A Review of Environmental Challenges Facing Martian Colonisation and the Potential for Terrestrial Microbes to Transform a Toxic Extraterrestrial Environment

Daniel Keaney 1,*, Brigid Lucey 1 and Karen Finn 2

1 Department of Biological Sciences, Munster Technological University, Bishopstown, T12 P928 Cork, Ireland; brigid.lucey@mtu.ie
2 Department of Analytical, Biopharmaceutical and Medical Sciences, Atlantic Technological University Galway City, Old Dublin Road, H91 T8NW Galway, Ireland; karen.finn@atu.ie
* Correspondence: daniel.keaney@mycit.ie

Abstract: Mars is a focus of New Space Age exploration and colonisation, but there are significant challenges to successful colonisation by humankind. Environmental microbes play a key role in supporting the ecosystems of Earth, especially within the biodegradation and bioremediation sectors. However, the repurposed roles of microbes on Mars and their associated uses to colonists remain incompletely defined. The aim of this review was to examine the key roles of microbes on Earth and how they have been employed by humans to tackle four pivotal environmental challenges associated with the colonisation of Mars, namely the physical environment, the creation of a hospitable environment via terraforming, environmental sustainability and life support, and finally, renewable processing technologies. Some species of microbes were found to be tolerant of the ever-changing physical environment on Mars (freeze–thaw and UVC exposure) making them useful for bioremediation applications. Employing perchlorate-remediating microbes for their ability to bioremediate the soils of sodium perchlorate, which is present in Martian soils, in addition to their innate ability to cycle nutrients through the biosphere showed promise in establishing sustained crops to support colonists. The employment of terrestrial environmental microbes is a necessary part of overcoming key environmental challenges to successfully colonise Mars. Without this, future New Space exploration is unlikely to be successful.

Keywords: bioremediation; terraforming; nitrogen fixation; decomposition; new space age; Mars; perchlorate

1. Introduction

Microbes contribute to the fundamental foundations upon which our global biosphere is built [1–4]. Throughout history, microbes have played key roles in supporting human existence on planet Earth, from the creation of exotic flavours via fermentation [5] in ancient civilisation, to the modern-day use of genetically modified microbes for the creation of biopharmaceutical drugs [6]. As we enter the New Space Age with future manned missions planned to distant planets (Mars being the focus of not only this review but forthcoming NASA missions in the 2030s), it is important to question the reimagined roles of microbes and examine where they belong within the creation of new civilisations on Mars and beyond. While this review focusses on the challenges of colonising Mars, we acknowledge the numerous global challenges to planetary health on Earth, including climate change, environmental destruction, war, famine, and gross social and economic injustices. This raises important ethical debates about investing Earth’s finite resources into the exploration and the terraforming of distant planets, potentially at the expense of improving our own planetary health first. At the same time, exploration of technologies and ecological solutions (including novel application of microbes) is likely to support many terrestrial systems on...
Earth as well as future strategies to terraform of Mars, thus benefiting both avenues of development alike. Fortunately, planetary exploration and the terraforming of Mars can help support terrestrial systems in many ways thus benefiting Earth and Martian colonisation initiatives alike.

The colonisation of Mars presents challenges that could drive the advancement of cutting-edge technologies. The need for solutions in areas including life support, habitat construction, resource utilisation, and space travel have the potential to spur technological breakthroughs with applications on Earth. These innovations could positively impact two prominent fields causing issues to Earth’s planetary health: renewable energy and healthcare.

Considering the physical environment of Mars, the three main renewable sources of energy consist of solar power, wind power and geothermal energy. Current research into the development of photovoltaic technologies has shown promise for human exploration of Mars [7]. Photovoltaics is the conversion of light into electricity using semiconducting materials that exhibit the photovoltaic effect. As a result, the continual development and reimagination of photovoltaic technology has allowed for the creation of cheap solar energy on Earth, which is both more accessible and greener than fossil fuel to people on Earth [8]. Meanwhile, despite winds on Mars being less powerful than on Earth, this has forced the development of a low Reynolds number vertical axis wind turbine for Mars [9]. This turbine has the added advantage of being able to function midst Martian dust storms. This technology has the potential to revolutionise sub-Saharan communities plagued by drought and consequent dust storms. While geothermal technologies for Mars are yet to emerge for use terrestrially, the continual exploration of geothermal energy on Mars [10] is sure to be the cause of innovative environmentally friendly and accessible technologies on Earth. The insights gained from Mars missions can be applied to address Earth’s resource challenges, including water scarcity and responsible mining practices [11–13]. Research into sustainable housing and power provision on Mars, often utilising solar energy, may lead to advancements in efficient space-based housing and energy production, with broader applications on Earth. These technologies contribute to the development of more efficient and sustainable energy solutions [14].

On a humanistic level, the drive to push the boundaries of humanity through space exploration and science can often inspire a new generation of creative thinkers, determined to make global change. Uniting nations with a common goal (for example mRNA vaccine development during the SARS-CoV-2 pandemic [15]) has the potential to revolutionise planetary health, both on Earth and beyond [16]. As of 2023, NASA has funded 8 new studies based on furthering our understanding of human health in relation to space travel. The titles of some of the studies funded include “A time course of bone microarchitectural and material property changes in male and female mice during simulated unloading and spaceflight.” and “Effects of Acute and Protracted Proton Radiation Exposure on Bone Health.” [17]. Meanwhile, rethinking the challenges associated with easily cured first-world human health issues while in space poses its own set of problems, yet the solutions, such as improved telehealth consultations between astronauts and physicians, can aim to have positive knock-on effects terrestrially in communities lacking necessary healthcare [18].

The colonisation of Mars itself is a multi-faceted operation with many different thematic areas that must be considered, from cost considerations to human health. This review examines the potential of microbes within the Martian environment as we look towards the future of the colonisation of Mars. This review describes some of the key roles that microbes play in supporting the terrestrial ecosystem, emphasising modern bioremediation applications and their abilities to ensure environmental sustainability. Much of the current literature about Mars is currently focused on the human aspect of colonisation, from astronaut’s mental health and psychological challenges [19,20], to physical hazards such as radiation exposure and the dangers associated with the natural environment of Mars [21,22]. However, there is a paucity of focus on the challenges of environmental sustainability on Mars and methods to overcome these challenges.
Therefore, this review aims to understand how humankind has employed microbes over time and the roles of microbes themselves within nature. The knowledge gained from this cumulative information aims to address four pivotal environmental challenges linked to the initial colonisation of Mars: The Physical Environment, The Creation of a Hospitable Environment Via Terraforming, Environmental Sustainability and Life Support and Renewable Processing Technologies, through the application of key environmental microbes.

2. A Brief History of Anthropogenic Uses of Bacteria

Throughout history, the relationship between humans and microbes has been dynamic and multifaceted. From co-evolution in prehistory [23] to the profound impacts of the Agricultural Revolution and the challenges of infectious diseases in ancient civilisations [24,25], humans and microbes have influenced each other’s evolution. The Middle Ages witnessed devastating pandemics, while the Scientific Revolution and the 19th century marked breakthroughs in understanding microbiology and disease transmission. The 20th century brought about advancements including vaccines and antibiotics, altering the course of infectious diseases. In the contemporary era, ongoing research of the human microbiome highlights the intricate interplay between microbes and human health.

2.1. Bacterial Uses in Ancient Civilisation

Evolving alongside bacteria, humans have been using the microbial fermentation process to produce foods for thousands of years [26]. Without micro-organisms the human race would not have had the complex flavours and fragrances created as a result of the chemical by-products of microbial fermentation [27]. This process of strategic enzymatic digestion dates back to at least 3150 B.C. as the Egyptians used *Saccharomyces cerevisiae* in winemaking. Specifically, Cavalieri (2003) describes taking samples of wine residue found in ancient Egyptian wine jars and subjecting them to molecular sequencing. Through ribosomal DNA extraction, amplification, and sequencing, it was possible to identify the presence of *Saccharomyces cerevisiae* [26].

2.2. Modern Uses of Bacteria

*Food*: Currently, fermented foods are increasingly highlighted as an important dietary benefit. These foods include yoghurt, sour cream, sauerkraut, kimchi, and miso, which are produced via modern microbial fermentation techniques using *Streptococcus thermophilus*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *Tetragenococcus halophilus*, respectively [28]. These foods facilitate the creation of reportedly bioactive and nutritive compounds, aiming to help target obesity and inflammation, and to provide a source of beneficial probiotics, as reported by Marco et al. (2017). While the area of probiotics is seen as a controversial topic amongst researchers due to many unsubstantiated claims, some studies suggest they provide beneficial effects by competing with gastrointestinal pathogens, such as *E. coli*, for adhesion to the gut lining [29] by excluding and displacing pathogens such as food-borne biofilm-forming pathogens [30] restoring epithelial barrier function [31] and even have the potential for beneficial bacteriocin production [32].

Bacteriocins can be described as ribosomal synthesised peptides that exhibit antimicrobial activities. These peptides can either kill or inhibit closely related strains of bacteria, or non-related bacteria, without harming itself, or any other bacteria in the surrounding microbial community [33]. They are used more commonly in today’s society in food technology as preservatives. Due to their peptide nature and protease sensitivity, once ingested they become inactivated by the body’s digestive processes and are generally regarded as safe [34,35]. In 1928, nisin, produced by *Lactococcus lactis*, was the first of these food-preserving peptides discovered and has since been commercialised and marketed as Nisaplin® [36]. While useful as a sustainably produced food preservative to New Space Age colonists, nisin variants are under constant construction via genetic modification, thus expanding its range of use to future colonists [37].
**Genetic Engineering & Recombinant Biopharmaceuticals:** While engineered bioremediation microbes have shown promise in the field of oil spill remediation, removal of heavy metals, pesticide remediation and even plastic waste degradation [38–41], their direct application in nature has not been pursued due to the ethical risks associated with the release of genetically modified organisms into the environment and their potential for associated horizontal gene transfer. Nonetheless, the therapeutic products associated with contained modified microbes have revolutionised the pharmaceutical industry. The global pharmaceutical market was worth $934.8 billion in 2017, with expectations that it will reach $1.9 trillion by 2027, according to IQVIA global contract research organisation [42]. In 1978, Genentech’s David Goeddel created the world’s first recombinant human insulin, using *Escherichia coli* K12 as the host system to express both the insulin α-chains and β-chains to create a safe and effective form of human insulin. The creation of Humulin R (rapid acting) and Humulin N (NPH, intermediate acting) ensued and heralded in a new age of medicine, by means of recombinant DNA biopharmaceuticals produced using bacterial vectors [43].

**Medicine:** A pivotal moment within the 21st century’s medical history was the discovery of antibiotics. The year of 1928 brought with it a worldwide revolution as Sir Alexander Fleming first discovered penicillin [44]. Over the coming decades, novel and effective antibiotics were being discovered from soil samples worldwide and this era (between 1950–1970) is now referred to as “The Golden Age of Antibiotic Discovery”.

In today’s modern world, the human race has entered the termed “Age of Disenchantment” as anti-microbial resistance (AMR) is increasing [45]. According to the Centre for Disease Control (CDC) in the U.S.A, at least 2 million people, each year, become infected with antibiotic resistant bacteria, and of those 2 million, at least 23,000 people die. It is speculated that by 2050 antibiotic resistant ‘superbugs’ will be responsible for the deaths of over 10 million people each year, surpassing the death toll caused by cancer [45]. While modern antibiotic discovery may be slowing down, the use of recombinant pharmaceuticals serves as a promising and sustainable option for New Space Age societies.

Throughout human history microbes have played a significant role in supporting humanity by providing key life-building services such as food and medicine production, yet the question remains regarding their potential role in the colonisation of Mars.

### 3. The Role of Bacteria in Combatting New Space Age Challenges

The Mars Exploration Programme consisting of previous missions including The Mars Reconnaissance Orbiter, The Mars Exploration Rovers, The Curiosity Rover, The Phoenix Lander, and in more recent times, The Opportunity Rover, have all paved the way in increasing knowledge of the Martian landscape. Recent and future missions to Mars include NASA’s 2020 Rover, which aims to study Martian astrobiology, the Mangalyaan Orbiter 2 in 2022, and the Martian Moons Exploration in 2024, which aims to collect samples from the orbiting moons Phobos and Deimos. There are also plans to have manned missions to Mars as early as the 2030s. Key areas that pose a challenge to the colonisation of Mars include:

(A) The Physical Environment.
(B) The Creation of a Hospitable Environment Via Terraforming.
(C) Environmental Sustainability & Life Support.
(D) Environmentally Sustainable Renewable Processing Technologies.

The use of bacteria in facing these Martian colonial challenges (see Figure 1) will be crucial in achieving these goals.
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Figure 1. The transferable uses of bacteria on Mars, with examples of associated bacteria and their abilities.

3.1. The Physical Environment

Mars, characterised by its arid and desolate landscape, holds considerable potential for future human colonisation. A significant portion of Mars’ surface is covered in a thick layer of dust primarily composed of iron (III) oxide [46]. The Martian soil, constituting the fine regolith and dust that makes up the surface, is toxic due to elevated concentrations of perchlorate compounds (0.5–1%) [47,48]. Perchlorate, typically found as the anion component of salts associated with cations including ammonium, sodium, or potassium, is considered both a potent oxidizing anion and a health hazard to humans [49]. The future application of bioremediation bacteria holds promise for detoxifying Martian soils, enabling crop cultivation, and facilitating successful human colonization.

The surface temperatures on Mars, fluctuating over its approximately 24.65-h day, vary based on location and reporting sources [50,51]. At the equator, noon temperatures can reach 30 °C, while at the poles, they can plummet to −140 °C, averaging at −63 °C [52]. Mars’ present-day UV wavelengths (200 nm–400 nm) closely resemble those reaching Earth’s surface (290 nm–400 nm) [53]. Notably, Mars’ UV radiation environment features shorter wavelengths in the UVC (200 nm–280 nm) and UVB (280 nm–315 nm) spectra, with UVC posing biological risks due to its DNA-disrupting capabilities [54]. UVC energy absorption by DNA leads to the dimerization of nucleic acid bases, including cyclobutane pyrimidine species and pyrimidine (6–4) pyrimidone compounds, along with their Dewar isomers [55].

One study that highlighted the effects of both freeze-thaw and UVC radiation individually and in tandem on bioremediation strains, demonstrated that some bacteria can withstand up to 20 freeze-thaw cycles in the absence of a cryoprotectant, along with large UVC doses, while the combination of these environmental assaults can either make these strains more UV resistant or sensitive, species dependent [56].
One way to protect these valuable bacteria from thermal fluctuations and exposure is to introduce bioreactors capable of withstanding extreme temperatures, while keeping the enclosed bacteria at a constant temperature. The ISA has recently employed a photobioreactor housing a species of algae called *Chlorella vulgaris*, which is responsible for both oxygen production and for providing a source of food [57].

To protect against solar UV exposure, the implementation of a physical UV filter that blocks harmful wavelengths could be employed, or a biological filter. One biological filter is pityriacitrin, a potent UV filter produced by *Malassezia furfur*, hypothesised to help reduce fungal UV sensitivity and thereby act as an advantage while residing commensally on the skin [58].

Biofilms are an extra-cellular polymeric matrix secreted by bacteria. These allow for adherence to a surface, provide protection against external forces and facilitate effective proliferation [59] thereby serving as a protection to the physical environment. Experimental evidence gathered from the EXPOSE-R2 facilities onboard the ISS detail how both hot and cold desert strains of *Chroococcidiopsis* survive when exposed to space vacuum, Martian atmosphere, UVC radiation and temperature extremes, due to an ability to form biofilms [60]. Communal biofilms are those which consist of two or more species of bacteria, and they behave symbiotically. Certain strains of *E. coli* can produce strong biofilms, which could be used in a similar way as *Chroococcidiopsis*, in order to produce a shielding effect to other bacterial species from stimuli such as UVC rays, when grown in a communal biofilm [61,62]. One promising bacterium for communal-extraterrestrial biofilms is *Deinococcus radiodurans*, a non-pathogenic coccus, well known for its ability to withstand more than 1500 kilorads of gamma radiation without dying or mutating [63]. Owing to the fact it carries multiple copies of its genome, *D. radiodurans* can repair any DNA strand breakages caused by ionising radiation and thus might be expected to ensure the long-term survival of a communal biofilm. It is also highly freeze-thaw resistant [56].

In addition to using technological and naturally occurring mechanisms of protecting bacteria against the harsh Martian climate, genetic engineering opens a new frontier of possibilities and brings with it a consideration of bioforming, or of genetically modifying all inhabitants [64]. However, while it is a valid option, the power of genetics is not fully realised and large-scale manipulations to bacterial genomes, or the creation of a perfectly suited synthetic microbe, may prove to be more dangerous to human-life than helpful.

What was originally reported to be a modern adaptation in response to the selective pressures of antibiotic overuse, has since been proven to be a biological and natural trait found in bacteria as a recent metagenomic analysis of 30,000-year-old Beringian permafrost bacterial DNA details the discovery of genes encoding for resistance to β-lactam, tetracycline, and glycopeptide antibiotics. Many antimicrobials are found in nature—for example β-lactam antibiotics are a broad group of molecules that are naturally produced by different organisms (moulds belonging to *Penicillium* spp. and *Cephalosporium* spp. for penicillins and cephalosporins, respectively), and bacteria belonging to different species for monobactams and carbapenems [65], so it might be expected that antimicrobial resistance might predate the antibiotic era.

Interestingly, antimicrobial resistance stems beyond the realms of Earth and has also been found in outer space, as bacteria subjected to experimentation on the International Space Station (ISS) have yielded potentially concerning results. Comparisons of biofilm-formation, conjugative transfer and antibiotic resistance was carried out in *Staphylococcus* and *Enterococcus* isolates from the ISS, and it was found that biofilm formation was observed in 83% of the isolates; they also possessed multiple genes encoding the resistance of chloramphenicol and erythromycin, along with a higher gene transfer capacity than their terrestrial counter-parts due to the presence of three *vir* signature genes; *virB1, virB4* and *virD4* [66]. It is speculated that this is a function of how bacteria react at both a physical and biomolecular level under zero-gravity conditions, along with the absence of a process known as fluid shear. Within the human body, bacteria encounter many different fluids, respective of their location, such as blood, mucous or stomach fluid. These fluids exert a
mechanical force on the bacteria, causing shear of their cell walls. It has been postulated that this shear within a bacterium’s surroundings dictates how it behaves [67,68]. It was noted how areas within the body including the intestinal tract, the respiratory area, and the urogenital area, are all areas with low fluid shear, but are also very common sites of infection [69]. Coincidentally, areas of microgravity, such as those found extra-terrestrially, happen to be low-shear environments.

3.2. Terraforming

Terraforming, translated as Earth-shaping, can be described as the deliberate modification of a planet, moon or celestial body’s atmosphere, biosphere (temperature and ecology), and surface topography, to create a habitable environment similar to that of Earth. The terraforming of Mars is a multi-faceted operation, which, according to Haynes and McKay (1992), involves three main steps, namely the human/robotic exploration of Mars (which has been achieved through various Mars landers); the planetary engineering step, designed to warm the planet, liberate liquid water and produce a thick carbon dioxide atmosphere; and the introduction of pioneering microbial communities able to proliferate in the Martian environment [70]. While there are numerous, and technologically feasible, answers as to how humankind would conduct the planetary engineering steps, such as using orbital mirrors, redirecting ammonia asteroids and producing halocarbons [71], the introduction of suitable bioremedial microbial communities is an emerging and important area of research.

3.2.1. Microbes & Bioremediation

Bioremediation has been defined as “a biological response to environmental abuse” [72]. It is the use of micro-organisms to degrade pollutants caused by human activities that pose a threat to humans and the environment, such as oil spills.

The study of the suitability of bioremediation microbes for use within the New Space Age suggests a fundamental question, namely whether these bacteria always possessed the power to bioremediate, or whether the release of xenobiotic compounds into the environment by anthropogenic activities has caused these bacteria to evolve to the modern world.

Understanding the Origins of Bioremediation

It has been thought that archaea are the oldest known forms of life to exist on this planet [73]. Indeed, a fossil was found in Canada’s Arctic Circle comprising of bacterial remnants found in stromatolites that originated from submarine-hydrothermal environments at least 3770 million years ago [74]. The evidence of oxidised biomass detected within these rock tubules suggests the presence of biological activity caused by archaea. Furthermore, 2700-million-year-old fossilised archaean lipids have also been found in shale samples from the Pilbara Craton, Australia [75], thus providing more conclusive evidence which aids in the creation of the microbial timeline. Bacteria/Eukarya produce membrane lipids with hydrophobic fatty acid tails that are ester-bound to glycerol-3-phosphate whereas Archaea synthesise membrane lipids with hydrophobic isoprenoid lipid tails that are ether-linked to glycerol-1-phosphate with the opposite chirality [76].

Deep sea vents have remained relatively untouched by the activities of mankind; therefore, any analysis of the inhabiting chemosynthetic bacteria could give an indication of whether ancient bacteria had already possessed these remediating qualities, or whether they had evolved in response to anthropogenic stresses. Many deep-sea bacteria are thermophilic which enables them to survive amongst deep-sea vents. Owing to sulphide deposits rich in copper, gold, silver, and zinc–among other chemical elements [77], that originate from these vents, it appears that these deep-sea bacteria excel at metal bioremediation. Species such as Thermosipho melanesiensis BL429, Pyrolobus fumarii and Mariniloga iezophile KA3 have all been observed in deep-sea vents worldwide. Their genomes have been characterised revealing that these microbes specialise in heavy metal transportation (cobalt, chromium, zinc and mercury), sulphur oxidation, and sulphur reduction, respectively [78]. Their ability to withstand the toxic heavy metals emerging from beneath the Earth’s crust acts
as an advantage to suit their environment. These micro-organisms existed long before the anthropogenic activity ensued, therefore, there is a strong possibility that bacteria naturally evolved the abilities to utilise xenobiotic compounds, or closely related naturally occurring compounds, to aid their own survival.

Recently, heavy-metal-resistant thermophilic bacteria such as *Geobacillus stearothermophilus* and *Bacillus sphaericus*, isolated from hot spring environments, have been employed to bioremediate heavy metals present in the waste waters of the Jordan Rotogravure Establishment [79]. From these findings, bioremediation is not likely to be solely a modern occurrence or adaptation.

Xenobiotics can range from food additives to environmental pollutants and drugs. With the introduction of modern man-made xenobiotic compounds to the environment, there is a strong suggestion that bacteria were able to adapt, by means of specialised enzymatic and metabolic pathways, along with horizontal gene transfer, as detailed by Van Der Meer [80], and by Springael [81]; thus, allowing for the metabolism of xenobiotic compounds. Thus, understanding the origins of bioremediation helps us to understand the role bioremediation microbes could play in the colonisation of Mars and the restoration of its environment, similar to their modern-day uses.

**Transferable Environmental Uses of Microbes in the Modern World**

Owing to the generation of xenobiotic compounds through human activity, the roles of bacteria and archaea within today’s environment have been used in ways beyond that of their duties as natural organic decomposers of dead flora and fauna, and instead, they have been employed by humans for use in proactive environmental initiatives. While there are many different types of bioremediating initiatives, as seen in Table 1, those that will be discussed in this review and as such are applicable to the colonisation of Mars comprise; bioremediation, biostimulation and bioaugmentation. Arguably, both biostimulation and bioaugmentation belong to the subject of bioremediation, but nonetheless, they will be treated as individual environmentally conscious initiatives that detail the uses of bacteria to generate/restore/maintain pristine environments.

**Table 1. Bioremediation Initiatives, descriptions, and applications. Adapted from the Asian Journal of Pharmacy and Life Science [82].**

<table>
<thead>
<tr>
<th>Initiative</th>
<th>Description</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-Situ Bioremediation</td>
<td>The use of bioremediation “on site” where the pollution has occurred.</td>
<td>Treatment of wastewater, groundwater, soil/sludge, gas.</td>
<td>[83]</td>
</tr>
<tr>
<td>Ex-Situ Bioremediation</td>
<td>The bioremediation of foreign substances by indigenous bacteria.</td>
<td>Composting, Land farming.</td>
<td>[84]</td>
</tr>
<tr>
<td>Biosparging</td>
<td>The injection of air into a contaminated area in order to stimulate the bioremediation properties of inhabiting bacteria.</td>
<td>Treatment of polluted soils and groundwater.</td>
<td>[85,86]</td>
</tr>
<tr>
<td>Bioaugmentation</td>
<td>The introduction of non-indigenous bacteria to a site of pollution in order to stimulate bioremediation.</td>
<td>Treatment of polluted soils and xenobiotics.</td>
<td>[86]</td>
</tr>
<tr>
<td>Bioreactors</td>
<td>The introduction of liquid-state polluted material into an enclosed controlled environment, home to bioremediation microbes.</td>
<td>Wastewater treatment.</td>
<td>[87]</td>
</tr>
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</table>

Soil bioremediation is an important consideration in ensuring that the environment remains pristine, with an emphasis on environments that contain soils which are used to grow crops. The most frequent contaminant of European soils are heavy metals and mineral oils. These, in turn, can be taken up through the soil by plants and crops, which
are then ingested [88]. One study from the Middle East detailed how aviation kerosene contaminated clay soils, caused by the Merox process, was subjected to bioremediation [89]. The bioremediation of the soil was carried out in lab-scale and field-scale microcosms. Both *Pseudomonas* spp. AP and *Pseudomonas* spp. CK (DSMZ; Braunschweig, Germany) were isolated from kerosene contaminated clay soils. It was found that the soils that had artificially higher populations of these bacteria, remediated more than 90% of the kerosene within the soil, in comparison to 50% remediation obtained by natural attenuation after seven weeks [89].

Antibiotics are released into the environment through farm manure and through its use in medical treatment, often polluting the environment and persisting thereby selecting for increasing antimicrobial resistance [90,91]. In particular, for example, tetracycline antibiotics tend to remain largely unchanged when expelled from the body [92]. Fortunately, these xenobiotics are subject themselves to bioremediation as detailed in a study involving researching the use of bacterial methods to remove antibiotics, including biodegradation, from public areas and drinking water [93]. Another report suggests that the treatment of β-lactamase effluent and wastewater treatment of cephalexin, ceftaroline, ampicillin, and amoxicillin has been shown to be successful when *B. subtilis* 1556WTNC is applied [94].

Contaminated environments, such as soils, that are naturally inhabited by bioremediating micro-organisms such as *Paraburkholderia fungorum*, *Dechloromonas aromatica* and *Pseudomonas putida*, for example, as seen in Table 2, can also be subjected to biostimulation, which is the manner in which native micro-organisms are purposely stimulated through environmental changes such as pH, moisture, aeration, or the addition of nutrients. This provides micro-organisms with the optimal conditions and requirements needed for their bioremediation capabilities [95]. Kanissery and Sims detail how the biostimulation of natural inhabiting micro-organisms, such as species of the genus *Pseudomonas*, in fields containing herbicide-contaminated soils can enhance the degradation of these chemicals [96].

<table>
<thead>
<tr>
<th><strong>Bacterium</strong></th>
<th><strong>Substrate</strong></th>
<th><strong>Uses</strong></th>
<th><strong>References</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas stutzeri</em></td>
<td>Xylene, toluene, phenol and polycyclic aromatic hydrocarbons.</td>
<td>Universally in soils.</td>
<td>[97]</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Toluene, phenanthrene and naphthalene.</td>
<td>Universally in soils.</td>
<td>[98]</td>
</tr>
<tr>
<td><em>Dechloromonas aromatica</em></td>
<td>Benzoate, perchlorate, chlorobenzoate, and toluene.</td>
<td><em>In situ</em> bioremediation, especially in ground/surface water environments.</td>
<td>[99]</td>
</tr>
<tr>
<td><em>Deinococcus radiodurans</em></td>
<td>Ionic mercury and toluene.</td>
<td>Radioactive mixed waste environments.</td>
<td>[100]</td>
</tr>
<tr>
<td><em>Alcanivorax borkumensis</em></td>
<td>Fuel hydrocarbons.</td>
<td>Marine environments; used during the Deepwater Horizon oil spill in the Gulf of Mexico.</td>
<td>[101]</td>
</tr>
<tr>
<td><em>Paraburkholderia phymatum</em></td>
<td>Xylene, toluene, phenol and polycyclic aromatic hydrocarbons.</td>
<td>Universally in soils.</td>
<td>[102]</td>
</tr>
<tr>
<td><em>Paraburkholderia fungorum</em></td>
<td>Dibenzothiophene, fluorene, naphthalene and phenanthrene, along with polycyclic aromatic hydrocarbons other than condensed thiophenes.</td>
<td>Universally in soils.</td>
<td>[103]</td>
</tr>
</tbody>
</table>

Bioaugmentation is the means by which selected micro-organisms, chosen for their prevalent bioremediation abilities, are cultured, and introduced to an environment, in order to aid the bioremediation process [95]. There are many abiotic factors influencing bioaugmentation, including pH, temperature, the soil’s organic content, moisture content, nutrient content and soil type [104]. For example, Hong (2007) studied the effect of temperature and pH on the degradation levels of a nitrophenolic pesticide by *Burkholderia* spp. It
was found that optimal activity came from a temperature of approximately 30 °C with a slightly alkaline pH (pH 7.5), whereas at 10 °C and 50 °C, along with a highly acidic pH (pH 1.5–3.5) it was found that these parameters were not suitable for the soil detoxification of the pesticide [105].

One of the most publicised examples of where biostimulation and bioaugmentation were combined to enhance an environment’s microbial bioremediation powers, was at the BP (British Petroleum) Deepwater Horizon oil spill of 2010, when the oil drilling rig “Deepwater Horizon”, located in the Macondo Prospect in the Gulf of Mexico, exploded [106]. There followed the world’s largest ever oil spill and the environmental effects were devastating; multiple species of pelagic, tidal, and estuarine organisms, including sea turtles, birds and oceanic mammals were severely affected and over 200,000 km² of the Gulf was inaccessible to fishermen and those conducting similar primary economic activities [107]. With the estimated equivalent of approximately 4.9 million barrels of oil leaking into the Gulf of Mexico, BP had to act fast in determining how they would fix this issue. The deep-sea oil cloud was found to contain a dense microbial community (up to 10⁵ CFU/mL), feeding on the petroleum. Using 16S rRNA microarrays, it was determined that 951 subfamilies of bacteria were detected from 62 phyla, with 3 families of Oceanospirillales found to be particularly dominant [108,109]. Officials chose to use different bioremediation strategies in order to combat the influx of oil affecting the shoreline and surrounding waters, instead of physically washing and collecting the surface oil. Biostimulation was conducted using specially curated fertilisers, similar to the oleophilic fertiliser and the slow-release fertilisers used in the Exxon Valdez Spill in Prince William Sound [109]. This encouraged the growth of the inhabiting bioremediation microbes, such as Alcanivorex borkumensis, as seen in Table 2, along with added microbes through the process of bioaugmentation. As a result, the concentrations of polynuclear hydrocarbons detected dropped significantly (<1.0 ppb) upon the release of the microbes [109]. Furthermore, the release of methanotrophs cleared up almost all organic gaseous deposits emitted from the oil line, with meta-genomic analysis detailing how the microbial communities evolved and declined in response to the available nutrients, thus utilising the available hydrocarbons [109,110].

3.3. Environmental Sustainability via Microbial Action

One initiative in ensuring human survival during space travel and the establishment of human and microbial colonies on the Martian landscape is the use of self-sustained and controlled environments, built upon the foundation of nutrient cycling.

3.3.1. A Brief Overview of the Roles of Microbes in Nature

Bacteria are ubiquitous in nature and have diversified and evolved to survive in many different environments, including those of extreme temperatures, pressures, and high levels of radiation.

Every bacterial species that makes up the global population has a specific role to play in maintaining the world’s ecosystems, both terrestrial and aquatic. Vital roles carried out by the majority of micro-organisms within the biosphere include the nutrient cycling of organic materials such as carbon, sulphur and nitrogen. Bacteria have evolved many different mechanisms suited to their environment in order to aid the cycling of nutrients, using compound-dependant pathways. These activities include decomposition, nitrogen fixation, denitrification, and chemosynthesis [111]. The ongoing evolution of bacterial processes extends in some cases to be able to use man-made compounds for nutrient cycling [112].

3.3.2. Microbial-Based Decomposition

Decomposition is the process or act of breaking down an organic material or substance into smaller constituent parts, especially by the action of decomposers (e.g., fungi and bacteria). In deceased animals, decomposition is broken down into 5 stages; algor mortis, bloat, active decay, advanced decay and dry remains [113]. Microbial decomposition begins almost instantly during algor mortis, as the body cools and an anaerobic internal envi-
Decomposition microbiology itself can be divided into 2 classes, namely the decomposition of plant-based organisms, and the decomposition of cadavers and/or carcasses [113]. If not for the action of decomposers, organic carbon, in the form of dead and decaying organic matter would soon exhaust atmospheric carbon dioxide (CO$_2$). This would have a subsequent impact on photosynthesising plants, which in return provide an oxygen source and a food source for humans.

The world’s supply of organic carbon is dispersed throughout the globe and is assimilated into the environment in a number of ways. It is found atmospherically, in animal and plant matter, and is also captured by the world’s oceans; but the main source of trapped organic carbon is within soil [114]. The burning of fossil fuels and release of greenhouse gases into the atmosphere acts as an atmospheric insulator, trapping the sun’s heat. Over time this results in an increase in global temperatures, i.e., global warming. The rise in atmospheric CO$_2$ levels and warmer conditions stimulates plant growth, which leads to increases in biomass and stocks of carbon found within the soils, therefore, lessening the rate at which atmospheric CO$_2$ levels increase [115]. Meta-analysis has found that microbial populations have shown an increased rate of decomposition as a result of elevated atmospheric CO$_2$ levels. As a result of the lower levels of equilibrium between the rates of decomposition and CO$_2$ capture, this limits the amount of carbon that accumulates over time. Ultimately, with the liberation of more CO$_2$ from the soil, comes an increase in the effect of global warming [114,116].

In the world’s oceans, as the temperatures rise, so does the sea level as the water begins to warm. The oceans hold 45% of the world’s sequestered organic carbon and they have a carbon cycle of their own [117]. Oceanic microbes, including phytoplankton, use this carbon and are thought to have created 50–85% of the world’s atmospheric oxygen, with 5% of that being created solely by the genus cyanobacteria *Prochlorococcus* [118]. It has been found that the oceans are beginning to store more atmospheric CO$_2$ than is being released, due to natural changes in ocean currents and weaker upper-ocean overturning [119]. An increased level of carbon dioxide results in the water becoming more acidic and, coupled with the rise in global temperatures, this puts significant strain on the entire oceanic food chain beginning with the microbial decomposers. The study of bacterial organic decomposers may be key in ensuring the creation of a pristine environment not only in a terrestrial setting, but also in an extra-terrestrial environment.

The Nitrogen Cycle: Fixation, Ammonification, Nitrification and Denitrification

Along with microbial decomposition, another fundamental pillar involved in Earth-based microbial life processes is the nitrogen cycle. The nitrogen cycle is essentially a biogeochemical process by which nitrogen is converted from its many forms and returned back into the biosphere, and from there it can be re-used by living organisms. The main components of the nitrogen cycle are fixation, ammonification, nitrification, and denitrification.

Nitrogen fixation is an important and key element in plant growth and survival. Nitrogen makes up a large component of chlorophyll which in turn is needed for photosynthesis, but plants are unable to absorb nitrogen directly from the atmosphere. Originally discovered in 1901 by Beijerinck [120], biological nitrogen fixation is the process by which a specialised group of prokaryotes can utilise free atmospheric nitrogen (N$_2$) and convert it into ammonia (NH$_3$) by means of a catalytic enzyme, namely nitrogenase. This, in turn, allows for the uptake of nitrogenous matter by plants. Nitrogen-fixing bacteria range from symbiotic aquatic cyanobacteria [121], to soil-dwelling bacteria including *Rhizobium* which forms a symbiotic relationship with legumes [122]. Consequentially, these symbiotic nitrogen-fixing-bacteria are important in the growth of certain legume crops and could play a necessary role in extra-terrestrial human nutrition. With modern biotechnological advances comes the possibility that scientists could over-express the nitrogen-fixation genes (such as the *nif* genes [123]) that encode for the respective enzymes in these symbiotic bacteria (*Rhizobium*, and cyanobacteria such as *Anabaena, Nostoc*, and *Spirulina*), as seen in Table 3. This transferable biotechnological advancement could be applied to many different
environments and scenarios and could even allow humans to establish extra-terrestrial crop production and come one step closer to successfully inhabiting distant planets. This has already been shown to be possible as Jeong and Jouanneau detailed how they successfully over-expressed, and therefore increased, nitrogenase activity in strains of Rhodobacter capsulatus by 50–100% in comparison to the wild type strain [124]. More recent studies show the overexpression of the NifH gene in Azotobacter vinelandii which was responsible for twice the level of acetylene reduction than the wild type cell [125].

In present-day times, nitrogen-consuming bacteria have found ways to benefit from the output of excess nitrogen-containing compounds into the ecosystem by mankind, resulting in adverse consequences. Since the pre-industrial era, global atmospheric nitride (N$_2$O) levels have increased from ~270 nmol/mol to ~319 nmol/mol in 2005, with human activities accounting for more than one third of all N$_2$O emissions [126]. Most of this pollution stems from agricultural use. When fertilisers travel from the soils and enter local waterways and systems, it provides the inhabiting bacteria with an abundance of nutrients. This causes the bacteria to reproduce exponentially, in what is known as eutrophication. These respiring bacteria deplete the water of available oxygen–asphyxiating any oxygen-depandent organisms, and when the bacteria die, they pollute the water quality. Over time this results in habitat degradation, a loss in biodiversity and chemical pollution of both aquatic and terrestrial environments [127,128].

Table 3. Nutrient cycling bacteria within the environment, their associated roles and the associated key genes and how the respective phenotypic expression traits.

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Role</th>
<th>Associated Gene(s)</th>
<th>Expression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>Decomposition</td>
<td>ureR, fis, hybG, zapB, and PROSTU_03490</td>
<td>The swarming of P. mirabilis on decaying organic material upregulates genes that produce a strong odour that specifically attracts Bot Flies (Lucilia sericata) which aid in the decomposition process.</td>
<td>[129]</td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td>Decomposition</td>
<td>phoX</td>
<td>Alkaline phosphatase activity</td>
<td>[130]</td>
</tr>
<tr>
<td>Rhizobium meliloti</td>
<td>Nitrogen Fixation</td>
<td>nifHDK operon</td>
<td>nifHDK encodes for nitrogenase, an enzyme commonly used by bacteria in legume plant nodules which reduces free nitrogen (N$_2$) into ammonia (NH$_3$), which is used by the plant.</td>
<td>[131]</td>
</tr>
<tr>
<td>Bradyrhizobium japonicum</td>
<td>Nitrogen Fixation</td>
<td>FixL</td>
<td>A haemoprotein sensor histidine kinase to aid in fixation</td>
<td>[123]</td>
</tr>
<tr>
<td>Geobacillus kaustophilus</td>
<td>Chemosynthetic reduction of Chromium</td>
<td>ChrA</td>
<td>ChrA helps in the reduction of chromium from deep sea thermal vents, into less toxic forms of the metal</td>
<td>[78]</td>
</tr>
</tbody>
</table>

Chemosynthetic bacteria (e.g., Pyrodictium abyssi and Green Sulphur bacteria) are found in deep sea hydro-thermal vents and are able to use inorganic substrates as a food source. They are often known as extremophilic bacteria due to their ability to withstand deep sea pressure and high amounts of magmatic heat that flows from the Earth’s crust through these vents. Not to be confused with chemolithotrophs and chemosau-trophs; chemolithotrophs are organisms that generate energy by the oxidation of inorganic molecules for biosynthesis or energy conservation via aerobic or anaerobic respiration,
while chemoautotrophs are organisms that obtain their energy from a chemical reaction, but their source of carbon is the most oxidized form of carbon, carbon dioxide (CO$_2$) [132–134].

In an environment where sunlight cannot penetrate, bacteria have developed an unique way of surviving; converting compounds such as hydrogen sulfide, hydrogen gas, ferrous iron, and ammonia (which all lack carbon) into compounds that can be utilised by the bacteria and other surrounding organisms, in a process known as chemosynthesis [135].

These bacteria are fundamental to the ecosystems of the deep-sea thermal vents and the food webs that extend up through the ocean from the sea floor. Studying these bacteria, and how they behave, could provide scientists with a greater understanding as to how extra-terrestrial life could evolve. A better understanding of extremophilic bacteria could prove to be useful in numerous terrestrial sectors, including new space age biotechnologies.

With the understanding of the beneficial processes associated with environmental microbes comes the development of sustainable environmental technologies for use within the New Space Age and the colonisation of Mars, with emphasis on The MELiSSA. The MELiSSA (Micro-Ecological Life Support Alternative) project, launched in 1989 by the European Space Agency (ESA) was intended to act as a support in the understanding of closed-life support systems along with aiding the understanding of long-term regenerative life support systems. MELiSSA is based off of the principles of the beneficial roles of bacteria in nature.

As shown in Figure 2, the project goals consisted of repurposing organic wastes, including CO$_2$ and urea, into food, oxygen and water [136]. Based on a terrestrial aquatic ecosystem, MELiSSA is a loop system comprised of five various compartments—each with a specific microbial role. Compartment one houses the thermophilic anaerobic bacteria that break down crew waste and degrade fibre from the non-edible parts of higher plants. This produces volatile fatty acids, minerals and ammonium. These products move into compartment two, containing photoheterotrophic bacteria such as _Rhodospirillum rubrum_. At this point, these products are utilised and minerals and ammonium is produced. These products are utilised in compartment three by nitrifying bacteria (_Nitrosomonas/Nitrobacter_) which create nitrate ions. These ions are used in the final compartment, which is separated into ‘A’ and ‘B’ and are used by the inhabiting higher plants, such as grains, along with photoautotrophic bacteria (_Arthrospira plantensis_). These compartments allow for the production of food, water, oxygen and also absorb carbon dioxide from the atmosphere [136]. While these microbiological life support systems are ideal for new age space travel, they could also be employed during the colonisation of Mars to help ensure sustainability.

The establishment of self-sustaining biodomes on the Martian landscape could potentially house the first wave of new space age explorers. Implementation of self-sustaining ecosystems, such as those seen in MELiSSA, would allow for long-term indefinite inhabitation by astronauts. One study carried out on the feasibility of a biodome was the biosphere 2 project, whose goal was to recreate and study Earth’s biomes within an enclosed loop system with implications for future non-terrestrial life support systems. Located in the deserts of Arizona, _biosphere 2_ was a closed experimental system and colony site, where the crew endured two years of experimentation, using recycled air and water supplies. What they observed were high fluctuation in CO$_2$ levels; a loss in biodiversity of flora and fauna due to the decline in pollinators and their associated plants, and the intense proliferation of certain species of insects [137,138]. While the _biosphere 2_ project was eventually re-envisioned, a lot of valuable information was obtained which can be used to inform the ecology of future biodome developments.
Figure 2. The MELiSSA closed loop system, adapted from the ESA website (2015). https://www.esa.int/spaceinimages/Images/2015/06/MELiSSA_loop_diagram (accessed on 17 September 2023).

3.4. Renewable Processing Technologies

While looking towards the future uses of microbes on Mars resources are finite and colonisation requires renewable microbial-based technologies. If mankind colonises Mars, these colonists must utilise all available resources. As a result, biological processing technology must be developed for different kinds of resources found within the environment; mainly for solid waste (produced by the actions of those onboard spacecrafts), volatiles (mainly occurring with the life support systems) and minerals/geological materials (from the planet’s surface itself, and asteroids). The anaerobic process of breaking down human waste by bacteria has revealed the production of nitrous oxide, a fuel for space travel [139].

Volatiles such as carbon dioxide can be utilised by either higher plants or cyanobacteria, as discussed earlier, which can be found in the life support systems. The use of engineered bacteria to precipitate calcium/iron from regolith and other geological materials, in order to make bio-cements and construct bio-polymers for in situ manufacturing is another possible use of resources by means of bacterial action [140]. This in turn will allow for the steady colonisation of Mars as buildings can be constructed and the landscape will be altered to fit human needs in the new space age.

In the only publication to date on the subject it has been calculated that during a 916-day mission to Mars, the use of *Arthrobacter maxima* and *Arthrobacter platensis* over a 496-day period could produce enough biomass on Mars to decrease the shipped wet-food mixed-menu on a one-way-journey by 38%; using *Cupriavidus necator* over 202 days for Martian polyhydroxybutyrate synthesis can lower the total mass shipped to Mars by 85%, and instead allow for the production of a 120 m³ six-person habitat by means of 3-D printing; the use of *Methanobacterium thermoautotrophicum* could reduce manufacturing mass of Martian fuel (methane) by 56% based on 205 days of Martian bioproduction. Lastly, as an example, a few days of acetaminophen production by *Synechocystis* sp. PCC 6803 could restock used pharmaceuticals or those which have been exposed to irradiation or have become expired, thus eliminating the pressures faced by the conservation of certain medical supplies while waiting for future shipments [141].

The Martian landscape, in terms of its suitability for crop cultivation, lacks nitrogen which is necessary for crop growth. Despite a recent discovery by *The Curiosity Rover* at Gale
crater, Mars, which detailed the discovery of sedimentary nitrogen deposits most likely caused by ancient volcanic plume lightning fixation [142], the main source of available nitrogen on Mars is found atmospherically at 2.7% [143]. The use of bacteria which are encoded to over-express the \textit{nif} genes for nitrogen fixation could help significantly in Martian crop cultivation.

New space age travel to extra-terrestrial colonial sites requires rockets, and rockets require fuel. Another by-product of space shuttle soli-propellants is sodium perchlorate [49,144,145]. Sodium perchlorate is an inorganic, water-soluble compound which has also been found on Mars, as detailed by previous rovers, from inferred brine seeps—a remnant of Mars’ prehistoric past where rivers once carved the topography of the Red Planet [47,146]. Perchlorate poses a significant risk to the health of future colonists as not only is it toxic but can be passed through the food chain via bioaccumulation. This would pose a threat of perchlorate poisoning to colonists growing crops within perchlorate-contaminated soils [147]. One way to combat this threat is the employment of either wildtype perchlorate-reducing bioremediation microbes, such as \textit{Dechloromonas aromatica} or \textit{Azospira oryzae}, or even the use of a genetically modified organism, which has been engineered to withstand both the harsh conditions of Mars and is also able to reduce perchlorate. Both of these methods feed into the terraforming stages of Mars. At present, analytical methods for perchlorate sampling and detection within a system are confined to cumbersome and large pieces of equipment such as ion chromatography [148,149]. Recently, a more compact and novel method for sampling and detecting perchlorate within a system has been identified by means of Raman Spectroscopy [150]. Combined with the use of perchlorate-reducing strains, Raman spectral analysis could allow colonists to monitor remediated soils to ensure the complete absence of perchlorate, thus reducing the risk of perchlorate bioaccumulation within crops grown in Martian soils.

Nonetheless, these potential initiatives mentioned to protect bacteria, mainly biological, technological, and even bio-technological, showcase a variety of options that might assist survival, increasing the possibility of one day leaving the Earth for Mars.

4. Conclusions

To conclude, microbes have been proven to be the foundation on which all biological life resides. From allowing the cycling of nutrients and organic matter through the biosphere and their relationship with ancient civilisation in the creation of fermented foods and drinks, to their modern employment within the environment in the form of bioremediation to help restore pristine environments; microbes have proven their worth. In line with this, as we enter the New Space Age, we are confronted with dynamic, multi-faceted, and cross-disciplinary challenges in facing the issue of the colonisation of Mars; in particular, the physical environment, the creation of a hospitable environment via terraforming, environmental sustainability and life support and renewable processing technologies. This review highlights at least some of the range of potential microbes have to offer in tackling the challenges associated with environmental sustainability and longevity. While the colonisation of Mars is advanced and poses infinite multi-disciplinary issues that could be argued distracts from those effecting our own planetary health, the act of pursuing planetary exploration and terraforming provides us with constant new technologies and innovative solutions to the same issues faced on Earth as are faced on Mars. When viewed through the lens of microbes within the Martian environment, their innate ability to withstand freeze–thaw and the creation of UV filters and biofilms, to their ability to detox Mars’ perchlorate laden landscape for cultivation and create potentially self-sustainable enclosed life support loops and processing technologies, the answer to the question of \textit{where microbes belong in the creation of a new civilisation on Mars} is clear: at the very foundations of all biological processes, as ever. It can be concluded that microbial processes are necessary to the success of colonising Mars. To exclude microbes from the early colonisation of Mars would be detrimental to the production of renewable life-supporting resources, and in turn, the colonists of the New Space Age.
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