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Biofilm contamination in confined space stations: reduction, coexistence or an opportunity?

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The prolonged human permanence in confined environments in space, such as in the case of the International Space Station, has inadvertently fostered conditions leading to uncontrolled microbial proliferation on surfaces, known as biofilm. Biofilm presence represents a challenge in critical spacecraft systems, that can lead to contamination issues and systems loss of function due to biofouling phenomena. This scenario is further complicated by microgravity that has a controversial role on biofilm growth and formation. Biocontamination can be a limiting factor in human long-term mission in outer Earth orbit and an economic and health issue on ISS. This study addresses the pressing need for effective antimicrobial strategies against such resilient biofilms in confined environments where the usage of biocidal chemical compounds is strictly controlled due to toxicity dangers. Traditional methods can be complemented by advanced antimicrobial coatings techniques. A promising approach is based on the oxygen plasma as coating platform. The technology can be potentially extended to a wide range of antibiofilm agents (e.g., peptides, bacteriophages, nanoparticles, quorum sensing disrupting agents, etc.) and substrates (e.g., metal, plastic, ceramic) showing an exceptional flexibility. An alternative vision of the biofilm challenge can be inspired by the dual nature of biofilms, addressed as “good” or “bad” depending on the specific application. Indeed, biofilm have a great potential in closed systems as small space habitat (e.g., ISS) that can be inspired by their role in “large space habitat” as planet Earth itself. The replication of such a complex biological equilibrium is an open challenge.

KEYWORDS

biofilm, nanoparticles, antimicrobial coatings, space, biofilm detection, management strategies, wetting, bacteria

1 Introduction

Biofilms are aggregates of microorganisms that proliferate at interfaces by self-synthesizing extracellular polymeric substances (EPS) (Hall-Stoodley et al., 2004). Biofilms formation is ubiquitous constituting a risk due to microbial pathogen presence in food related facilities, drinking water systems colonization, and medical devices and implants contamination (Novikova, 2004a; Hall-Stoodley et al., 2004; Høiby et al., 2015). Moreover, biofilm is a resilient structure due to its enhanced resistance to chemical compound employed for surfaces detergency, but also for its active role in microbial resistance against antibiotics (Penesyanyan et al., 2020).

Microbial contamination on solid surfaces can significantly compromise the lifespan and functionality of industrial equipment, including heat exchangers, air, and water recycling systems (Stoodley et al., 1999; Zea et al., 2018). Notably, the presence of biofilms has been detected aboard the International Space Station (ISS) (Venkateswaran et al., 2014; Zea et al., 2018; Zea et al., 2020; Marra et al., 2023a), highlighting the challenges of equipment maintenance in space and emphasizing broader concerns in human space exploration. Prolonged habitation in the confined environments of spacecraft, such as the ISS, inadvertently creates ideal conditions for microbial growth (Coil et al., 2016), posing a significant risk to both equipment and human health. This situation is exacerbated by the unique microgravity conditions, combined with the presence of atmospheric moisture and nutrients in habitable pressurized cabins (Klaus et al., 1997; Kim et al., 2013; Venkateswaran et al., 2014). Such environmental conditions prove to be ideal for biofilm formation leading to critical spacecraft systems loss of functions. In general biofilm can develop at different types of interfaces (liquid-air, solid-air or solid-liquid). Liquid-air interfaces require the presence of free liquid surfaces, not often encountered, unless explicitly needed, and are typically easily removed together with the contaminated liquid. In HEPA filters, solid-air interfaces can be colonized by bacterial and fungi, as reported on Mir orbital station (Checinska et al., 2015). These are called airborne biofilm. Diverse species of microorganisms have been identified in the aforementioned environments with prevalent genera including *Staphylococcus sp.*, *Corynebacterium sp.*, and *Bacillus* species. Airborne fungi also were isolated, with Penicillium and Aspergillus as the dominant genera. Opportunistic pathogenic bacteria species were also isolated, including *Flavobacterium meningosepticum*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus sp* (Novikova, 2004b). Regrettably, certain of these species have been linked to infectious diseases affecting the respiratory and digestive systems.

A recurring type of biofilm contamination is the one occurring at solid-liquid interface. An example is the wastewater collection reservoirs unit, also known as the Water Process Assembly (WPA) a part of the Water Recovery System (WRS). This component was fouled by biofilm growth in several occasions, and replacement material were furnished by Earth to solve this problem (Weir et al., 2012). Most common microbial organisms isolated from this biomass are *Ralstonia pickettii*, *Bulkholderia sp.* and *Cupriavidus metallidurans* (Zea et al., 2020). The WRS is one of the so-called Environmental Control and Life Support System (ECLSS), a network of facility dedicated to astronauts' life support aboard the ISS. The ECLSS is necessary for water recycling, thermal control mechanisms, and waste management. One of the few advantages of confined environments is the ability to control physical conditions of wastewater, such as temperature, pH, and ionic strength, or at least be aware of those parameters (Flemming, 2011). In the case of biofilm management, the right combination of these conditions can either promote or inhibit biofilm growth. However, the control of these conditions is limited by the need for compatibility with human life. Additionally, the use of chemical compounds to control biofilm is restricted by concerns about human toxicity. Currently, on the ISS, biocontrol is achieved by adding iodine to drinking water (Wang et al., 2021); however, the long-term effects

on astronaut health are a concern, and alternative approaches to reduce toxicity are currently under investigation and discussed in this perspective.

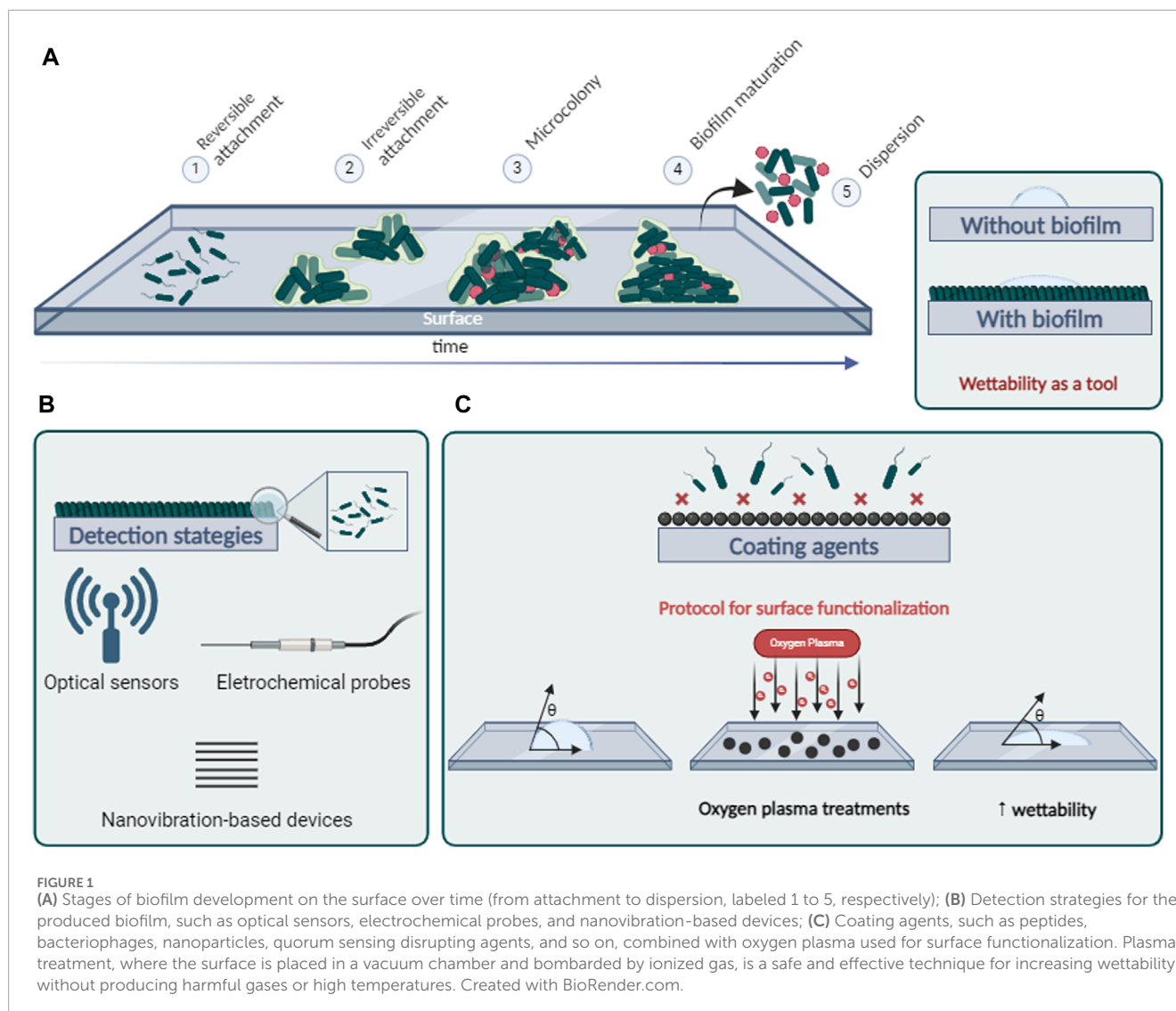
This rise in microbial colonization and the emergence of antibiotic-resistant pathogens aboard spacecraft have profoundly shifted our understanding of microbial behavior in the realm of space exploration. This shift underscores an urgent need to develop novel and effective antimicrobial strategies, particularly considering the altered immune responses of astronauts in-flight, which heightens their susceptibility to infectious diseases (Siems et al., 2021).

Microgravity conditions further complicate this scenario. It is still unclear its role in biofilm enhanced abroad as it tends to enhance microbial activity and biofilm robustness, increasing the risk of microbial-induced corrosion and biofouling and compromising long-term space missions (Benoit and Klaus, 2007; Horneck et al., 2010). Moreover, recent works highlight how biofilm spatial organization heavily affects bacterial gene expression, suggesting a hidden role of gravity (Sanchez-Vizueté et al., 2021; Dergham et al., 2023; Flores et al., 2023).

Addressing these challenges calls for a spectrum of new antimicrobial strategies aimed to prevent as long as possible biofilm formation (Flemming, 2020). While traditional methods like biocides, ionizing radiation, and biofilm detachment techniques have been employed, their effectiveness is often limited against the resilient nature of biofilms, especially in confined environment where only few substances categories can be employed (Wang et al., 2021). This limitation has spurred the exploration of more innovative approaches, particularly in the development of advanced antimicrobial coatings. These coatings are increasingly seen as promising solutions due to their diverse mechanisms of action, including anti-adhesion/microbe-repelling, antimicrobial agent release, and contact-killing properties (Campoccia et al., 2013; Wang et al., 2021; Yazdani-Ahmadabadi et al., 2022).

Recent advancements in this domain have been groundbreaking, particularly the integration of metal ions and nanoparticles into these coatings (Marra et al., 2023b). These elements target essential microbial structures and processes, enhancing the coatings' antimicrobial efficacy. Moreover, the generation of reactive oxygen species through photoexcitation has emerged as a potent method for combating a broad spectrum of microorganisms, adding another layer of defense against microbial threats.

Despite these advancements, a significant technological gap remains in the scale-up from lab-scale to specific testing on real situations, particularly in space environments. The integration of these advanced antimicrobial coatings, is still poorly tested aboard ISS (Wang et al., 2021), and the synergistic implementation alongside other sterilization technologies, can significantly improve biocontamination management. However, to fill this gap, the need for further research focused on the space field is mandatory. As human ventures into space continue to advance, the evolution and adaptation of antimicrobial strategies will play a crucial role in safeguarding astronaut health and ensuring the integrity of spacecraft systems under these challenging conditions.



2 “Promising coating” technologies: nanoparticle and peptides

Recent advancements in nano-coatings methodologies improved the efficacy of antimicrobial and antibiofilm agents against biofilm growth on surfaces (Dhandapani et al., 2012; Lim et al., 2015; Marra et al., 2023b). Biofilm growth is summarized in five key steps illustrated at the top of Figure 1.

A primary approach is based on preventing bacterial adhesion on surfaces, delaying the first step of biofilm formation (Speziale et al., 2009; Gorth et al., 2012; Campoccia et al., 2013; Wang et al., 2021). A promising work following this approach demonstrated an antiadhesion properties of lubricant impregnated surfaces aboard the ISS (Flores et al., 2023). Another complementary or synergistic route is the implementation of bacterial-killing agents to prevent microbial proliferation after the first phase of adhesion (Campoccia et al., 2013).

Notable focus is recently placed on metal oxide nanoparticles (MNPs), and in particular on TiO₂ (Alavi et al., 2019; Liu et al., 2019). TiO₂ nanoparticles demonstrate significant photocatalytic

activity, generating reactive oxygen species that effectively penetrate and disintegrate biofilm matrices (Jalvo et al., 2017). Iron oxide nanoparticles show best results when coated with biocompatible materials such as dextran (Naha et al., 2019). In parallel to nanoparticle-based strategies, antimicrobial peptides (AMPs) offer a promising alternative for biofilms control (Gaglione et al., 2017; Di Somma et al., 2020; Hancock et al., 2021). Their capacity to penetrate and destabilize biofilms at multiple levels makes them a versatile and effective tool against biofilm-related infections (de la Fuente-Núñez et al., 2012; Lim et al., 2015; Hancock et al., 2021). AMPs disrupt microbial membranes through various mechanisms, which often leads to cell death. Beyond these actions, AMPs can impair intracellular signaling systems, thereby influencing critical biofilm control pathways. Notably, families of AMPs like Cathelicidins (Van Harten et al., 2018) and their derivatives, including LL-37, have shown considerable anti-biofilm activity (Bandurska et al., 2015). These peptides effectively inhibit both the formation and growth of biofilms. Furthermore, peptides derived from amphibians have demonstrated robust anti-biofilm properties, in some instances surpassing the efficacy of conventional

antibiotics (Ding et al., 2020). The reason under the interest of both TiO₂ and AMPs in space field, is their proven low toxicity towards humans (Warheit et al., 2007; Nordström and Malmsten, 2017). Others unconventional biobased antimicrobial agents can be represented by bacteriophages and phages. Phages and phage-derived products have gained scientists interest as an alternative to antibiotics in preventing and treating biofilms and associated infections (Amankwah et al., 2021). The development of efficient phage-based treatments requires a deeper understanding of specific bacteria strain resistance to phages and the co-evolutionary mechanisms between phages and bacteria. Phage-based treatments for bacterial biofilm destruction includes the use of mono phages, phage cocktails, genetically engineered phages, and phage-derived enzymes (Pires et al., 2017).

Whichever antimicrobial agent is chosen to ensure a stable bond between antimicrobial agent and a desired substrate, the easiest way is to use the right chemical linker (e.g., in the case of peptides, dopamine shows excellent properties (Lim et al., 2015)). However, toxicity and additional costs must be considered, and in the case of confined environment are not negligible. Following this field of view, a promising technique is represented by Cold Plasma (CP) employment (Lieberman and Lichtenberg, 1994; Fridman, 2008; Vandenabeele and Lucas, 2020). CP was already employed as an effective methodology to bond nanoparticles to a variety of surfaces with technological relevance, such as glass (Marra et al., 2023b), and metal (Dong et al., 2019). This innovative method eliminates the need for high temperatures and chemical precursors, thus preserving the integrity of the surfaces. The performance of these nanoparticle coatings is rigorously evaluated through comprehensive wetting analysis (Evgenidis et al., 2017; Zabiegaj et al., 2021; Kampouraki et al., 2022) and biofilm growth assessments, employing techniques like confocal laser scanning microscopy (CLSM) (Di Somma et al., 2020). The results, depicted at the bottom of Figure 1, from these evaluations reveal a dual benefit: a robust contact-killing effect and enhanced anti-adhesion properties. These properties contribute to a significant reduction in both live and dead biofilm biomasses (Recupido et al., 2020). Moreover, the omniphilic behavior observed during the wetting analysis suggests a strong correlation with the coatings' anti-adhesion capabilities. In the pursuit of employing coatings for space applications, Wang et al. (Wang et al., 2021) conducted a comprehensive review of various coatings suitable for testing within the specified parameters of space environments. Among the most promising candidates is the silver nanocluster–silica composite coating developed by Balagna et al. (Balagna et al., 2012), currently proposed as an antimicrobial coating in the aerospace sector. The efficacy of this coating was evaluated on a commercial polymeric film, utilized in space applications to construct the inner section of inflatable modules that come into contact with crew members. Notably, this coating exhibits inherent air impermeability. Various bacteria (such as *Staphylococcus aureus*, *B. cereus*, *Morganella*, *Klebsiella pneumoniae*, *E. coli*) and fungi (*Candida parapsilosis*, *Candida albicans*, *Candida glabrata*) identified on board the ISS and Mir station have exhibited sensitivity to the silver nanocluster–silica composite coating (Zea et al., 2020; Marra et al., 2023a).

Durability tests further demonstrated the coating's resilience, remaining active for the formation of an inhibition halo and displaying an absence of microorganism growth even after 84 days

of air-aging. These findings underscore the potential of coating as a robust and durable solution for antimicrobial protection in space applications. In the pursuit of enhancing the durability of coatings for extended space missions, a particular type of coating warrants consideration: self-healing coatings. Inspired by the regenerative processes observed in living organisms, self-healing materials have the ability to autonomously or non-autonomously repair damage, effectively restoring the original structure and function (Wang et al., 2020). Intrinsic self-healing materials, which are cross-linked by supramolecular non-covalent bonds (e.g., hydrogen bonds, host-guest interactions, ionic interactions, etc.), can repair damaged regions through dynamic recombination of these supramolecular bonds (Shchukin et al., 2006; Chen et al., 2015).

A groundbreaking development in antimicrobial strategies is the synergistic integration of metal nanoparticles (MNPs) and AMPs, leveraging their distinct properties to devise a comprehensive and highly effective treatment approach (Ding et al., 2020). This synergy arises from the complementary mechanisms of MNPs and AMPs, each targeting different facets of bacterial physiology, thereby culminating in an overarching antimicrobial effect. MNPs primarily exert their antibacterial impact by disrupting the structural integrity of bacterial cell membranes and inducing the generation of reactive oxygen species (ROS) (Campoccia et al., 2013). This activity increases the permeability of bacterial cells, rendering them more vulnerable to external agents. Conversely, AMPs engage bacterial cells through various mechanisms, including the direct disruption of cell membranes, inhibition of critical enzymes, and interference with DNA, RNA, and protein synthesis processes (Campoccia et al., 2013; Wang et al., 2021).

Despite those promising techniques must be fully explored, at the best of our knowledge both for Earth and space applications, a definitive antibiofilm coating is far from being realized, and the term “promising” is still too vague.

3 Redefining biofilm challenges in space: from reduction to coexistence strategies

In the ever-evolving field of antimicrobial and antibiofilm research, a significant shift has been observed in the general approach. This evolution intertwines traditional practices with cutting-edge scientific approaches. Given the billions of years over which microorganisms have adapted to a variety of stresses, it becomes evident that completely eradicating them is an unfeasible goal. In our vision, the emphasis should be focused on understanding how to deal with biofilm presence in complex confined environments. In all efforts to combat biofouling (Cirillo et al., 2021), it is crucial to recognize that biofilms have evolved versatile defense mechanisms against various stresses, including those induced by toxic metals, irradiation, antibiotics, and host immune systems. Expecting an easy and enduring victory over biofouling is unrealistic. Rather, a possible and reasonable achievement is to prolong the period during which biofilms do not pose problems with the final goal to obtain a steady state coexistence with these complex biological systems, as indeed it is in natural environment. Any anti-fouling technology is time-dependent and not permanent. The temporal requirements vary,

ranging from hours to days for applications like removable catheters and the food, beverage, and pharmaceutical industries, to months and years for systems like desalination plants, membrane systems, steam condensers. Following this point of view in the peculiar case of the ISS or other spacecraft environment, a final solution against biofouling is unachievable. Unfortunately, to the best of our knowledge, no coating, antibiotics, detergent operation can be applied to permanently solve the issue of biofilm growth on surfaces.

Nevertheless, the biofilm's indomitable resilience should not deter our efforts. As mentioned earlier, biofilm growth is probably impossible to permanently eradicate, but it can be slowed to the extent that it ceases to be a significant concern. In the context of extended space missions, its growth must be decelerated, aiming to minimize the frequency and invasiveness of extraordinary cleaning operations.

Possible approaches to handle biofouling taking into account the peculiarities of space exploration needs should include:

- Redesigning the WPA to withstand organic loads, preventing complete blockage by biofilm. Not all flow systems are susceptible to biofilm; in fact, there are instances of systems successfully coexisting with biofilms (Flemming, 2011). The occurrence of biofouling is strictly dictated by flow conditions. As a general law, an increase in hydrodynamic forces promotes both the attachment of planktonic cells to surfaces, and the detachment of sessile cells from biofilm (Stoodley et al., 1999; Stoodley, 2016; Fanesi et al., 2021). Typically, an enhancement of flow increases disposal of metabolites, delivery of nutrients and oxygen promoting biofilms. However, after a critical value the detachment process overcome the attachment process and the transport phenomena benefits (Stoodley, 2016). The redesign of the WPA should consider these two processes, considering potential organic loads and, critically, the significant unknown factor of the system: microgravity. Further studies are imperative to comprehend how microgravity influences biofilm growth (Marra et al., 2023a).
- The application of specific coatings with antimicrobial and antibiofilm properties aims to manage and delay the growth of microorganisms. The introduction of substances required for coating materials is meticulously regulated aboard the International Space Station (ISS). The unique and confined environment poses considerable challenges. Any chemical compound used must not only be deemed safe to ensure the health and safety of astronauts but also exhibit sufficient effectiveness against bacterial proliferation to obviate the need for periodic reintroduction processes. All coating techniques must adhere to the European Cooperation for Space Standardization's (ECSS) standards, which govern the materials permitted in confined space stations and specifically address requirements for antimicrobial materials (European Cooperation for Space Standardization, <https://ecss.nl/> (accessed: December 2020)).
- Detection strategies to identify early stages of biofilm formation on surfaces. Monitoring biofilms formation is a valuable method for timely recognition of fouling layer development and can be crucial for cleaning operation timing, in order to guarantee a good anti-fouling protocol.

An ideal monitoring device should offer fast, accurate, real-time, non-destructive, on-line, and cost-effective information about fouling layer development. To the best of our knowledge this ambitious goal has not been reached, but several techniques have been proposed, including nanovibration-based devices (Salazar et al., 2023) and spectroscopy technique for online biofilm monitoring (Kampouraki et al., 2024), nanoparticles probes for detection purpose (Bazsefidpar et al., 2023).

- Smart data analysis and mathematical model. The implementation of machine learning and/or artificial intelligence in bioinformatics can outclass human ability to manage vast amount of data (Srivastava et al., 2020). This instrument can be especially useful for biofilm growth for long-period of time. In general, in literature there is a huge viability of data regarding biovolume, surface coverage, strains, substrate, and coating implementation on biofilm. However, due to the great biofilm heterogeneity there is a lack of connection and relationship between those features. The implementation of IT tools can potentially lead to mathematical model useful to predict for example, the fouling phenomena in systems like pipes or tubes. These novel methods can identify crucial parameters to be identify a can be as useful in microgravity conditions as on Earth application. For instance, the complexity of biofilm structures can be compared to other three-dimensional or bi-dimensional biological structures like tumours or skin (Lowengrub et al., 2009; Deisboeck et al., 2011), where mathematical models, such as cellular automata (Migliaccio et al., 2023), have already been successfully employed.

The combination of the aforementioned interdisciplinary approaches would lead to a holistic management of biofilm prevention within a specific field of application. A particular attention should be placed in the case of complex environments such as the ISS, or possible future permanent human colonies on the Moon or Mars, where a major attention should be paid to investigate the role of modified gravity conditions on evolution of complex biological systems. The next steps towards achieving possible coexistence with biofilm should focus on designing specific biofilm sensors to schedule maintenance operations for ECLSS facilities. Future research should also encompass the redesign of liquid and air flow systems to identify the stress conditions range (e.g. wall shear stresses) that trigger biofilm formation. Most of available literature is focused on the study of biofilm in static condition in lab-environments, such as Petri dishes or agarose gels, stressing only the role of biological or chemical stimuli as shaping driving force of biofilm. We suggest to extend this investigation and consider also mechanical stimuli, such as flow or bulk stresses, as sources of microbial three-dimensional organization. In this vision it is important to extend experimental investigation to conditions mimicking biofilm growth in real conditions, such as pipes, tubes and reservoirs, where submerged biofilm is growth typically under the presence of an external flow, on materials such as metals, glass or plastic.

Furthermore, it is imperative for future research to focus on identifying a range of antimicrobial and antibiofilm agents to treat surfaces prone to contamination. When evaluating these coatings,

critical considerations should include their low toxicity, durability, and the ability not to cause damage to the substrate on which they are applied.

4 Conclusion

Biofilm in the peculiar conditions typical of confined space habitats represents a potential criticism for long term space exploration. A full comprehension of the role of external stresses on biofilm formation is still not available, new approaches are now being used to investigate the role of anisotropic and bulk stresses, such as gravity, on bacteria. It is now evident that a complete eradication of biofilm is not feasible. Moreover, even its reduction poses significant complexities, given the resilience of these microbial communities and the unique constraints of space environments. This understanding necessitates a paradigm shift in our approach to space biofouling: from attempting total elimination to seeking innovative ways of coexistence that minimize risks to human health. In this perspective, we have emphasized potential guidelines to mitigate biofilm formation, recognizing its presence as a dual health and technical risk in confined environments. This consideration is particularly applicable to what is often referred to as ‘bad’ biofilms as counterposed to ‘good’ ones where biofilms are used as a biotechnological tool for applications such as bioreactors or bioremediation. In particular, the concept of “good” biofilms finds practical application in the recent Biorock project (Cockell et al., 2021). Biorock, is a European Space Agency (ESA) project conducted aboard the ISS, that explores the feasibility of large-scale biomining in space. The study focuses on the bioleaching of vanadium from basalt rock, showcasing the advantageous aspects of utilizing biofilms in space (Sheoran et al., 2009). This approach has as aim the utilization of *in-situ* resources to support space exploration in extraterrestrial planet. Strains considered potential candidate for this task are here reported: *Acidithiobacillus ferrooxidans*, *Bacillus subtilis*, *Cupriavidus metallidurans*, *Shewanella oneidensis*, and *Sphingomonas desiccabilis* (Cockell et al., 2021). Aside from biomining, biofilms can be utilized in space exploration as a part of the life-support systems (Fahrion et al., 2021). For example, bacteria can be involved in the production of methane, needed as fuel for some propulsion systems. Cyanobacterial biofilms have been considered suitable candidates for methane production and subsequent use as fuel (Justiniano et al., 2023).

Nature provides numerous examples where biofilm is crucial for environmental homeostasis, as seen in aquariums (as mentioned earlier). On a long time perspective, it would be good to reproduce in confined space environments this complex equilibrium, giving to biofilm a positive role in the overall biological balance of the system. This coexistence strategy not only seeks to minimize the adverse impacts of biofilm but also explores the prospect of leveraging biofilm as a beneficial tool. By comprehending the intricate dynamics of biofilm formation and its interaction with diverse surfaces and environments, especially in microgravity, we will be able to design systems where biofilms can be harnessed for our advantage. This could have implications for

various space applications, including life support systems, waste management, and other critical operations. The knowledge acquired thanks to the space application research will also have sound implications in standard non-space conditions, providing novel approaches that will improve sustainability of human technology on Earth.

In conclusion, the journey of managing biofilm in space is not just about combating a challenge, but also about embracing an opportunity. It invites a broader perspective where biofilm, once seen as a formidable foe, can be transformed into a valuable ally in our quest for sustainable and long-term space exploration. This shift in perspective opens up new avenues for research and innovation, paving the way for more effective and holistic approaches to biofouling management in space.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

DM: Conceptualization, Writing–original draft. RF: Visualization, Writing–original draft. SC: Conceptualization, Funding acquisition, Project administration, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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