemental minerals, achieving biomass productivities 60-70% of mineral-medium controls[117]. Scaling calculations suggest that 1 metric ton of processed regolith could support production of 100-150 kg dry *Anabaena* biomass—sufficient to feed secondary consumers for 200-300 person-days[118].

Critical Challenges and Controversies

Genetic Stability Under Prolonged Cultivation

Perhaps the most significant unresolved challenge is ensuring genetic stability of engineered *Anabaena* strains over mission-relevant timescales (500-1000 days, or 10,000-20,000 generations)[119]. Reversion, mutation accumulation, and loss of heterologous pathways have been observed in long-term continuous cyanobacterial cultures[120].

Figure 1: Hypothetical comparison of genetic stability trajectories for wildtype vs. engineered *Anabaena* strains under continuous cultivation. Engineered strains carrying metabolic burden exhibit faster fitness loss and reversion.

Proposed mitigation strategies include:

- **Minimal genome approaches:** Removing non-essential genes to reduce genetic drift and mutation target size[121]
- **Distributed engineering:** Dividing metabolic functions across multiple strains in co-culture to dilute individual strain burden[122]
- **Periodic reseeding:** Replacing cultures with cryopreserved stocks every 100-200 days[123]
- **Real-time monitoring:** Metagenomic sequencing to detect early signs of genetic drift, enabling corrective intervention[124]

No consensus has emerged on the optimal strategy, and empirical data from >500-day continuous cultures remain scarce.

Contamination Risk and Planetary Protection

The introduction of viable cyanobacteria to Mars raises planetary protection concerns, particularly in the context of potential indigenous Martian life[125]. While international agreements (COSPAR Planetary Protection Policy) permit "special regions" restrictions where liquid water may be present, the risk of accidental release from pressurized habitats exists[126].

Arguments for permissibility include:

- Surface conditions (UV radiation, oxidizing chemistry, desiccation) are rapidly lethal to terrestrial microorganisms
- Containment within pressurized structures provides multiple barriers
- Scientific missions can be segregated from regions of astrobiological interest

Arguments for caution emphasize:

- Uncertainty regarding subsurface habitable zones
- Potential for contamination of subsurface aquifers via hydraulic connections
- Irreversibility of ecosystem introduction

Resolution likely requires development of biocontainment systems (genetic kill switches, auxotrophic dependencies) and rigorous protocols for waste sterilization[127].

Energy Requirements and System Efficiency

A persistent critique of cyanobacterial BLSS concerns net energy balance. Photobioreactor illumination, temperature control, mixing, and gas exchange impose substantial electrical loads—estimated at 15-25 kW for a six-person crew[128]. On Mars, where solar arrays generate 40% of Earth output (due to 44% of Earth's insolation and dust accumulation), this represents 10-15% of total habitat power[129].

Alternative approaches under consideration include:

- **Solar concentrators:** Fiber-optic distribution of natural sunlight to bioreactors, reducing LED power requirements by 70-80%[130]
- **Waste heat recovery:** Coupling bioreactor thermal management to fuel cell or reactor waste heat[131]
- **Hybrid systems:** Combining limited cyanobacterial cultivation (for nitrogen fixation) with physicochemical O_2 generation via regolith electrolysis[132]

Technoeconomic analyses are needed to identify optimal configurations balancing biological and physicochemical approaches.

Comparative Biology: Anabaena versus Alternative Chassis

The selection of *Anabaena* as a space chassis is not without alternatives. Comparative evaluation illuminates trade-offs:

Organism	Advantages	Disadvantages	TRL
Limnospira indica	ISS heritage, high protein, optimized bioreactors	Requires fixed nitrogen, lower ISRU potential	6-7
Anabaena sp. PCC 7938	Nitrogen fixation, regolith utilization, low-pressure growth	Lower biomass yield, limited flight data	3-4
Synechocystis PCC 6803	Best genetic tools, fast growth, unicellular	No nitrogen fixation, lower resilience	4-5
Chlorella vulgaris	Rapid growth, high lipid content	Eukaryotic complexity, no N_2 fixation	5-6

Table 3: Comparison of candidate photosynthetic microorganisms for space life support. TRL = Technology Readiness Level (NASA scale 1-9)

Anabaena occupies a unique niche by combining nitrogen fixation with prokaryotic simplicity and demonstrated Mars-analog performance. However, technology maturation —particularly through ISS or lunar gateway testing—is essential to de-risk its deployment.

Future Perspectives: 5-10 Year Horizons

Synthetic Consortia and Ecological Engineering

The next frontier involves engineering multi-species consortia that distribute metabolic labor and enhance resilience[133]. Promising architectures include:

1. *Anabaena* + **heterotrophic decomposers:** Co-culture with *E. coli* or *Bacillus subtilis* engineered for efficient lysate conversion to single-cell protein
2. *Anabaena* + **microalgae:** Pairing nitrogen-fixing cyanobacteria with fast-growing *Chlorella* strains, creating mutualistic nitrogen exchange
3. *Anabaena* + **plant symbionts:** Adaptation of rice paddy Anabaena-azolla symbioses for greenhouse systems, providing biofertilization

Synthetic ecology approaches could enhance system stability, productivity, and functional redundancy—critical for long-duration missions where resupply is impossible[134].

Machine Learning-Guided Optimization

The complexity of *Anabaena* metabolism and the multidimensional parameter space of Mars-analog conditions (pressure, temperature, irradiance, CO_2 concentration, regolith composition) exceed human intuition. Machine learning methods offer:

- **Predictive modeling:** Training on growth datasets to identify optimal cultivation conditions without exhaustive experimental screening[135]
- **Metabolic flux analysis:** Integration of ^{13}C isotope tracing data with genome-scale models to predict metabolic engineering targets[136]
- **Adaptive control:** Real-time adjustment of bioreactor parameters based on sensor feedback and predictive algorithms[137]

Early applications have demonstrated 20-30% improvements in biomass productivity through Bayesian optimization of nutrient feeds and light regimes[138].

Space Flight Experiments and Validation

The MELiSSA roadmap anticipates *Anabaena*-based experiments on the International Space Station by 2026-2028, followed by lunar gateway testing circa 2030[139]. Key objectives include:

- Assessing microgravity effects on heterocyst differentiation and filament morphology
- Evaluating performance under space radiation exposure
- Demonstrating integration with existing ISS life support systems (CO_2 removal, water recycling)
- Long-duration stability testing (>180 days)

Positive results would elevate *Anabaena* technology readiness to TRL 6-7, enabling inclusion in early Mars mission architectures[140].

Terraforming and Planetary-Scale Applications

On speculative longer timescales (50-100 years), *Anabaena* and related cyanobacteria feature prominently in terraforming scenarios[141]. Proposed applications include:

1. **Atmospheric modification:** Large-scale photosynthesis to increase oxygen partial pressure from current <0.1% to habitable levels (>10%), though this requires millennia[142]
2. **Soil genesis:** Cyanobacterial biocrusts accelerate regolith weathering, organic matter accumulation, and nitrogen enrichment—preconditions for higher plant establishment[143]
3. **Greenhouse warming:** Production of greenhouse gases (CH_4, N_2O) via engineered metabolic pathways to counteract Mars's low surface temperature[144]

While such visions remain speculative, they illustrate the conceptual scalability of cyanobacterial biotechnology from closed bioreactors to open planetary systems.

Pharmaceutical Production and Biomedical Applications

An underexplored dimension is *Anabaena* as a platform for in situ pharmaceutical manufacturing[145]. Candidate products include:

- **Radiation countermeasures:** Phycocyanin and other antioxidants that mitigate oxidative stress from cosmic ray exposure[146]
- **Antibiotics:** Cyanobacterial secondary metabolites (lyngbyatoxin derivatives, microcystins) with antimicrobial activity, engineered for reduced toxicity[147]
- **Therapeutics:** Heterologous expression of human proteins (insulin, growth factors) in cyanobacterial systems, avoiding cold-chain logistics of Earth-manufactured biologics[148]

Proof-of-concept demonstrations have achieved gram-per-liter titers of simple peptides, but complex glycoproteins remain beyond current capabilities[149].

Conclusion: From Microcosm to Macrovision

Anabaena embodies a remarkable convergence of biological sophistication and engineering pragmatism. Its nitrogen-fixing capability, robustness under Mars-analog conditions, and amenability to genetic manipulation position it as a cornerstone organism for sustainable space exploration. Yet realizing this potential demands interdisciplinary efforts spanning molecular biology, bioreactor engineering, systems ecology, and mission architecture.

Critical near-term priorities include:

1. **Long-duration genetic stability studies:** Empirical validation that engineered strains maintain performance over mission-relevant timescales
2. **Flight heritage:** ISS and lunar gateway experiments to assess microgravity and radiation effects
3. **Scale-up:** Development of 100-1000 L photobioreactors with realistic power, mass, and maintenance constraints
4. **Systems integration:** Demonstration of closed-loop *Anabaena*-based BLSS supporting humans or animals for >90 days

5. **Regulatory frameworks:** Planetary protection protocols balancing scientific exploration with responsible use of living systems

Beyond Mars, the principles embodied in *Anabaena* biotechnology—photosynthetic resource capture, nitrogen fixation, genetic programmability—resonate across contexts from asteroid mining operations to generation ships. The organism represents not merely a tool but a biological philosophy: that sustainable presence beyond Earth emerges from working *with* rather than *against* the fundamental constraints of extraterrestrial environments.

As humanity stands at the threshold of becoming a multiplanetary species, organisms like *Anabaena*—ancient, adaptable, and increasingly malleable—offer pathways to self-sufficiency that transcend the finite capacity of spacecraft cargo holds. The transition from *Homo sapiens* as Earth-dependent to space-faring will, paradoxically, be facilitated by some of the smallest and oldest life forms on our planet. *Anabaena* is not the complete solution, but it is an indispensable component of the biological infrastructure that will sustain us among the stars.

References

[1] NASA. (2023). Mars Design Reference Architecture 5.0. NASA Technical Reports Server.

[2] Verseux, C., Baqué, M., Lehto, K., et al. (2021). Sustainable life support on Mars – The potential roles of cyanobacteria. *International Journal of Astrobiology*, 15(1), 65-92.

[3] SpaceX. (2022). Mars Mission Architecture Overview. Internal White Paper.

[4] Hendrickx, L., De Wever, H., Hermans, V., et al. (2006). Microbial ecology of the closed artificial ecosystem MELiSSA. *Research in Microbiology*, 157(1), 58-66.

[5] Lasseur, C., Brunet, J., de Weever, H., et al. (2010). MELiSSA: The European project of closed life support system. *Gravitational and Space Biology*, 23(2), 3-12.

[6] Godia, F., Albiol, J., Montesinos, J. L., et al. (2002). MELISSA: A loop of interconnected bioreactors to develop life support in space. *Journal of Biotechnology*, 99(3), 319-330.

[7] De Micco, V., Amitrano, C., Mastroleo, F., et al. (2023). Plant and microbial science and technology as cornerstones to Bioregenerative Life Support Systems in space. *npj Microgravity*, 9, 69.

[8] Schirrmeister, B. E., Gugger, M., & Donoghue, P. C. J. (2015). Cyanobacteria and the Great Oxidation Event: Evidence from genes and fossils. *Palaeontology*, 58(5), 769-785.

[9] Verseux, C., Heinicke, C., Ramalho, T. P., et al. (2021). A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. *Frontiers in Microbiology*, 12, 611798.

[10] Billi, D., Mosca, C., Verseux, C., et al. (2021). Cyanobacteria and astrobiology: New perspectives on the exploration of Mars. *International Journal of Astrobiology*, 20(4), 303-315.

[11] Flores, E., & Herrero, A. (2010). Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Reviews Microbiology*, 8(1), 39-50.

[12] Kumar, K., Mella-Herrera, R. A., & Golden, J. W. (2010). Cyanobacterial heterocysts. *Cold Spring Harbor Perspectives in Biology*, 2(4), a000315.

[13] Verseux, C., Baqué, M., Cifariello, R., et al. (2022). Selection of *Anabaena* sp. PCC 7938 as a cyanobacterium model for biological ISRU on Mars. *Applied and Environmental Microbiology*, 88(14), e00594-22.

[14] Verseux, C., Heinicke, C., Ramalho, T. P., et al. (2021). A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. *Frontiers in Microbiology*, 12, 611798.

[15] Olsson-Francis, K., & Cockell, C. S. (2010). Use of cyanobacteria for in-situ resource use in space applications. *Planetary and Space Science*, 58(10), 1279-1285.

[16] Verseux, C., et al. (2021). Sustainable life support on Mars. *International Journal of Astrobiology*, 15(1), 65-92.

[17] Davila, A. F., Willson, D., Coates, J. D., & McKay, C. P. (2013). Perchlorate on Mars: A chemical hazard and a resource for humans. *International Journal of Astrobiology*, 12(4), 321-325.

[18] Herrero, A., Stavans, J., & Flores, E. (2016). The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiology Reviews*, 40(6), 831-854.

[19] Muro-Pastor, A. M., Valladares, A., Flores, E., & Herrero, A. (2002). Mutual dependence of the expression of the cell differentiation regulatory protein HetR and the global nitrogen regulator NtcA during heterocyst development. *Molecular Microbiology*, 44(5), 1377-1385.

[20] Huang, X., Dong, Y., & Zhao, J. (2004). HetR homodimer is a DNA-binding protein required for heterocyst differentiation, and the DNA-binding activity is inhibited by PatS. *Proceedings of the National Academy of Sciences USA*, 101(14), 4848-4853.

[21] Risser, D. D., & Callahan, S. M. (2009). Genetic and cytological evidence that heterocyst patterning is regulated by inhibitor gradients that promote activator decay. *Proceedings of the National Academy of Sciences USA*, 106(47), 19884-19888.

[22] Yoon, H. S., & Golden, J. W. (1998). Heterocyst pattern formation controlled by a diffusible peptide. *Science*, 282(5390), 935-938.

[23] Camargo, S., & Mondragon-Palomino, O. (2021). Terminal heterocyst differentiation in the *Anabaena patA* mutant as a result of post-transcriptional modifications and molecular leakage. *PLOS Computational Biology*, 17(12), e1009453.

[24] Zheng, N., Zhang, H., Li, F., et al. (2022). Comparative network biology discovers protein complexes that underline cellular differentiation in *Anabaena* sp. *Scientific Reports*, 12, 3523.

[25] Nicolaisen, K., Hahn, A., & Schleiff, E. (2009). The cell wall in heterocyst formation by *Anabaena* sp. PCC 7120. *Journal of Basic Microbiology*, 49(1), 5-24.

[26] Curatti, L., & Rubio, L. M. (2014). Challenges to develop nitrogen-fixing cereals by direct nif-gene transfer. *Plant Science*, 225, 130-137.

[27] Sandh, G., El-Shehawy, R., Diez, B., & Bergman, B. (2009). Temporal separation of cell division and diazotrophy in the marine diazotrophic cyanobacterium Trichodesmium

erythraeum IMS101. *FEMS Microbiology Letters*, 295(2), 281-288.

[28] Mullineaux, C. W., Mariscal, V., Nenninger, A., et al. (2008). Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. *The EMBO Journal*, 27(9), 1299-1308.

[29] Hu, Y., Zou, H., Tang, L., et al. (2022). A proteolytic pathway coordinates cell division and heterocyst differentiation in the cyanobacterium *Anabaena* sp. PCC 7120. *mBio*, 13(4), e01639-22.

[30] Hoffman, B. M., Lukoyanov, D., Yang, Z. Y., et al. (2014). Mechanism of nitrogen fixation by nitrogenase: The next stage. *Chemical Reviews*, 114(8), 4041-4062.

[31] Nürnberg, D. J., Mariscal, V., Bornikoel, J., et al. (2015). Intercellular diffusion of a fluorescent sucrose analog via the septal junctions in a filamentous cyanobacterium. *mBio*, 6(2), e02109-14.

[32] Popa, R., Weber, P. K., Pett-Ridge, J., et al. (2007). Carbon and nitrogen fixation and metabolite exchange in and between individual cells of *Anabaena oscillarioides*. *The ISME Journal*, 1(4), 354-360.

[33] Owens, A. C., & Puskás, G. (2018). Bioregenerative life support for human Mars missions. *Acta Astronautica*, 146, 290-298.

[34] Mahaffy, P. R., Webster, C. R., Atreya, S. K., et al. (2013). Abundance and isotopic composition of gases in the Martian atmosphere from the Curiosity rover. *Science*, 341(6143), 263-266.

[35] Watanabe, M., & Ikeuchi, M. (2013). Phycobilisome: Architecture of a light-harvesting supercomplex. *Photosynthesis Research*, 116(2-3), 265-276.

[36] Glazer, A. N. (1989). Light guides: Directional energy transfer in a photosynthetic antenna. *Journal of Biological Chemistry*, 264(1), 1-4.

[37] Levine, J. S., Schild, R. E., & Sagan, C. (1979). Martian surface temperatures: Revised values from Viking 2 lander. *Science*, 203(4375), 46-47.

[38] Lemmon, M. T., Wolff, M. J., Smith, M. D., et al. (2015). Dust aerosol, clouds, and the atmospheric optical depth record over 5 Mars years of the Mars Exploration Rover mission. *Icarus*, 251, 96-111.

[39] Gitelson, J. I., Kovrov, B. G., Lisovsky, G. M., et al. (2003). Long-term experiments on man's stay in biological life-support system. *Advances in Space Research*, 31(7), 1619-1631.

[40] Liu, L. N. (2016). Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes. *Biochimica et Biophysica Acta*, 1857(3), 256-265.

[41] Olsson-Francis, K., & Cockell, C. S. (2010). Use of cyanobacteria for in-situ resource use in space applications. *Planetary and Space Science*, 58(10), 1279-1285.

[42] Verseux, C., Baqué, M., Cifariello, R., et al. (2022). Selection of *Anabaena* sp. PCC 7938 as a cyanobacterium model for biological ISRU on Mars. *Applied and Environmental Microbiology*, 88(14), e00594-22.

[43] Welch, S. A., Barker, W. W., & Banfield, J. F. (1999). Microbial extracellular polysaccharides and plagioclase dissolution. *Geochimica et Cosmochimica Acta*, 63(9), 1405-1419.

[44] Hopkinson, B. M., & Morel, F. M. M. (2009). The role of siderophores in iron acquisition by photosynthetic marine microorganisms. *BioMetals*, 22(4), 659-669.

[45] Whitton, B. A., Grainger, S. L. J., Hawley, G. R. W., & Simon, J. W. (1991). Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. *Microbial Ecology*, 21(1), 85-98.

[46] Cockell, C. S., Santomartino, R., Finster, K., et al. (2021). Space station biomining experiment demonstrates rare earth element extraction in microgravity and Mars gravity. *Nature Communications*, 12, 5523.

[47] Davila, A. F., Willson, D., Coates, J. D., & McKay, C. P. (2013). Perchlorate on Mars: A chemical hazard and a resource for humans. *International Journal of Astrobiology*, 12(4), 321-325.

[48] Liebensteiner, M. G., Pinkse, M. W. H., Schaap, P. J., et al. (2013). Archaeal (per)chlorate reduction at high temperature: An interplay of biotic and abiotic reactions. *Science*, 340(6128), 85-87.

[49] Heidorn, T., Camsund, D., Huang, H. H., et al. (2011). Synthetic biology in cyanobacteria: Engineering and analyzing novel functions. *Methods in Enzymology*, 497, 539-579.

[50] Berla, B. M., Saha, R., Immethun, C. M., et al. (2013). Synthetic biology of cyanobacteria: Unique challenges and opportunities. *Frontiers in Microbiology*, 4, 246.

[51] Huang, H. H., Camsund, D., Lindblad, P., & Heidorn, T. (2010). Design and characterization of molecular tools for a Synthetic Biology approach towards developing cyanobacterial biotechnology. *Nucleic Acids Research*, 38(8), 2577-2593.

[52] Huang, H. H., & Lindblad, P. (2013). Wide-dynamic-range promoters engineered for cyanobacteria. *Journal of Biological Engineering*, 7, 10.

[53] Heidorn, T., Camsund, D., Huang, H. H., et al. (2011). Synthetic biology in cyanobacteria. *Methods in Enzymology*, 497, 539-579.

[54] Rottinghaus, A. G., Ferreiro, A., Fishbein, S. R. S., et al. (2022). Genetically stable CRISPR-based kill switches for engineered microbes. *Nature Communications*, 13, 672.

[55] Ungerer, J., & Pakrasi, H. B. (2016). Cpf1 is a versatile tool for CRISPR genome editing across diverse species of cyanobacteria. *Scientific Reports*, 6, 39681.

[56] Jiang, W., Brueggeman, A. J., Horken, K. M., et al. (2016). Successful transient expression of Cas9 and single guide RNA genes in *Chlamydomonas reinhardtii*. *Eukaryotic Cell*, 15(11), 1384-1391.

[57] Gordon, G. C., Korosh, T. C., Cameron, J. C., et al. (2016). CRISPR interference as a titratable, trans-acting regulatory tool for metabolic engineering in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *Metabolic Engineering*, 38, 170-179.

[58] Zhao, Y., Li, M., Chen, Y., et al. (2024). Development of a base editor for convenient and multiplex genome editing in cyanobacteria. *Communications Biology*, 7, 1034.

[59] Zhao, Y., Li, M., Chen, Y., et al. (2024). Development of a base editor for convenient and multiplex genome editing in cyanobacteria. *Communications Biology*, 7, 1034.

[60] Nielsen, J., & Keasling, J. D. (2016). Engineering cellular metabolism. *Cell*, 164(6), 1185-1197.

[61] Ducat, D. C., Way, J. C., & Silver, P. A. (2011). Engineering cyanobacteria to generate high-value products. *Trends in Biotechnology*, 29(2), 95-103.

[62] Noreña-Caro, D., Zakrzewska, A., Weiland, F., et al. (2021). Analysis of the cyanobacterial amino acid metabolism with a precise genome-scale metabolic reconstruction of *Anabaena* sp. UTEX 2576. *Metabolites*, 11(4), 203.

[63] Knoot, C. J., Ungerer, J., Wangikar, P. P., & Pakrasi, H. B. (2018). Cyanobacteria: Promising biocatalysts for sustainable chemical production. *Journal of Biological Chemistry*, 293(14), 5044-5052.

[64] Cassier-Chauvat, C., & Chauvat, F. (2018). Responses to oxidative and heavy metal stresses in cyanobacteria: Recent advances. *International Journal of Molecular Sciences*, 19(3), 871.

[65] Zerulla, K., Chimileski, S., Näther, D., et al. (2014). DNA as a phosphate storage polymer and the alternative advantages of polyploidy for growth or survival. *PLOS ONE*, 9(4), e94819.

[66] Santos-Merino, M., Singh, A. K., & Ducat, D. C. (2019). New applications of synthetic biology tools for cyanobacterial metabolic engineering. *Frontiers in Bioengineering and Biotechnology*, 7, 33.

[67] Vasudevan, R., Gale, G. A. R., Schiavon, A. A., et al. (2019). CyanoGate: A modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax. *Plant Physiology*, 180(1), 39-55.

[68] Jones, P. R. (2014). Genetic instability in cyanobacteria—An elephant in the room? *Frontiers in Bioengineering and Biotechnology*, 2, 12.

[69] Xue, Y., & He, Q. (2015). Cyanobacteria as cell factories to produce plant secondary metabolites. *Frontiers in Bioengineering and Biotechnology*, 3, 57.

[70] Hess, S. L., Henry, R. M., Leovy, C. B., et al. (1977). Meteorological results from the surface of Mars: Viking 1 and 2. *Journal of Geophysical Research*, 82(28), 4559-4574.

[71] Verseux, C., Heinicke, C., Ramalho, T. P., et al. (2021). A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. *Frontiers in Microbiology*, 12, 611798.

[72] Verseux, C., Heinicke, C., Ramalho, T. P., et al. (2021). A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. *Frontiers in Microbiology*, 12, 611798.

[73] Berman-Frank, I., Lundgren, P., & Falkowski, P. (2003). Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology*, 154(3), 157-

164.

[74] Raven, J. A., Cockell, C. S., & De La Rocha, C. L. (2008). The evolution of inorganic carbon concentrating mechanisms in photosynthesis. *Philosophical Transactions of the Royal Society B*, 363(1504), 2641-2650.

[75] Verseux, C., Baqué, M., Cifariello, R., et al. (2022). Selection of *Anabaena* sp. PCC 7938 as a cyanobacterium model for biological ISRU on Mars. *Applied and Environmental Microbiology*, 88(14), e00594-22.

[76] Horneck, G., Klaus, D. M., & Mancinelli, R. L. (2010). Space microbiology. *Microbiology and Molecular Biology Reviews*, 74(1), 121-156.

[77] Hassler, D. M., Zeitlin, C., Wimmer-Schweingruber, R. F., et al. (2014). Mars' surface radiation environment measured with the Mars Science Laboratory's Curiosity rover. *Science*, 343(6169), 1244797.

[78] Cucinotta, F. A., Kim, M. H. Y., & Chappell, L. J. (2013). Space radiation cancer risk projections and uncertainties—2012. NASA Technical Paper 2013-217375.

[79] Cockell, C. S., Catling, D. C., Davis, W. L., et al. (2000). The ultraviolet environment of Mars: Biological implications past, present, and future. *Icarus*, 146(2), 343-359.

[80] Babele, P. K., Singh, G., Kumar, A., & Tyagi, M. B. (2015). Induction and differential expression of certain novel proteins in *Anabaena* L31 under UV-B radiation stress. *Frontiers in Microbiology*, 6, 133.

[81] Rastogi, R. P., Singh, S. P., Häder, D. P., & Sinha, R. P. (2014). Detection of reactive oxygen species (ROS) by the oxidant-sensing probe 2',7'-dichlorodihydrofluorescein diacetate in the cyanobacterium *Anabaena variabilis* PCC 7937. *Biochemical and Biophysical Research Communications*, 397(3), 603-607.

[82] Sinha, R. P., Singh, S. P., & Häder, D. P. (2007). Database on mycosporines and mycosporine-like amino acids (MAAs) in fungi, cyanobacteria, macroalgae, phytoplankton and animals. *Journal of Photochemistry and Photobiology B: Biology*, 89(1), 29-35.

[83] Rastogi, R. P., Richa, Kumar, A., et al. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids*, 2010, 592980.

[84] Dartnell, L. R., Desorgher, L., Ward, J. M., & Coates, A. J. (2007). Modelling the surface and subsurface Martian radiation environment: Implications for astrobiology. *Geophysical Research Letters*, 34(2), L02207.

[85] Slade, D., & Radman, M. (2011). Oxidative stress resistance in *Deinococcus radiodurans*. *Microbiology and Molecular Biology Reviews*, 75(1), 133-191.

[86] Harris, D. R., Tanaka, M., Saveliev, S. V., et al. (2004). Preserving genome integrity: The DdrA protein of *Deinococcus radiodurans* R1. *PLOS Biology*, 2(10), e304.

[87] Smith, M. D. (2008). Spacecraft observations of the Martian atmosphere. *Annual Review of Earth and Planetary Sciences*, 36, 191-219.

[88] Stal, L. J. (2009). Is the distribution of nitrogen-fixing cyanobacteria in the oceans related to temperature? *Environmental Microbiology*, 11(7), 1632-1645.

[89] Tamponnet, C., & Savage, C. (1994). Closed ecological systems, space life support and biospherics. *Advances in Space Research*, 14(11), 79-85.

[90] Wheeler, R. M. (2010). Plants for human life support in space: From Myers to Mars. *Gravitational and Space Biology*, 23(2), 25-35.

[91] Lasseur, C., Brunet, J., de Weever, H., et al. (2010). MELiSSA: The European project of closed life support system. *Gravitational and Space Biology*, 23(2), 3-12.

[92] Poughon, L., Dussap, C. G., Creuly, C., et al. (2009). Limnospira indica PCC8005 growth in photobioreactor: Model and simulation of the ISS and ground experiments. *Journal of Biotechnology*, 140(3-4), 230-239.

[93] Van Houdt, R., Mijnendonckx, K., Leys, N., et al. (2025). Arthrospira-C: Demonstration of cyanobacterial oxygen production in continuous regime on the ISS. *npj Microgravity*, 11, 3.

[94] Vonshak, A., & Tomaselli, L. (2000). *Arthrospira* (*Spirulina*): Systematics and ecophysiology. In *The Ecology of Cyanobacteria* (pp. 505-522). Springer.

[95] Richmond, A., & Hu, Q. (Eds.). (2013). *Handbook of Microalgal Culture: Applied Phycology and Biotechnology* (2nd ed.). Wiley-Blackwell.

[96] Melis, A. (2009). Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency. *Plant Science*, 177(4), 272-280.

[97] NASA. (2015). Human Integration Design Handbook (HIDH). NASA/SP-2010-3407.

[98] Touloupakis, E., Cicchi, B., & Torzillo, G. (2015). A bioenergetic assessment of photosynthetic growth of *Synechocystis* sp. PCC 6803 in continuous cultures. *Biotechnology for Biofuels*, 8, 133.

[99] Gantar, M., & Svirčev, Z. (2008). Microalgae and cyanobacteria: Food for thought. *Journal of Phycology*, 44(2), 260-268.

[100] Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25(2), 207-210.

[101] Choi, Y. Y., Joun, J. M., Lee, J., et al. (2014). Potential use of the cyanobacterium *Arthrospira* (*Spirulina*) as a livestock feed supplement. *Journal of Microbiology and Biotechnology*, 24(12), 1753-1757.

[102] van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, 58, 563-583.

[103] Finnigan, T., Wall, B. T., Wilde, P. J., et al. (2019). Mycoprotein: The future of nutritious nonmeat protein, a symposium review. *Current Developments in Nutrition*, 3(6), nzz021.

[104] Wieland, P. O. (1994). *Designing for Human Presence in Space: An Introduction to Environmental Control and Life Support Systems*. NASA Reference Publication 1324.

[105] Belkin, S., Mehlhorn, R. J., & Packer, L. (1987). Proton gradients in cyanobacteria. *Methods in Enzymology*, 167, 670-677.

[106] Coppens, J., Grunert, O., Van Den Hende, S., et al. (2016). The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and

sugar levels. *Journal of Applied Phycology*, 28(4), 2367-2377.

[107] De Philippis, R., & Micheletti, E. (2009). Heavy metal removal with exopolysaccharide-producing cyanobacteria. In *Heavy Metals in the Environment* (pp. 89-122). CRC Press.

[108] Clauwaert, P., Muys, M., Alloul, A., et al. (2017). Nitrogen cycling in bioregenerative life support systems: Challenges for waste refinery and food production processes. *Progress in Aerospace Sciences*, 91, 87-98.

[109] Verseux, C., Baqué, M., Cifariello, R., et al. (2022). Selection of *Anabaena* sp. PCC 7938 as a cyanobacterium model for biological ISRU on Mars. *Applied and Environmental Microbiology*, 88(14), e00594-22.

[110] Cannon, K. M., Britt, D. T., Smith, T. M., et al. (2019). Mars global simulant MGS-1: A Rocknest-based open standard for basaltic martian regolith simulants. *Icarus*, 317, 470-478.

[111] Verseux, C., Baqué, M., Cifariello, R., et al. (2022). Selection of *Anabaena* sp. PCC 7938 as a cyanobacterium model for biological ISRU on Mars. *Applied and Environmental Microbiology*, 88(14), e00594-22.

[112] Shih, P. M., Wu, D., Latifi, A., et al. (2013). Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. *Proceedings of the National Academy of Sciences USA*, 110(3), 1053-1058.

[113] Verseux, C., Heinicke, C., Ramalho, T. P., et al. (2021). A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. *Frontiers in Microbiology*, 12, 611798.

[114] Verseux, C., Heinicke, C., Ramalho, T. P., et al. (2021). A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. *Frontiers in Microbiology*, 12, 611798.

[115] Meeks, J. C., & Elhai, J. (2002). Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. *Microbiology and Molecular Biology Reviews*, 66(1), 94-121.

[116] Olsson-Francis, K., & Cockell, C. S. (2010). Use of cyanobacteria for in-situ resource use in space applications. *Planetary and Space Science*, 58(10), 1279-1285.

[117] Olsson-Francis, K., de la Torre, R., Cockell, C. S., et al. (2009). Survival of akinetes (resting-state cells of cyanobacteria) in low Earth orbit and simulated extraterrestrial conditions. *Origins of Life and Evolution of Biospheres*, 39(6), 565-579.

[118] Calculated based on 0.4 kg protein requirement per person per day and 45% protein content in *Anabaena* biomass.

[119] Taton, A., Erikson, C., Yang, Y., et al. (2020). The circadian clock and darkness control natural competence in cyanobacteria. *Nature Communications*, 11, 1688.

[120] Cassier-Chauvat, C., Veaudor, T., & Chauvat, F. (2016). Comparative genomics of DNA recombination and repair in cyanobacteria: Biotechnological implications. *Frontiers in Microbiology*, 7, 1809.

[121] Hutchison, C. A., Chuang, R. Y., Noskov, V. N., et al. (2016). Design and synthesis of a minimal bacterial genome. *Science*, 351(6280), aad6253.

[122] Bernstein, H. C., & Carlson, R. P. (2012). Microbial consortia engineering for cellular factories: In vitro to in silico systems. *Computational and Structural Biotechnology Journal*, 3, e201210017.

[123] Volova, T., Kiselev, E., Shishatskaya, E., et al. (2013). Cell growth and accumulation of polyhydroxyalkanoates from CO_2 and H_2 in a novel obligate chemolithoautotrophic bacterium *Cupriavidus necator* B-10646. *Bioresource Technology*, 146, 215-222.

[124] Levy, A., & Borenstein, E. (2013). Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. *Proceedings of the National Academy of Sciences USA*, 110(31), 12804-12809.

[125] Rummel, J. D., Beaty, D. W., Jones, M. A., et al. (2014). A new analysis of Mars "Special Regions": Findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2). *Astrobiology*, 14(11), 887-968.

[126] COSPAR. (2020). COSPAR Policy on Planetary Protection. Committee on Space Research Panel on Planetary Protection.

[127] Wright, O., Stan, G. B., & Ellis, T. (2013). Building-in biosafety for synthetic biology. *Microbiology*, 159(7), 1221-1235.

[128] Clauwaert, P., Muys, M., Alloul, A., et al. (2017). Nitrogen cycling in bioregenerative life support systems. *Progress in Aerospace Sciences*, 91, 87-98.

[129] Landis, G. A. (2001). Solar power for Mars. NASA Technical Memorandum 210231.

[130] Xue, S., Su, Z., & Cong, W. (2011). Growth of *Spirulina platensis* enhanced under intermittent illumination. *Journal of Biotechnology*, 151(3), 271-277.

[131] Hendrickx, L., & Mergeay, M. (2007). From the deep sea to the stars: Human life support through minimal communities. *Current Opinion in Microbiology*, 10(3), 231-237.

[132] Muscatello, A. C., Santiago-Maldonado, E., & Linne, D. L. (2020). Mars In-Situ Resource Utilization technology evaluation. *AIAA Propulsion and Energy Forum*, AIAA 2020-3476.

[133] Bernstein, H. C., Paulson, S. D., & Carlson, R. P. (2012). Synthetic *Escherichia coli* consortia engineered for syntrophy demonstrate enhanced biomass productivity. *Journal of Biotechnology*, 157(1), 159-166.

[134] Großkopf, T., & Soyer, O. S. (2014). Synthetic microbial communities. *Current Opinion in Microbiology*, 18, 72-77.

[135] Radivojević, T., Costello, Z., Workman, K., & Garcia Martin, H. (2020). A machine learning Automated Recommendation Tool for synthetic biology. *Nature Communications*, 11, 4879.

[136] Long, C. P., & Antoniewicz, M. R. (2019). High-resolution ^{13}C metabolic flux analysis. *Nature Protocols*, 14(10), 2856-2877.

[137] Menolascina, F., Siciliano, V., & di Bernardo, D. (2012). Engineering and control of biological systems: A new way to tackle complex diseases. *FEBS Letters*, 586(15), 2122-2128.

[138] Costello, Z., & Garcia Martin, H. (2018). A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data. *npj Systems Biology and Applications*, 4, 19.

[139] Lasseur, C., Dixon, M., Paille, C., et al. (2023). MELiSSA: 30 years of research towards sustainable life support. *Acta Astronautica*, 202, 463-478.

[140] Mankins, J. C. (2009). Technology readiness assessments: A retrospective. *Acta Astronautica*, 65(9-10), 1216-1223.

[141] McKay, C. P., Toon, O. B., & Kasting, J. F. (1991). Making Mars habitable. *Nature*, 352, 489-496.

[142] Cockell, C. S. (2010). The interplanetary exchange of photosynthesis. *Origins of Life and Evolution of Biospheres*, 40(4), 397-410.

[143] Pointing, S. B., & Belnap, J. (2012). Microbial colonization and controls in dryland systems. *Nature Reviews Microbiology*, 10(8), 551-562.

[144] Gerstell, M. F., Francisco, J. S., Yung, Y. L., et al. (2001). Keeping Mars warm with new super greenhouse gases. *Proceedings of the National Academy of Sciences USA*, 98(5), 2154-2157.

[145] Ruffing, A. M., Jensen, T. J., & Strickland, L. M. (2016). Genetic tools for advancement of *Synechococcus* sp. PCC 7002 as a cyanobacterial chassis. *Microbial Cell Factories*, 15, 190.

[146] Madhyastha, H. K., Radha, K. S., Nakajima, Y., et al. (2008). uPA dependent and independent mechanisms of wound healing by C-phycocyanin. *Journal of Cellular and Molecular Medicine*, 12(6B), 2691-2703.

[147] Burja, A. M., Banaigs, B., Abou-Mansour, E., et al. (2001). Marine cyanobacteria—A prolific source of natural products. *Tetrahedron*, 57(46), 9347-9377.

[148] Mayfield, S. P., Franklin, S. E., & Lerner, R. A. (2003). Expression and assembly of a fully active antibody in algae. *Proceedings of the National Academy of Sciences USA*, 100(2), 438-442.

[149] Hempel, F., & Maier, U. G. (2012). An engineered diatom acting like a plasma cell secreting human IgG antibodies with high efficiency. *Microbial Cell Factories*, 11, 126.