Biofilm 2 (2020) 100026

Contents lists available at ScienceDirect

Biofilm

journal homepage: www.elsevier.com/locate/bioflm

Potential biofilm control strategies for extended spaceflight missions

Luis Zea [a,*,1], Robert J.C. McLean [b,1], Tony A. Rook [c], Geoffrey Angle [d], D. Layne Carter [d], Angela Delegard [e], Adrian Denvir [e], Robin Gerlach [f], Sridhar Gorti [d], Doug McIlwaine [g], Mononita Nur [d], Brent M. Peyton [f], Philip S. Stewart [f], Paul Sturman [f], Yo Ann Velez Justiniano [d]

[a] BioServe Space Technologies, University of Colorado, Boulder, CO, USA
[b] Texas State University, San Marcos, TX, USA
[c] Sherwin-Williams, Cleveland, OH, USA
[d] NASA Marshall Spaceflight Center, Huntsville, AL, USA
[e] NCH Corporation, Irving, TX, USA
[f] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[g] Chem Treat, Inc., Richmond, VA, USA

ARTICLE INFO

Keywords:
Mars
Moon
Water
Biofilm
Spacecraft
Life-support

ABSTRACT

Biofilms, surface-adherent microbial communities, are associated with microbial fouling and corrosion in terrestrial water-distribution systems. Biofilms are also present in human spaceflight, particularly in the Water Recovery System (WRS) on the International Space Station (ISS). The WRS is comprised of the Urine Processor Assembly (UPA) and the Water Processor Assembly (WPA) which together recycles wastewater from human urine and recovered humidity from the ISS atmosphere. These wastewaters and various process streams are continually inoculated with microorganisms primarily arising from the space crew microbiome. Biofilm-related fouling has been encountered and addressed in spacecraft in low Earth orbit, including ISS and the Russian Mir Space Station. However, planned future missions beyond low Earth orbit to the Moon and Mars present additional challenges, as resupplying spare parts or support materials would be impractical and the mission timeline would be in the order of years in the case of a mission to Mars. In addition, future missions are expected to include a period of dormancy in which the WRS would be unused for an extended duration. The concepts developed in this review arose from a workshop including NASA personnel and representatives with biofilm expertise from a wide range of industrial and academic backgrounds. Here, we address current strategies that are employed on Earth for biofilm control, including antifouling coatings and biocides and mechanisms for mitigating biofilm growth and damage. These ideas are presented in the context of their applicability to spaceflight and identify proposed new topics of biofilm control that need to be addressed in order to facilitate future extended, crewed, spaceflight missions.

1. Introduction. Biofilms and spacecraft Environmental Control and Life Support Systems (ECLSS)

Biofilms are surface-adherent accumulations of microorganisms in an extracellular polymeric substance (EPS) matrix. The EPS is mostly composed of polysaccharides, proteins, lipids, and nucleic acids that provide them with a scaffold to form a three-dimensional structure and which enables them to adhere to surfaces. Biofilms associated with microbial corrosion often have a variety of minerals present in the matrix [1]. This extracellular biofilm matrix improves cell-to-cell communication and can protect the microbes from mechanical stresses, biocides, antimicrobials, and ultraviolet radiation, among other types of stresses [2,3]. Biofilms have an important role on multiple types of infections in humans, including medical device-associated infections, dental caries, cystitis, pulmonary infections associated with cystic fibrosis and endocarditis [4]. They can also degrade the surface upon which they grow, including corrosion of metals and mineralization and weakening of polymers [5]. Furthermore, they can accumulate to the point of causing structural and/or functional damage to mechanical parts (biofouling). The first investigations regarding controlled biofilm growth in microgravity (with *Burholderia cepacia* and *Pseudomonas aeruginosa*) were first reported in 1999 and 2001, respectively[6,7], although there were

* Corresponding author.
E-mail addresses: Luis.Zea@colorado.edu (L. Zea), McLean@txstate.edu (R.J.C. McLean).
[1] These two authors contributed equally.

https://doi.org/10.1016/j.bioflm.2020.100026
Received 12 January 2020; Received in revised form 8 May 2020; Accepted 24 May 2020
Available online 30 May 2020

certainly earlier indications of biofouling problems in spacecraft. For example, the Soviet Salyut 6 and 7 and the Mir space stations experienced problems derived from microbial contamination on piping, behind panels, water recycling systems, electrical connectors, radiators, air conditioning, oxygen electrolysis block, a navigation window, an extra-vehicular activity (EVA) suit's headphone, and thermal control system [8]. Similarly, the International Space Station (ISS) has had challenges arise from microbial contamination and biofilm formation, notably in the wastewater collection reservoir component of the Water Recovery System (WRS), which is a part of the Environmental Control and Life Support System (ECLSS) [9]. As seen in Table 1, some of the most common microbial organisms isolated from the WRS (namely on the filter immediately downstream of the WPA wastewater tank), are *Ralstonia picketii*, *Bulkholderia sp.* and *Cupriavidus metallidurans* [10]. More recently several metagenomic studies have been performed in the ISS (e.g. Ref. [11,12]). In the case of the US-segment of the ISS, the WPA's wastewater tank – the component that has shown the most problems related to biofilm – receives crew urine (treated with an oxidizer and an inorganic acid) distillate, cabin humidity condensate, and water produced from CO_2 and H_2 by the Sabatier reactor (when in operation). The WPA processes the contents of the wastewater tank into potable water for the crew and multiple other systems [9]. Biofilm formation can be problematic in any spacecraft system, however, it is of particular importance when it occurs in the ECLSS, and the WRS in particular, given that this key life-support system serves to provide the crew and other critical systems with potable water [13]. To maintain this critical function, an improved method for biofilm control must be developed and implemented on future missions. Based on experiences to date with the WRS in the ISS [9] and similar biofilm occurrence seen with municipal drinking water distribution systems [14], and systems involving grey-water recycling [15]; total biofilm eradication in the WRS of spacecraft does not appear feasible. The working mitigation strategy is to control and not eradicate biofilm growth, since the latter is likely not feasible particularly in the context of an extended mission beyond low Earth orbit. The ultimate goal is to simply prevent biofilm growth from impacting the mechanical functionality of the system via corrosion, fouling, or some component organisms bypassing the disinfection processes and affecting the potable water. Consistent with this strategy is the requirement to maintain biofilm control in the WRS with initial system operation, rather than attempting to regain control of biofilm growth by using methods to destroy and/or detach an existing biofilm. Detecting and monitoring bacterial and biofilm levels will be necessary in key ECLSS components, so that appropriate life support functions are maintained. While the bulk of the identified biofilm issues in spacecraft relate to the WRS component of ECLSS [10], one might also anticipate microbial growth on surfaces prone to water condensation or absorption. Volatile organic compound condensation onto surfaces has been proposed as a strategy for extracting airborne compounds during analytical chemical approaches [16] and so one might assume that a similar process along with associated biofilm formation would occur on surfaces or

Table 1
Microbial isolates collected from the wastewater tank (WW), portable water bus (PWB), or condensate.

Microbial species	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	System Component		
												WW	PWB	Condensate
Acidovorax temperans		x						x				x		x
Burkholderia multivorans	x	x	x	x	x	x	x	x	x			x	x	
Burkholderia species					x	x		x		x	x	x	x	
Cupriavidus basilensis		x					x	x	x		x	x	x	x
Cupriavidus metallidurans	x	x	x	x	x	x	x	x		x	x	x	x	x
Curvibacter lanceolatus							x			x	x	x	x	
Flexibacter species								x	x			x		x
Lecyhopora species						x	x					x		
Lecythophora mutabilis								x				x		
Microbacterium laevaniformans						x						x		
Novosphingobium species				x			x					x		
Paecilomyces species								x				x		
Ralstonia insidiosa		x	x	x	x	x	x	x	x	x	x	x	x	x
Ralstonia pickettii	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Shingobium yanoikuyae								x				x		
Sphingobium xenophagum								x				x		x
Unidentified Gram-negative rod				x	x	x		x	x	x	x	x	x	x
Acinetobacter species					x								x	
Afipia species										x			x	
Bradyrhizobium species						x							x	
Burkholderia kururiensis											x		x	
Burkolderia kururiensis										x			x	
Caulobacter vibrioides								x						x
Chitinophaga arvensicola						x							x	
Chitinophaga species				x	x								x	x
Chryseobacterium gleum													x	
Cryptococcus laurentii			x	x									x	
Curvibacter lanceolatus										x			x	
Leifsonia species					x								x	
Mesorhizobium species						x			x				x	
Methylobacterium species			x	x									x	
Microbacterium species													x	
Pelomonas species					x								x	
Phyllobacterium myrsinacearum			x										x	
Rhodopseudomonas species										x				x
Sphingobium yanoikuyae					x								x	
Sphingomonas asaccharolytica		x											x	
Sphingomonas capsulata		x											x	
Sphingomonas paucimobilis	x												x	
Sphingomonas sanguinis		x											x	
Staphylococcus epidermidis						x							x	
Wautersia metallidurans													x	

materials prone to water accumulation on spacecraft. In non-ECLSS situations, prevention or removal of moisture accumulation represents a feasible strategy for biofilm control.

Furthermore, the problems resulting from biofilm formation on future spacecraft may be exacerbated by observed changes on bacterial phenotype and gene expression when grown in microgravity [17,18]. For example, an *in-vitro* investigation performed in space using *Burkholderia cepacia* resulted in (*i*) larger cell counts of bacterial biofilms grown in stainless steel submerged in water and a (*ii*) decreased sensitivity to iodine (which is commonly used as potable water disinfectant [19]), with respect to matched Earth controls [7]. Another investigation that used *Pseudomonas aeruginosa* cultured in modified artificial urine medium (mAUM) in space, and with respect to matched Earth controls, showed an increase in (*i*) number of viable cells, (*ii*) biomass, and (*iii*) mean biofilm thickness, and (*iv*) a 'column-and-canopy' biofilm structure, unlike the flat mats observed on the ground samples [20] (reviewed in Refs. [21, 22]).

One notable condition that is encountered during spaceflight is microgravity. Although short duration microgravity conditions can be encountered by parabolic aircraft missions or drop tower experiments, prolonged microgravity studies need to be performed during spaceflight including the International Space Station. Flight opportunities are rare and so there has been the development of a number of microgravity simulation approaches, most notably random positioning (RP) devices [23,24] and clinostat technology involving rotating wall vessels (RWVs) [25]. Microgravity analog devices such as RWV and RP devices do mimic many but not all aspects of spaceflight (see reviews by Refs. [21,23, 25–27]). The main advantage of analog experiments is one of accessibility and relatively low cost when compared to space flight. In spite of limitations, microgravity analog approaches represent an important, accessory to space biology research.

2. Moon and Mars crewed missions

While spare parts can and are sent to ISS for system maintenance, this approach will be more complicated on missions to the Moon and prohibitive on a mission to Mars. Hence the importance of proactively mitigating the risks derived from biofilm formation on future spacecraft, namely on key components of the WRS. The magnitude of this challenge is different between missions to the Moon and Mars and it heavily depends on two things: orbital mechanics and mission architecture.

There are three major categories of trajectories that can be taken for Earth-Moon and Earth-Mars missions: (*i*) ballistic, (*ii*) low-thrust, and (*iii*) cyclers. Given that the second and third options take longer for the spacecraft to arrive to its destination, ballistic trajectories are the preferred option for crewed-mission planners. In the case of lunar missions, a ballistic trajectory requires a minimum 3.125 km/s velocity to leave Earth, and under these circumstances, it takes about 5 days (~120 h) to reach the Moon [28]. In fact, Apollo missions took between 66 and 90 h for the crew to arrive to lunar orbit. These short-duration flights enable the implementation of relatively low-complexity ECLSS, for example, dumping the urine outside the spacecraft instead of recycling it. One proposal being considered is for future lunar missions to stop at a Lunar Orbital Platform – (Gateway), a space station orbiting the Moon on a near-rectilinear halo orbit (https://www.nasa.gov/topics/moon-to-mars/lunar-gateway), before descending to the surface. Unlike the ISS, Gateway would not be planned to be permanently crewed but to receive ~1 month-long visits at least once per year. In contrast to the permanently inhabited ISS, the intermittent habitation on Gateway places unique challenges for its ECLSS; namely, surviving complete or partial dormancy periods during uncrewed phases. The next vehicle under this mission architecture is the lunar lander, a vehicle used to descend from Gateway to the surface and which will be used for short periods of time, therefore having relatively less stringent ECLSS requirements compared to Gateway. Finally, future crewed facilities on the lunar surface will likely be operational for years to decades, potentially starting with temporary and eventually transitioning to permanent inhabitation.

While the Moon is, on average, 384,400 km away from the Earth, Mars can be anywhere between ~55 million and ~225 million km away, depending on the relative position of the planets as they orbit the Sun. Mars-mission planners can choose from three subcategories of ballistic trajectories: (*i*) flyby, (*ii*) opposition class, or (*iii*) conjunction class; the latter two refer to the location of Earth and Mars with respect to themselves and the Sun in the middle of the mission. Conjunction class trajectories have shorter flight times, longer Mars stay times, and longer mission duration than opposition class [29]. The aspect of having shorter interplanetary transit durations makes this type of trajectory reduce crew exposure to deep space radiation and microgravity [30]. Given Earth's and Mars orbital mechanics, these types of missions can occur at a ~2 year frequency during specific launch periods. A mission that launches in July 2020 is used here as an example to describe representative durations. In this case, it would take seven months to arrive to the red planet, requires a 17-month stay (to wait for the planets to be in the desired locations), and a six-month return, for a total mission duration of around 30 months [31]. Shorter transfer times or mission durations are achievable at the expense of higher propulsion requirements [29]. The longer time away from Earth warrants higher systems reliability and reparability for Mars missions compared with those staying in low Earth orbit (LEO).

Regardless of the mission architecture chosen for a human mission to Mars, the ECLSS will need to be operable for at least ~30 months to sustain the crew. How long a given spacecraft's ECLSS needs to remain fully functional depends on the mission architecture, however. For example, a single-spacecraft approach for Earth-Mars transit, stay on Mars, and Mars-Earth transit, as proposed in Ref. [32], means that this spacecraft would need to remain completely viable for ~30 months. An architecture based on the use of a Mars transit vehicle to get to Martian orbit, a lander to descend to the surface, stay on the Martian surface in a habitat, return to Martian orbit on the same lander, and return to Earth on the same transit vehicle has its own, unique, ECLSS requirements. As currently envisioned at the time of writing this paper, the Mars transit vehicle needs to have an operable ECLSS for seven months during transit from Earth to Mars, be able to remain viable during a 17-month partial or total dormancy period and be active again for another six months during the return flight to Earth. Similarly, to the lunar lander, the Martian lander would have relatively-low complexity ECLSS requirements as it would not have to be closed-loop. The Martian habitat's ECLSS, however, shall remain fully functional for at least 17 months. All of this would be further exacerbated if these systems would need to be able to support more than one fast-transit mission – thus instead of being ~30 months, they would need to be operable for more than five years – and/or be reusable. We present a series of potential biofilm mitigation approaches that can be implemented on future interplanetary-transit spacecraft ECLSS – namely on the WRS due to the inherent sensitivity with biofilm growth, with a focus on the most stringent requirements from the previously described microgravity scenarios: Gateway and the Martian transit vehicle. The basis of this work comes from a NASA-Montana State University joint biofilm workshop, which took place in Bozeman, MT on July 18, 2019, and subsequent research into each topic discussed. This workshop provided a unique opportunity whereby representatives from NASA, the academic community, and industries with an impressive background in biofilm research could provide input. Potential control strategies for extended spaceflight missions were discussed, which were here grouped under six different categories. (*a*) Biocides; (*b*) Coatings to prevent fouling; (*c*) Ionizing radiation to reduce the microbial load; (*d*) Signal manipulation to either interfere with biofilm formation or induce biofilm detachment; (*e*) Biocontrols, in which viruses or other organisms may be employed to combat biofilms; (*f*) Removal of nutrients to inhibit microbial growth and associated biofilm formation; and (*g*) Other strategies including combining physical and chemical treatment or equipment replacement that might be considered. In the following sections, we will explore some of these concepts.

3. Biofilm control strategies

3.1. Biocides

There are two applications for biocides in the WRS, including the potable water and the wastewater. Each application has a fundamentally different role for the biocide function. In WPA product water, microbial growth is primarily maintained by the sterilization process in the WPA's catalytic reactor, the low organic content (typically less than 0.5 mg/L), and the stringent processing requirements implemented during assembly. As such, biofilm growth has not been an issue for the potable water plumbing. The function of the biocide is to provide residual microbial control in response to any atypical microbial presence. The ISS has historically used iodine as the potable water bus biocide via an iodinated resin initially developed by the Umpqua Research Company for use on NASA Space Shuttle program. This technology currently disburses 1–4 mg/L of iodine into the WPA product water [33]. New options such as silver are being considered as replacements for potable water [34]. While Ag nanoparticles have shown promise against biofilms in some tests (e.g. Ref. [35]), other investigators have shown that Ag nanoparticles can induce a change in *P. aeruginosa* biofilms to a non-culturable but metabolically active state, which reduces effectiveness [36]. Certainly, other biocides (shown in Table 2) could be considered, however the effectiveness in spacecraft as well as a low potential for volatile (potentially harmful) byproducts does need to be considered. In contrast, no biocide is currently used in the WPA wastewater (urine distillate and humidity condensate). During the July 2019 workshop at Montana State University, industrial and academic experts agreed that a biocide would be essential in maintaining biofilm control in the WRS of future NASA missions, though likely in conjunction with another method. Many variables must be understood before selecting a biocide for the wastewater application, such as material compatibility, effective concentration, shelf life, and kill spectrum. These variables are currently being reviewed on multiple biocides under consideration for use in the WPA wastewater tank (summarized in Table 2). Biocide impact on biofilm formation as a byproduct of planktonic cell growth disruption is the center for future technology analysis.

As Li et al. [79] explain in their literature review, silver biocides are broad spectrum and would require very little maintenance in long-duration missions. Aside from this, it is pointed out that combined physical and chemical methods, such as sonication and a biocide, would be sufficient to inhibit biofilm growth. Separately, chlorine and bromine have been biocidal options for the potable water bus and may also have application for the wastewater. However, iodine's lower vapor pressure and chlorine and bromine's ability to form byproducts are the main reasons for which iodine was chosen for the potable water bus. Nevertheless, chlorine and bromine remain common disinfectants used in industrial private and public water systems as regulated by the Environmental Protection Agency (EPA) [77,80,81]. Rodriguez et al. [82]. tested multiple metallic materials involved in the water processor assembly, such as corrosion resistant steel, titanium, and hastelloy, and some non-metallic polymer materials. The materials were tested against multiple biocides, some of them especially popular in the industrial use of clean rooms, mainly against spore-forming bacteria, such as peracetic acid, hydrogen peroxide, and sodium hypochlorite.

One of the most common biocidal treatments of water for microbial control is the use of oxidizing chemicals, which can be categorized as either halogenated or non-halogenated. The most typical halogenated oxidizing biocides employ chlorine or bromine. The addition of a chlorinated biocide to water creates a mixture of hypochlorous acid and hypochlorite ions where the disinfecting properties of the mixture are attributed to the hypochlorous acid portion. Biocides which depend on hypochlorous acid for disinfecting properties are most effective within pH ranges of 6.0–7.5. Since the stability of hypochlorous acid is pH dependent, the disinfecting properties are quickly lost at ranges of 8.0 and higher. Consideration should also be given to the residual organic

Table 2
Some of the biocides being considered for wastewater tank biofilm mitigation. Concentrations, kill-time, use and effectivity spectrum usually change by organism type in the literature.

Biocide	Concentration	Effectivity Spectrum	Common Use
Lysozymes and proteases [37,38]	Lysozyme is usually used in a concentration of 10mg/mL in 10mM Tris-Cl (pH 8.0). Stability of the aqueous solution is a problem. 10mg/mL = 10,000ppm.	Vegetative bacterial cells and some potency against non-vegetative cells	Lysozyme is used by academia to degrade bacterial cell wall peptidoglycan and spore cell walls prior to DNA purification and as a food cleaning product. Protease is used for lytic purposes in research, laundry detergents etc.
Tetrasodium EDTA [39]	4% of total volume	Bacterial cell wall, removes Mg2+ from gram negative outer membranes.	Chelating agent in cosmetics and personal care products as well as medical and veterinary equipment
Silver dihydrogen citrate [40,41]	1:80 dilution (30 ppm ionic silver)	Bacteria, fungi, and viruses	Commercial and residential disinfection/sanitization products; Deodorant active ingredient for personal care products; Antimicrobial active/preservative for personal care products; pharmaceutical; agriculture; industrial; biofilm control
Colloidal silver and AgF [34,42]	Maximum concentration of 400 ppb used for water disinfection in Russian module of ISS	Bacteria, fungi, and viruses	Commercial and residential disinfection/sanitization products; Deodorant active ingredient for personal care products; Antimicrobial active/preservative for personal care products; pharmaceutical; agriculture; industrial; biofilm control
Peracetic Acid [43]	Around 0.2% of total volume	Bacteria, fungi, bacterial spores, and viruses	Surface disinfection and sterilization
Hydrogen Peroxide [44,45]	Around 3% of total volume. A mixture of hydrogen peroxide and peracetic acid can be effective at 22% hydrogen peroxide and 4.5% peracetic acid	Bacteria, fungi, bacterial spores, and viruses	Mostly surface disinfections in the medical industry
Sodium bromide, dihydrate *[solid biocide]* [46]	*"about 10 to about 90% by weight of sodium chlorite; about 10 to about 90% by weight of sodium bromide; and about 5 to about 90%*	Bacteria, fungi, bacterial spores, and viruses	Mostly surface disinfections in the medical industry

(*continued on next page*)

Table 2 (*continued*)

Biocide	Concentration	Effectivity Spectrum	Common Use
	by weight of potassium monopersulfate" [Proprietary]		
Formaldehyde [47]	37% formaldehyde by weight in water. Disinfection activity seen with prolonged exposure to 4% (w/v) for 24h at room temperature	Bacteria, fungi, bacterial spores, algae, and viruses	Used in medical industry, however prolonged exposure >0.75 ppm for 8h associated with health risk (potential carcinogen and asthma-like respiratory problems)
Glutaraldehyde [47–52]	2% of total volume	Bacteria, fungi, bacterial spores, algae, and viruses	Mostly surface disinfections in the medical industry. Sometimes mixed with phenol and sodium phenate. Safety concerns with human exposure include skin irritation, mucous membrane irritation and respiratory irritation.
Isothiazolin [53, 54]	5–100 ppm	Bacteria and fungi	Used with high pH household and industrial cleaners. Also used in low concentrations (<15ppm) in personal care and cosmetic products
Sodium hypochlorite [55]	2% of total volume *"For free chlorine: When the pH values are within a range of 8 to 9, 0.*4 ppm *of chlorine must be added. When the pH values are within a range of 9 to 10, 0.*8 ppm *of chlorine must be added"*	Bacteria, fungi, bacterial spores, algae, and viruses	Surface and water disinfection
Cu^{2+} ions [56–60]	Copper concentration of 0.4–0.8 ppm	Bacteria, fungi, and viruses	Disinfection, antimicrobials, plant growth retardant and detergents
Quaternary ammonium compounds [47,61–64]	Around 200 ppm	Bacteria, fungi, and viruses	Medical industry, patient disinfection
Elemental iodine (I_2) [42,65]	1–5 ppm used to disinfect ISS drinking water in US module. Higher concentrations (~8ppm) used in some military applications	Bacteria, fungi, and viruses	Water disinfection and medical applications
Povidone-iodine [66–70]	Approximately 10% (w/v) solution used for skin antiseptic	Bacteria, fungi, and viruses	Skin and topical wound antiseptic, and surface disinfection
Chlorine [71,72]	"For free chlorine: When the pH values are within a range of 8–9, 0.4 ppm of chlorine must be added. When the pH values are within a range of 9–10, 0.8 ppm of chlorine must be added"	Bacteria, fungi, and viruses	Surface and water disinfection. Not as effective against protozoa.
Bromine [72–78]	0.5–220 mg/L of water	Bacteria, fungi, bacterial spores, and viruses	Surface and water disinfection.

content within the water, as hypochlorous acid will react with the organic content and deplete the disinfecting potential. Various forms of brominated biocides, such as bromine monochloride (BrCl), hypobromous acid (HOBr-) and bromodimethylhydantoin, have been applied as microbial control agents to treat water. Similar to chlorinated water treatments, a brominated biocide creates a mixture of hypobromous acid and hypobromite with the disinfecting portion being attributed to hypobromous acid, which is slightly more stable at increasing alkalinity, but begins to lose meaningful disinfectant efficacy at pH of 9.0 and higher. Hydrolysis of an activated bromide salt or bromine chloride will produce a mixture of hypobromous acid and hydrochloric acid with sodium chloride which can be utilized for water disinfection. Another chlorinated biocide which has been effectively employed to control microbial growth within water is chlorine dioxide (ClO_2). The major advantages of the use of chlorine dioxide include biocidal efficacy at broader pH ranges, improved stability within the presence of residual organic compounds, and efficacy at relatively low concentrations (e.g. <1 ppm ClO_2). Combining hydrochloric acid with a mixture of hypochlorite and sodium chlorite or mixing a sodium chlorite with a strong chlorine solution will produce chlorine dioxide in situ. Since ClO_2 gas can be explosive, the appropriate safety precautions should be considered when applying ClO_2 for microbial control of water. Ozone is another strong oxidizer employed to treat water systems for the purposes of microbial control. A major benefit to the use of ozone as a microbiocidal treatment of water systems is the lower potential of corrosivity compared to other oxidative chemistries. The factors which can negatively impact the disinfecting potential of ozone are pH, temperature, and organic content. Increasing levels of any of these factors may deplete the microbicidal effectiveness of ozone within water treatment applications.

Biocides are a commonly used strategy to combat biofouling. However, in the context of spaceflight several issues must be addressed. Mass restrictions would require biocides to be effective at low concentrations, to reduce payload mass. Alternatively, biocides, notably ozone, could be generated in situ, although equipment reliability would need to be addressed. Gaseous and volatile compounds would represent a potential safety risk to crew members in the event of an accidental release. This would be relevant to the original biocide as well as any chemicals that may result from biocide interactions with microorganisms or other compounds in the water [83]. At least one study has illustrated the impact of disinfectant exposure to promoting asthma exacerbation in susceptible health care workers [84]. Material compatibility and corrosion risks are described above. While biofilms are inherently tolerant to many antimicrobials, the potential for resistance to a single compound can be mitigated by the use of strategies employing multiple compounds [83].

3.2. Coatings

The utilization of surface coatings to prevent biofilm formation has been widely studied across a number of application areas. A review of literature from 1968 to 2010 using the quid software package (https://quid.com/) revealed 301 publications dealing with biofilm control and/or prevention on surfaces and representative technologies are summarized in Table 3. The areas of focus included fungal control of

Table 3
Listing of representative antibiofouling coating technologies in the literature. Strategies described include coatings used; potential for rechargeable coatings (to address need to regenerate antifouling surfaces); and incorporation of other strategies with antibiofouling coatings.

Coating	Characteristics	Effectiveness	Common Use
Representative coatings			
Metal ions (Ag^+, $Cu2^+$, tributyl tin) [87]	Release of Ag^+ or Cu^{2+} ions, or organic tin; and potential generation of reactive oxygen species.	Ag is used widely in biomedical and other applications. Cu is traditionally used in plumbing but susceptible to biofilm corrosion. Organic tin is an effective marine antifouling compound	Ag used widely in biomedical and other applications. Copper-containing biocides used in ship coatings. Tributyl tin is used in ship antifouling paint but has toxicity concerns.
Titanium alloys and mixtures [88,89]	Couples strength and corrosion resistance of Ti with antimicrobial properties of associated metals (e.g. Ag) and other compounds	Antimicrobial and biofilm prevention mainly due to materials added to Ti.	Used in medical and dental implants due to bone integration (osseointegration) corrosion resistance and low toxicity of Ti
Various synthetic polymers (e.g. polyethylene glycol (PEG), poly *N*-vinylpyrrolidone (PVP), zwitterionic materials [90]	Strategy is to inhibit surface adsorption of soluble proteins and other organic molecules onto surfaces (i.e. conditioning film prevention). Conditioning films normally promote biofilms.	PEG widely used in a number of situations. However, can be prone to oxidative damage.	PEG polymers used in a number of clinical trials. Other polymers including glycoproteins being investigated for biocompatibility and longevity.
Quorum signal disrupting chemicals and enzymes [91,92]	Interfere with or inactivate quorum signals, which are needed for biofilm growth	Common strategy by some organisms in nature, but larger scale studies needed to assess longevity, effectiveness, in different chemical conditions.	Some promising early results in experimental trials. Would need validation for use in long term spaceflight.
Surface modification by altering hydrophobicity [93]	Interferes with chemical interactions associated with initial bacterial adhesion.	Works well in lab situations with monocultures and defined bacterial strains. In complex chemical environments with mixed populations, not as effective.	May work in association with other technologies.
Silicone coatings [94]	Interferes with chemical reactions associated with initial bacterial adhesion or adsorption of proteins and other molecules (conditioning film)	Promising test results in food applications, although longevity after repeated use is not as apparent.	Promising initial trials, long term use is not apparent.
Slippage coatings (Lubricant-Impregnated Surfaces) [95–97]	Strategy is to reduce strength of adhesion of microorganisms to surface to promote detachment	Promising in initial trials with marine systems (ships) and long-term seawater immersion	Mechanical durability concerns as materials can be prone to shear forces.
Alterations of surface topography [98,99]	Several microscale alterations of surface topography (including shark skin similarities). May interfere with available adhesion points or interfere with surface mobility and bacterial aggregation.	Promising in some biomedically relevant trials with defined bacterial strains and culture conditions.	Technology has not been investigated in wastewater situation.
Rechargeable coatings	Antimicrobial characteristic capable of being regenerated by in situ chemical or physical treatment	Lab-based tests against model organisms show promise.	Based on literature, still in development stage, but notable potential
N-halamine [100,101]	Compounds contain nitrogen-halogen covalent bonds. Often used to coat polyurethane and other polymers	Similar disinfecting characteristics to hypochlorite. Regenerated by exposure to hypochlorite.	Experimental trials being conducted with various polymers. Safety concerns during spaceflight with potential Cl2 gas generation.
Silver nanoparticles [102–104]	Release of Ag + ions and potential generation of reactive oxygen species.	Used widely in biomedical and other applications.	Regeneration accomplished by cleaning surface with nitric acid then using AgNO3 to regenerate silver nanoparticles [104].
Coating coupled with other technology			
Copper and grooming [105]	Process whereby copper-based antifouling paint used on ships and is periodically cleaned using brushing or some other mechanical treatment.	Useful in control of macrofouling (i.e. barnacles) although microbial colonization can occur	Requires access to surfaces prone to biofouling, so likely not practical for spaceflight.
Aeration (bubble formation) and antifouling coating [106]	Aeration provides shear forces that mechanically remove loosely adherent biofilms	Shows promise in controlling macrofouling. Used in ships and also membrane bioreactors.	Phase separation requirements (air removal) not practical in microgravity.
Bioelectric or ultrasound augmentation of antifouling treatments [107,108]	Several mechanisms proposed including enhancement of biocide entry into biofilms, generation of reactive oxygen species or other electrochemically generated ions. Low intensity ultrasound enhances antimicrobial penetration, but in one study does not alter structure	Mixed results, depending on experimental conditions. Tests include attempts to prevent initial biofilm attachment, or removal of pre-existing biofilms	Lack of conclusive support would not merit investigations of this strategy during long-term spaceflight.

surfaces, efficacy of surface treatments on biofilm control, prevention of bacterial adhesion to surfaces, use of anti-fouling coatings to control biofilm within marine applications, biofilm control on medical devices, prevention of bio-influenced corrosion, and biofilm control within the paper industry, heat exchangers and within spaceflight systems. Fig. 1, analyzed by quid software (https://quid.com/) illustrates the relative density of publications within each of these applications of research over the past 51 years, as well as how closely these areas of biofilm control align with each other. Interestingly the cluster analysis of this literature record reveals the studies of coatings for biofilm control within spaceflight systems are not strongly aligned with other studies in this area. Fig. 2 illustrates how the number of studies related to biofilm control through the utilization of surface coatings has rapidly increased since the mid-1990s. The publications related to biofilm control within spaceflight systems were published between 1998 and 2010 [5,19,85,86]. Interestingly, while the studies into biofilm control within spaceflight systems fell off, an increasing number of studies focused on how surface coatings might play a role in controlling bacterial adhesion to surfaces, the efficacy of surface treatments, control of bio-fouling within marine environments, the impact of surface coatings to control biofilms on medical devices and the role of coatings to reduce bio-corrosion of surfaces. Investigations of biofilm control in other environments via improved surface-coating technology may help foster new research into the control of biofilms within spaceflight systems.

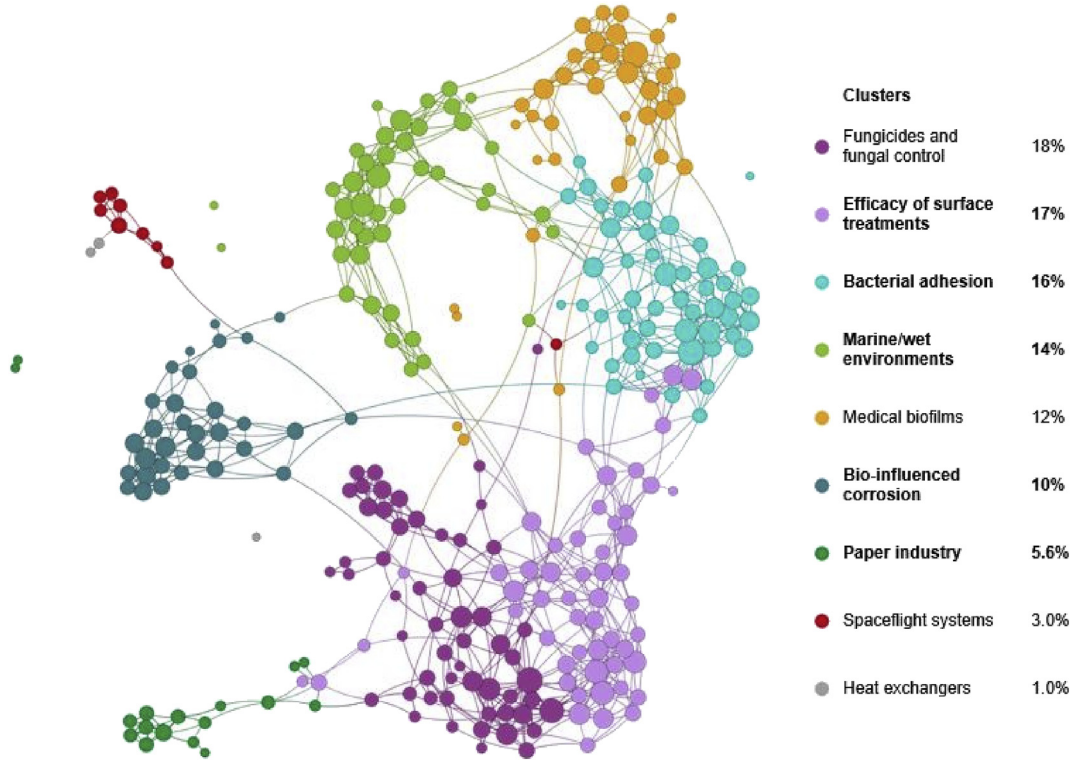

Fig. 1. Cluster analysis of publications on biofilm control and/or prevention on surfaces by quid software (https://quid.com/) from 1968 to 2019, showing the relative density of publications per application. This analysis indicates that studies of coatings for biofilm control within spaceflight systems are not strongly aligned to other applications.

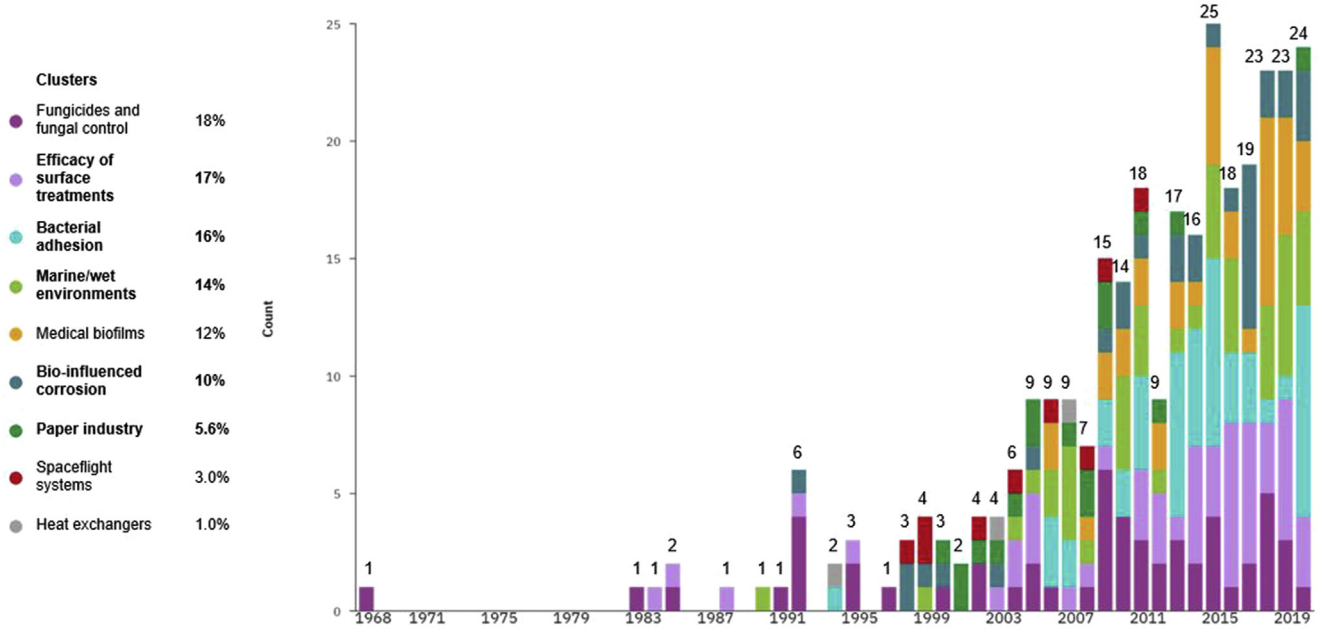

Fig. 2. Number of studies on the use of surface coatings to control biofilms showing a rapid increase in publications in the last two decades. Safeflight systems-related studies were published between 1998 and 2010.

Lubricant-impregnated surfaces (LIS), also known as slippage coatings, use the concept of the biofilm-resistant surfaces of the Nepenthes pitcher plant [96]. LIS incorporate both modifications of surface fine structure and the incorporation of a lubricant, so as to reduce initial adhesion of microorganisms and interfere with surface motility. In this technology a surface is roughened so that it can promote adhesion to a lubricating fluid. The lubricating fluid typically is immiscible with the liquid containing the microorganisms [97] so that it remains associated with the surface. LIS have shown promise in biofilm prevention in *P. aeruginosa* during lab culture [96,109] and the prevention of

biofilm-associated mineral deposition [110]. Recent investigations by Goodband et al. [97] showed that smoothing of the textured surface and loss of the oil lubricant diminished the effectiveness of LIS during prolonged use. There is an ongoing study currently underway on the ISS to investigate biofilm formation on different materials including LIS under microgravity conditions [22]. Another long-term experiment, conducted on the ISS from 2011 to 2016, investigated the relative susceptibility of various treated textile and metallic materials to biofilm formation [111]. Included in these tests were materials pre-treated with rhamnolipid biosurfactants, hydrogen peroxide, or silica and silver; as well as untreated materials (controls). Bacterial exposure resulted from the materials being exposed to cabin air followed by space crew members periodically touching or breathing on the various materials. Bacterial contamination was assessed by measurements of ATP levels, qPCR, and the composition of the microorganisms determined by 16S rRNA sequencing. These authors found low levels of organisms to be present and the organisms identified included the orders Actinomycetales, Bacillales, Enterobacteriales and Lactobacillales which agrees with previous studies of ISS flora [112–114]. Interestingly, pre-treatment of the materials did not have a significant benefit in terms of microbial load, however there were modest differences in microbial communities present.

One issue that is a concern with antibiofilm coatings is one of longevity. In the case of antimicrobial coatings that release active materials, there would be a finite time of effectiveness until the concentration of the inhibitory compound dropped beneath an effective level (reviewed in Refs. [115,116]). Another issue relates to the long-term mechanical and chemical stability of coatings. Certainly, a number of coatings give extremely promising results in the short term (e.g. Ref. [115]). However, the chemical and physical stability of prospective coatings may change over a prolonged period of time and diminish effectiveness [97]. Marine fouling is a global concern for shipping, and antifouling longevity and control mechanisms historically employed a variety of toxic, biocide-based antibiofouling compounds including tributyl tin and more recently copper- and zinc-based coatings [87]. Some of these biocide compounds, notably tributyl tin, have adverse environmental impacts; and due to toxicity considerations would be unsuitable for purification of drinking water ultimately intended for human use. Fouling release coatings including silicone and fluoropolymer coatings are being explored as an alternative marine biofouling control measure. The concept behind this approach is that modifications to surface chemistry or topography (i.e. sub-micrometer-scale patterns resembling shark skin or another pattern) either inhibit bacterial surface motility (aggregation into microcolonies) or reduce strength of adhesion [93,94,98,99]. In marine applications, fouling release coatings are typically employed along with physical approaches (removing loosely adherent microorganisms with brushing or some other mechanical approach). Biofilms are a major problem in the biomedical field being associated with medical device-associated infections including catheter-associated infections. Here, toxicity considerations for the human patient as well as effectiveness against biofilms are major considerations for the use [115]. Antimicrobial coatings are used in some cases, e.g. silver-containing urinary catheters [116], although the long-term effectiveness would be reduced due to silver leaching from the catheter. There have also been developments in the use of bacterial signal disrupting molecules (including furanones, nitric oxide, and other small molecules) (reviewed in Ref. [117]). Signal disruption does show considerable promise in biofilm prevention, but current technology in this area relies on the release of inhibitory compounds from coating materials and would have associated longevity concerns. In summary, the major issues confronting the use of coating technology involve the need for effectiveness over the anticipated length of the mission (3–5 years) as well as toxicity issues of released compounds in potable water, and chemical compatibility of the technology with other components of the WRS.

3.3. Ionizing radiation

Ionizing radiation, primarily ultraviolet (UV) light, has been used for some time to control microorganisms in wastewater [118]. With increasing drought and human population, a number of regions are now beginning to employ wastewater recycling as a key component of municipal drinking water. Aside from being used for non-potable uses such as crop and parkland irrigation, some recycled water is being used for potable water [119]. As well, UV light is used in many broad-distribution and also point of use water systems [120]. UV light is now frequently produced by UV-light-emitting diodes [120,121] and the most effective wavelengths ranging between 200 and 300 nm [120,122]. Nucleic acids absorb UV light around 260 nm [123] and one mechanism of cellular damage is the formation of cyclobutane pyrimidine dimers, notably thymine dimers that form between adjacent thymine residues on a single strand of DNA. Other UV-induced photoproducts also include binding of adjacent thymine and cytosine residues [124], as well as the formation of reactive oxygen species which can damage other cellular components and even result in small numbers of double-strand DNA breaks [125]. While bacteria do possess mechanisms for DNA repair, notably photoreactivation [124] and other repair mechanisms that do not require light but may be error-prone [125]; excess damage to nucleic acids and other key cellular components is lethal.

Higher energy ionizing radiation, notably gamma radiation, is used commercially as a sterilant, although considerable shielding is needed to protect humans working in the vicinity [126]. The higher energy of gamma radiation induces double-strand DNA breaks, damage to key proteins and lipids and generation of reactive oxygen species, all of which contributes to lethality [127]. A radiation dose of 25 kGy (2.5 Mrad) is used as a representative sterilization dose for materials to be used in a number of medical applications [128].

A number of factors influence the ability of ionizing radiation to reduce microbial populations. Turbidity certainly interferes with UV light penetration [118]. Organisms also vary in their capacity to repair radiation-induced DNA damage [127], so in that context it is not surprising that Hu et al. [129] observed a population shift in wastewater that was disinfected with UV. During spaceflight above the protective ozone layer of the Earth's atmosphere, solar radiation would be present, and may represent a natural source for disinfection. Table 4 describes series of experiments performed on the International Space Station (ISS) between 2008 and 2016 which examined the ability of various microorganisms to survive extraterrestrial UV radiation (summarized in Ref. [130]). During these experiments, organisms previously shown to be radiation resistant were exposed to solar radiation in low Earth orbit for 469 days (reviewed in Ref. [130]) and then returned to Earth for analysis. During the duration of the experiment, the UV flux ($\lambda = 100$ nm in one condition, and $\lambda =$ 200–400 nm in a second condition) was estimated between 4.58–4.92 x10^2 kJ/m^2 and 0.5 Gy of cosmic radiation [131]. In the first experimental condition, the organisms were exposed to space vacuum. In the second condition, organisms were exposed to simulated Mars light and atmospheric conditions. While biofilms did offer additional protection, the mechanism of the protection is not fully understood and may be due to altered pigmentation, matrix composition, or some unidentified mechanism [130]. Finally, in contrast to many biocides, UV does not have a residual effect in wastewater, meaning that biofilm growth is expected to continue in any region not directly exposed to the UV light.

In summary, ionizing radiation represents a potential mechanism whereby microbial populations could be reduced. However, a number of organisms have been shown to be resistant to UV-flux even under exposure to solar radiation (Table 4). There is at least one report showing sublethal doses of UVA (λ 365 nm, 25 W m^{-2}) enhanced biofilm formation under some culture conditions in *P. aeruginosa* PAO1 [134]. As a result, radiation if considered, would likely need to be combined with another approach such as biocide application for biofilm control.

Table 4
Summary of astrobiology experiments performed on the ESA EXPOSE facilities mounted outside the ISS.

Experiment and reference	Organisms present	Key objectives	Results
Biofilm Organisms Surfing Space (BOSS) using radioresistant organism [132]	*Deinococcus geothermalis*	Survival of biofilm and planktonic organisms exposed to space and Mars-like conditions	Desiccated *D. geothermalis* can survive in space and Mars-like conditions, with biofilms showing slightly better survival.
BOSS using cell aggregates of a cyanobacterium [133]	*Gloeocapsa* sp. and co-cultured α-proteobacteria isolate	Survival of biofilm and planktonic organisms exposed to space (including full solar spectrum at 1% light intensity) and Mars-like conditions.	Cell aggregates provide protection against UV radiation, and also provide a protective microhabitat for co-cultured α-proteobacterium
BOSS using desert isolates of *Chroococcidiopsis* spp [131].	*Chroococcidiopsis* spp.	Survival of biofilm and planktonic organisms exposed to space and Mars-like conditions and photosynthesis activity	Survival enhanced in biofilms compared with planktonic cells. Cells in bottom layer better preserved.

3.4. Biofilm detachment

During the formation of biofilms, bacteria go through several developmental stages (reversible and irreversible adhesion, aggregation and maturation, and finally dispersion) (reviewed in Refs. [135–137]) and there is evidence that biofilm-associated antimicrobial tolerance occurs at an early stage of biofilm development [138,139]. Biofilm control strategies typically address the first stages of biofilm formation (i.e. interfering with adhesion and aggregation) or involve various antimicrobial compounds (biocides and antibiotics) to combat established biofilms (Tables 2 and 3, addressed above). Adhesion interference strategies involve surface modification to reduce bacterial adhesion and coalescence into aggregates (biofilm microcolonies), or the development and testing of various antimicrobial compounds to combat established biofilms (addressed previously). One new approach that is being explored for biofilm control is an approach geared towards inducing biofilm detachment (final stage of the biofilm life cycle) [136,140–142]. When organisms leave biofilms and reenter the planktonic growth mode, antimicrobial susceptibility returns, although the rate of decline in biofilm-derived tolerance depends on the individual organisms as well as the process by which organisms leave biofilms (e.g. sloughing of cell populations, fragmentation of biofilms into individual cells, etc.) (reviewed in Refs. [136,143]). Both nutrient-based detachment stimuli [144–146] and specific detachment signals [147–149] have been proposed. While supplementation of some nutrients (e.g. succinate) have stimulated detachment in *Pseudomonas aeruginosa* [150], of more relevance to the proposed space mission is the potential role of starvation as several studies show that starvation induces detachment of biofilms (reviewed in Refs. [136,145]).

Biofilm detachment signaling is different from other types of signaling, notably quorum signaling (which is associated with biofilm formation) [136,151]. A key issue in biofilm physiology and associated genetic regulation is the role of a second signaling system, bis-(3′-5′)-cyclic dimeric GMP (c-di-GMP) [152]. During biofilm formation c-di-GMP levels become elevated approximately 3–4 fold when compared to planktonic cells due to an increase in diguanylate cyclase activity [153,154]. Among other things elevated c-di-GMP is associated with antimicrobial tolerance in biofilms and a loss of flagella. During detachment c-di-GMP levels are reduced by the activity of phosphodiesterase and as a consequence, bacteria within biofilms begin to lose antimicrobial tolerance, form flagella, and degrade the biofilm matrix prior to reentering the planktonic population. Most of these experiments have been performed under laboratory conditions and often employ monocultures. The advantage of a detachment-based strategy is the reduction of biofilm-mediated tolerance, while enabling current antimicrobial therapy. To the knowledge of the authors, no detachment experiments have been conducted during spaceflight (microgravity) or in microgravity analog experiments. Given the early stage of detachment research, it is likely premature to consider deliberate promotion of detachment as a biofouling control mechanism for extended spaceflight.

The most probable issue of biofilm detachment and population change is likely to occur during the dormancy phase. During the dormancy phase of a proposed spaceflight mission, exogenous nutrient input from space crew wastewater would cease, and the microbial community would initially rely on endogenous nutrients (use of metabolites and dead microorganisms) along with altered physiology, and then enter a period of starvation [155]. One would also anticipate that the population composition and physiology of both the biofilm and planktonic communities would change due to the stagnant water present during dormancy. Aside from nutrient limitation, oxygen consumption by microbial communities would potentially generate an anaerobic environment and alter microbial community composition, as has been shown in a domestic drinking water environment [156]. Detached biofilms may represent a potential clogging concern when ECLSS is reactivated following dormancy.

3.5. Biocontrol of biofilms

Control of microorganisms in anthropogenic water handling systems has been conventionally performed by the application of chemical biocides. However, with increased restrictions coming into place on the use of biocides and preservatives for industrial applications, there is an increasing interest into looking at naturally occurring or greener biocides. Biological control is defined as the “the use of a living organism to depress the population of an unwanted species or pest” has been practiced in the macro-biology world for many years [157,158]. But now there is a renewed interest in utilizing non-corrosion inducing and low slime producing microorganisms to combat the proliferation of other species that are considered harmful or damaging.

i. *Predatory Bacteria.* Bacterial predators such as *Bdellovibrio* and *Ensifer* have evolved a very unique survival strategy in which they obtain energy and other biosynthetic materials by taking them from other living bacteria. Sometimes described as a living antibiotic, they are considered a potentially safe alternative to antimicrobials for agriculture and water treatment applications [159,160]. There are four steps that must be completed for a bacterial cell to attack and consume another cell: (*i*) The predator bacteria finds its target prey, either through a chemotaxis mechanism or because the population of prey bacteria is sufficiently large so as to result in random collisions between the cells, (*ii*) the predator cell undergoes an irreversible interaction with the prey cell, (*iii*) the predator cell begins to degrade the prey cell by releasing specific macromolecules, (*iv*) the predator cell assimilates the released macromolecules which are used as nutrients in a specific and beneficial manner [161]. Similarly, there are different strategies for predation: (*i*) wolfpack or group predation, in which a number of predator cells release hydrolytic enzymes that degrade the cells of the near-by prey bacteria [162], (*ii*) epibiotic, individual cell to cell attack in which the predator bacteria attach to the outer surface of the prey cell, which assimilates the host molecules, (*iii*) direct invasion, where the predator bacteria enter the prey cell cytoplasm in a process called diacytosis, and (*iv*) periplasmic, where the predatory bacteria invade and grow within the periplasmic space found in Gram negative cells [161,163,164].

Unlike chemical biocide programs, researchers have shown that

predatory bacteria actually target prey bacteria in biofilms. For example, in the treatment of periodontitis, predatory bacteria were found to target and remove oxygen tolerant bacteria in the superficial layers of the biofilm and in the process expose anaerobic microorganisms deeper in the biofilm, making them susceptible to predatory attack [159,164]. The high bacterial densities within biofilms represents a very rich hunting ground for predatory bacteria. It is also interesting to note that in the Silva et al. [164] study, bacteria associated with gum health remained unaffected by the treatment. An additional advantage for the use of predatory bacteria for biocontrol is that the predatory bacteria can be non-pathogenic to humans.

Though predatory bacteria are viable for specific terrestrial applications, their usefulness for the NASA water treatment system may be limited. Predatory bacteria may not be effective against the entire microbial consortium in the WPA waste tank; thus, the remaining bacterial or fungal species would likely continue biofilm growth without competition from other microorganisms. A second consideration is whether predatory bacteria would maintain a functioning population in the WPA or if these organisms would need to be reinoculated. A third consideration is a question of whether predatory bacteria would also establish biofilms or enhance biofilm growth by prey bacteria. These considerations do not support the concept of biological control during spaceflight by predatory bacteria.

ii. *Bacteriophage.* A bacteriophage (phage) is a virus that infects and kills bacteria [165,166]. The bacteriophage attaches itself to a very specific site on the target bacterial cell wall and infects the host cell by injecting its DNA [167]. In doing this, the bacteriophage hijacks the host cellular machinery forcing it to make viral components, which ultimately form new bacteriophages. In the lytic cycle, new bacteriophages then lyse the cell, burst out of the host, and infect other bacteria. In terms of biofilm control and prevention, bacteriophages are known to have three different mechanisms. The first is a process where proteins known as EPS depolymerases are produced by the bacteriophage [168,169]. These enzymes break up the biofilm matrix through a chemical disruption mechanism. The second process involves bacteriophage infection of the bacteria within the biofilm causing direct cell lysing. The third process is where the cell walls are lysed as a result of the adsorption of phage virions and the onset of the phage lytic cycle [170].

While it has been shown that bacteriophage-induced lysis of targeted bacteria has the potential to break up biofilms, there are a number of limiting factors that may hinder this technology from being viable for NASA's biofilm control applications. Bacteriophages are generally specific to only one species of bacteria [171] so with biofilms that contain a large variety of different microorganisms a bacteriophage treatment would require a cocktail of bacteriophages to target each bacterial species in the biofilm [170]. Additionally, bacteriophage preparation requires culture of the phage with their host followed by a separation of bacterial cell remnants from the bacteriophage of interest. Phage titers can be measured using a bioassay (plaque-forming assay) or via quantitative PCR using phage-specific primers [172]. With the increasing onset of antibiotic resistance, phage therapy is being reexamined and would particularly need to be explored in a variety of environments (including wastewater), microbial growth conditions (bacteria tend to be more susceptible during active growth) and microbial populations including biofilms [173]. While one advantage of phage is that these viruses would replicate and so be able to reinfect their hosts, it is unclear whether resistance would develop. Resistance is certainly probable, given that bacterial biofilms and associated phage have co-evolved over several billion years [174].

iii. *Amoeba and other protozoa.* Amoeba are eukaryotic microorganism whose body most often consists of a single cell. Similar to other eukaryotic cells, their cytoplasm and cellular contents are enclosed within a cell membrane and their DNA is packaged into a central nucleus. Amoeba are known to consume bacteria and biofilm through a process known as phagocytosis. In this process receptors on the amoeba cell surface attach and bind to bacteria and are gathered and ingested within the amoeba. Larger amoeba will actually engulf their prey by gathering their pseudopods around the bacteria and ingest it in a process known as pseudopodia [175]. Other protozoa also ingest bacteria, although the specific mechanisms of ingestion may differ from those in amoeba [175,176]. As many protozoa routinely prey on bacteria, including biofilm-associated bacteria, there has been renewed interest in exploring amoeba and other protozoa as a biofilm control strategy. While this concept is certainly appealing several issues must be considered. Some bacteria, notably *Legionella pneumophila* and *Stenotrophomonas maltophila* have evolved mechanisms to persist within amoeba and presumably other protozoa wherein they can gain access to nutrients within the amoeba cytoplasm while inhibiting the host cell enzymatic digestive processes [177,178]. Several investigators have examined biofilm susceptibility to protozoa and other bacteria-ingesting organisms in monoculture and polymicrobial settings (e.g. Ref. [175,179,180]). While there has been some promise in pure culture (monoculture) lab trials, the results under more natural conditions are not as encouraging as individual microorganisms vary as to their susceptibility to predation by protozoa and other organisms. As well, bacteria, including those associated with biofilms, have been shown to evolve quickly and so the onset to increased protozoan resistance is certainly a possibility [180,181].

iv. *Tardigrades.* Tardigrades, commonly known as water bears, can be found in almost every habitat on Earth [182]. They are a phylum of small invertebrates that feed on the fluids from plant cells, animal cells, and bacteria. Their small size and relative ease to culture and obtain offspring make these hardy creatures an interesting target for biocontrol, particularly as at least two studies have shown their ability to survive radiation and temperature extremes during spaceflight [182,183]. A recent study showed that some metabolically-active tardigrades are heat sensitive [184]. However, there is limited research and development in the area of biofilm control and a thorough investigation into the use of tardigrades as a viable biofilm control strategy would need to be performed.

v. *Probiotics.* It is estimated that about 4 million adults in the United States use probiotics each month. Probiotics are live microorganisms intended to have health benefits but not induce disease, when consumed or applied to the body [185,186]. In the gastrointestinal tract, several strains of *Bacillus, Bifidobacterium, Escherichia coli, Lactobacillus,* and *Propionibacterium* have been used as probiotics (reviewed in Ref. [185]). Some probiotics, such as *Lactobacilli reuteri*, can produce a variety of compounds including reuterin and H_2O_2 that can react with biofilms, changing their structure and viability [187]. Other compounds produced by probiotics and normal flora can act as signaling molecules, which in some cases can promote biofilm growth. Examples of biofilm-promoting signals include quorum signals (QS) such as *N*-acylated homoserine lactones (QS in many gram negative bacteria) [188], small peptides (QS in many gram positive bacteria) [189] and autoinducer 2 (considered to be a universal QS in both gram positive and gram negative bacteria) [190], and polyamines [185]. However, the use of probiotics to control biofilms in water treatment system has not yet been systematically interrogated [187].

Each type of biocontrol has its limits and advantages, as summarized in Table 5. Further research will need to be done to evaluate the efficacy of these strategies and their potential for space applications. Biofilms are

Table 5
Examples of types of biocontrol and associated advantages and disadvantages.

Type of Biocontrol	Advantages	Disadvantages
Predatory Bacteria	Rapid growth Effective at removing resistant bacteria	Generally only attack Gram negative bacteria Not very active under anaerobic conditions
Bacteriophage	Non-toxic, many are well characterized Many produce EPS-degrading enzymes	Extremely specific Laborious culturing process
Amoeba and other protozoa	Preferentially thrive on biofilms	Ineffective against amoeba resistant bacteria
Tardigrades	Some may be extremely stress tolerant	Limited biofilm associated research available; stress tolerance is not conclusive
Probiotics	Well studied, effective at attacking health-related biofilms	Have potential to enhance new biofilm growth

now considered to be a very ancient form of life as they are associated within stromatolites from which fossils exist in the Precambrian era [191,192]. From an evolutionary standpoint, organisms within biofilms would have co-evolved with organisms (bacteriophage, other bacteria, eukaryotes, etc.) capable of preying on them. Perhaps the biggest challenge in biocontrol lies with the diversity of biofilms. Many of these biocontrol strategies are organism-specific, requiring previous knowledge on the composition of the biofilms being treated. One study with mixed *Escherichia coli* and *Pseudomonas aeruginosa* biofilms [171] observed that phage targeting one organism could be protected by the extracellular polymeric substance (EPS) produced by the non-target organism. Undoubtedly other mechanisms may also be involved. In summary, microbial competition or predation strategies may not be a suitable approach for control of spaceflight biofilms due to the complex interactions and probable evolution-based resilience of the polymicrobial populations present.

3.6. Chemical removal of nutrients

The composition of the wastewater (Table 6) can provide some insight into the amount of biomass that could be produced. The influent water stream contains organic carbon in the form of low molecular weight chemical species such as ethanol, acetic acid, 1,2-propanediol, and lactic acid [9]. These are all fine carbon sources to support microbial growth. Working from typical yield coefficients on similar carbon sources, the fouling potential of this stream can be estimated. For example, taking a typical biomass yield coefficient of 0.63 g biomass/g organic carbon for aerobic growth [193], the wastewater in Table 6 could support 72 mg/L of biomass if all of the carbon were consumed. In aerobic growth, oxygen is often limiting due to its relatively low solubility. For water saturated with oxygen in equilibrium with air at 1 bar, the oxygen concentration is approximately 8 mg/L. Taking a typical biomass yield of 0.39 g biomass/g oxygen [193], only 3 mg/L of biomass could be produced. This likely represents a lower bound on biomass production in the case of oxygen limitation because it assumes there is no additional aeration or entry of oxygen into the system. Obviously, many microorganisms are capable of anaerobic growth via anaerobic respiration (e.g. nitrate respiration) or fermentation and anaerobic microenvironments are likely within biofilm interiors (reviewed in Ref. [194]). Biological nutrient removal in systems having mixed aerobic and anaerobic conditions is reviewed elsewhere [195,196].

Table 6
Simplified approximate composition of water processor assembly (WPA) influent wastewater.

Constituent	Concentration (mg/L)	Concentration (μM)	Carbon to Element Molar Ratio
Total Organic Carbon (TOC)	114	9500	1.00
Ammonium (as N)	27	1930	4.9
Triethyl phosphate (as P)	0.083	2.68	3500
Sulfate (as S)	0.48	15.0	630

The known elemental composition of microbial biomass [197,198] provides a way to estimate the potential for limitation by other key constituents of biomass such as nitrogen, phosphorous, and sulfur. Certainly, micronutrients, notably iron and other key components are also required (e.g. Ref. [199]) but will not be addressed here. For the purposes of this preliminary inquiry, the average composition reported by Duboc et al. [200]; given by $CH_{1.728}O_{0.567}N_{0.169}P_{0.0184}S_{0.0032}$ will be used. By this formula, nitrogen is approximately 9.2% of biomass dry weight. If all of the ammonium in the wastewater were incorporated into biomass, the production of biomass would be 295 mg/L of microbial dry weight. This calculation suggests that nitrogen is present in excess and is unlikely to become limiting. If all of the phosphorous in triethyl phosphate, the predominant identified phosphorous source in the water, were converted into biomass, the approximate production of biomass would be just under 4 mg/L. If all of the sulfur in sulfate, the predominant identified sulfur source in the water, were converted into biomass, the approximate production of biomass would be 121 mg/L. Overall, these back-of-the-envelope calculations suggest that oxygen and phosphorous are most likely to limit microbial growth in the wastewater stream. These calculations are preliminary and should probably be considered less a definitive result than an illustration of an analytical strategy.

Researchers have found that nutrient availability, especially dissolved organic carbon along with phosphorous and nitrogen containing compounds, in the water or released from the materials used to construct the water handling system have played important roles in the establishment of biofilms of critical surfaces [201,202]. The molar ratio of carbon, nitrogen and phosphorous needed to allow microbial growth is approximately 100C: 10N: 1P [201]. With this being the case, removing or significantly reducing the availability of these nutrients should impact bacterial growth in the water and minimize biofilm formation. Volk et al. [202]. showed that by decreasing the dissolved organic carbon by half and coupling it with an ozone treatment resulted in a reduction in biofilm density that occurred over a 6 months period. We review current technologies that are available for chemical and physical removal of nutrients from water systems, which are summarized in Table 7. As well as the strategies shown here, source reduction of potential nutrients is also a viable option. Using terrestrial systems as an example, phosphate reduction in laundry detergents has lessened eutrophication in lakes and rivers receiving municipal wastewater [203].

i. *Removal of Biodegradable Dissolved Organic Carbon.* The concentration of Biodegradable Dissolved Organic Carbon (BDOC) is believed to be limiting to the growth and proliferation of bacterial species in water systems and therefore controlling the (BDOC) would benefit biofilm control by (i) reducing the microbial load, and (ii) reducing the parasitic demand on the biocide used in the treatment process.
ii. *Coagulation Processes.* A coagulation treatment process is one of the established methods for removal of BDOC from water. This chemical process uses the addition (either direct chemical addition or via an electrochemical dissolution of a metal anode) of soluble aluminum or iron based compounds to the water to facilitate the removal of a wide variety of materials including BDOC. Once in the water these dissolved compounds form colloidal species which then agglomerate into larger particles known as flocs. As the flocs form, they interact with the BDOC via complexation, precipitation, agglomeration and or adsorption mechanisms in which they are ultimately removed from the water through a clarification filtration process.

There are three different coagulation strategies (i) sweep flocculation, (ii) enhanced coagulation, and (iii) optimized coagulation [204]. With

Table 7
An overview of methods for BDOC reduction in water (modified from Ref. [204]).

Method	Effectiveness	Fraction(s) Targeted	Molecular Weight (MW) Range Targeted
Coagulation-based Systems			
Conventional	Moderate	Hydrophobic	All, particularly larger MW
Dissolved Air flotation (DAF)	Moderate	Hydrophobic	All
Direct Filtration	Moderate	Hydrophobic	All, particularly larger MW
Membrane-based Systems			
Microfiltration (MF) or ultrafiltration (UF)	Low		>10,000 Da
MF or UF. Coagulation	High	Hydrophobic	
Spiral-wound nanofiltration (NF) or reverse osmosis (RO)	High	All	>300 Da
Tubular NF	High	All	>300 Da
Oxidation-based Systems			
Ozone and Filtration	Moderate	Hydrophobic	All, particularly larger MW
Ozone + Slow Sand	Moderate		All, particularly larger MW
Advanced oxidation processes (AOPs)	Moderate	Hydrophobic	All, particularly larger MW
Adsorption-based Systems			
Activated Carbon	Low	Variable	Variable
Ion Exchange	High	Variable	Variable
Magnetic ion exchange (MIEX)	High	Variable	Variable

sweep flocculation the coagulant of choice is overdosed resulting in large amorphous flocs that encapsulate and capture not only the BDOC but microorganisms, heavy metals, and other contaminants. Enhanced coagulation, on the other hand, uses precise additions of the traditional coagulant to neutralize the charges normally present to keep the particles apart. Once these charges are neutralized the particles are encouraged to flocculate through agitation causing the particles to agglomerate so they can be removed by filtration or clarification [205]. Optimized coagulation is a technique similar to enhanced coagulation in which operational parameters such as pH control or the addition of bi-metallic nanoparticles along with polymer coagulants are added to maximize the efficiency of the coagulation process [206]. While all three methods have been shown effective the latter two are considered the most efficient.

The most common type of water treatment plant design is based around the "conventional" system where coagulation is followed by flocculation, settling, and finally filtration. While this method is effective at removal of turbidity, pathogens, and BDOC it is chemically intensive and is not suited to a microgravity type environment due to the requirement of gravity-assisted settling. Dissolved air flotation (DAF) is similar to the conventional process but the clarification process is accomplished using air to force flotation of the floc instead of sedimentation. Again, this method does not lend itself readily to a microgravity environment [207] since gravity is required for floc flotation and phase separation of the air bubbles. Finally, direct filtration can be used to clarify the water. In this process the treated water is passed through size exclusion media filters to remove the flocs. Unlike the other two methods direct filtration could be configured to be microgravity compatible. This method uses fewer chemical reagents than conventional systems as smaller floc sizes can be removed; however, filter fouling could be problematic, and this method might be questioned as increased backwashing of the filters would be required.

iii. *Membrane Filtration.* Membrane filters are common in the water treatment industry and they can be used in conjunction with the coagulation flocculation process or as a stand-alone treatment without any additional chemical treatment. There are 4 types of membrane processes: (i) Microfiltration (MF), (ii) Ultrafiltration (UF), (iii) Nanofiltration (NF), and (iv) Reverse Osmosis (RO). Both microfiltration and ultrafiltration are considered to be low pressure processes. MF filters have pore sizes ranging from 0.1 to 0.2 mm while UF filters have pore sizes ranging from 0.01 to 0.05 mm. The pores in the MF membrane are too large to reliably remove BDOC but UF membranes can remove some of the larger molecular weight BDOC compounds (20,000–100,000 MW) from water. These systems generally have a small footprint and low energy consumption; however, the inability to adequately remove BDOC makes them inadequate for effective nutrient removal. NF membranes and RO membranes are considered high-pressure and both are defined by their molecular weight cut off points. Both of these filter membrane systems have adequate pore size that can completely eliminate the passage of BDOC as well as metals and turbidity. However, they are easily fouled by both organic, inorganic, and biological mechanisms (i.e. biofilms) and are energy intensive while in operation [204].

iv. *Oxidation Based Systems.* Chlorine based oxidants, ozone, permanganate, and air are common oxidants used in the water treatment industry. While these chemicals primarily have been used to control the planktonic bacteria in the water, they will also oxidize larger particulate BDOC to smaller particles and molecules that can then be removed by filtration, slow sand filtration (which typically has associated biofilms [208]), or on activated carbon beds. The use of chlorine as an oxidant is considered problematic because of the potential to form harmful trihalomethane compounds. Ozone is a possible alternative to chlorine. It is a strong oxidant that will attack and break the double bonds in the more hydrophobic and aromatic BDOC species in the water. However, ozone is particularly difficult to handle in a microgravity environment as it requires an effective dispersion of microbubbles within the water to be effective [209]. It is also very reactive with rubber and yellow metals making it incompatible with the water processing and storage systems slated to be used on current and future space expeditions. Advanced oxidation processes (AOP) make use of the very reactive properties of hydroxyl radicals that are formed by the photocatalytic breakdown of ozone or hydrogen peroxide. UV-H_2O_2 systems have been shown to be more effective at eliminating BDOC than ozone treatment alone especially when used with an activated carbon filter. This method could be used in a microgravity environment; however, because of the short shelf life of hydrogen peroxide it would require an on-demand hydrogen peroxide generator and these systems are not currently commercially available.

v. *Adsorption and Ion Exchange.* There are numerous adsorbents that have been developed for the water treatment industry. These media work by attaching the species of interest to their surface through intramolecular forces. Granulated activated carbon (GAC) is known to be effective at removing most organics from water; however, pH and ionic strength of the solution can impact the efficacy of the process. For example, it was discovered that at pH 3 more BDOC will be adsorbed onto a GAC filter than will be adsorbed at pH 7 [210]. Adsorbents also tend to have limited capacity for the low molecular weight organics that are the primary nutrients for biofilm growth. Ion exchange is often referred to as "softening" and it works by preferentially replacing one ion from the water phase for another that is bound on the resin surface. Many of the organic species found in water are anionic in nature (they contain a carboxylic acid structure) and they can be removed by an anionic exchange resin. Once exhausted these resins are typically replaced with fresh resin, though they can also be recharged through the addition of salt allowing them to have a longer media lifetime. In recent years, a magnetic ion exchange resin (MIEX) system has been developed that uses beads rather than a traditional resin [211]. The presence of the magnetic beads

allows them to be easily removed and regenerated in a salt solution. While very effective at removing charged organics and inorganics from water, it is ineffective for uncharged compounds. Therefore, ion exchange would be used for removal of nitrogen, phosphorous, and sulfate-containing inorganic species that contribute to biofilm growth.

vi. *Removal of Nitrogen and Phosphorous Containing Compounds.* Table 8 lists the different techniques and methods that can be employed to remove nutrients such as phosphorous and nitrogen containing compounds. As can be seen in Table 8 many of the methods are similar to those described for the BDOC removal. Although chemical accumulation and precipitation are very effective for high phosphorous removal in terrestrial wastewater treatment, they are not appropriate for microgravity applications as settling would not occur. NF and RO membranes have been shown to produce concentrated nutrient effluent streams with over 80% retention of ammonia and nitrate in the concentrate.

One area of research and development that has gained considerable momentum is that of solid phase adsorbents for nitrate removal. Solid phase denitrification can be broken down into one of two processes, (i) heterotrophic denitrification, where bacteria use an external carbon source such as saw dust, wood chips, straw, and even dead and lysed cells as electron donors while nitrate functions as the electron acceptor reducing it to N_2, and (ii) autotrophic denitrification where the microbes fulfill their energy requirements by reducing nitrates using inorganic compounds as the electron donor species [212–214]. Researchers have demonstrated that using novel inorganic materials such as Mg/Cu bimetallic particles, nano zero valent iron (nZVI) and immobilized Pd/Cu catalysts when used in conjunction with adsorbents, and in some cases ion exchange resins, can improve nitrate reduction when compared to the system without these inorganic additives [215–218].

Biofiltration (reviewed in Ref. [208,219]) represent a mechanism whereby organic material and associated nutrients are removed by microbial communities growing as biofilms on a supporting matrix. Biofiltration may be coupled with other treatments such as ozonation to enhance the efficiency of the process [208]. During operation, microorganisms metabolize organic carbon and assimilate other nutrients including nitrogen and phosphorous, which leads to an increase of biomass (i.e. biofilms) that can clog the filtration system. Normally, the clogging is addressed by backwashing wherein the flow is reversed through the biofilter. Backwashing will remove some of the biomass and regenerate the filtration capability [220]. While biological assisted wastewater treatment including biofiltration is worthwhile investigating in the context of a long-term low gravity environment such as a future Moon or Mars base (addressed below in section G), the generation of biomass from backwashing or simple promotion of growth would create additional engineering problems in the context of space flight and is not a practical approach at present.

Bioelectrochemical systems represent another emerging technology for waste treatment and nutrient removal. The overall concept is that many metabolic reactions involve oxidation and reduction activities, and can be exploited to either generate a current, or else use an applied current to promote desired metabolic activities (reviewed in Refs. [221–223]). One example is the Enhanced Biological Phosphorous Removal (EBPR) process [222]. A key component of this process is the use of phosphate-accumulating organisms (PAO), which have the ability to sequester excess phosphate as intracellular polyphosphate granules in their cytoplasm [224]. The EBPR is an activated sludge process that relies on the ability of the PAO to take up, transform and store phosphate inside the cells. In these bioelectrochemical systems, iron released from the electrode and the electrochemical process frees up insoluble phosphates that are consumed by the PAO's contained in the activated sludge portion of reactor system, eliminating the phosphates from the water. Other methods to improve on the EBPR process as well as the denitrifying process is to combine microalgae with the bacterial consortium. This process allows for the biological removal of turbidity, nitrogen, phosphorous, and BOD/COD. This technology is suited to large industrial type applications where the algae can be grown in large ponds and fed to the treatment system when needed [225–227].

With magnetic separation adsorption materials tagged with magnetic particles are used as the collection and carrier material for removal of nitrates and phosphates in water. These adsorption materials are the same as the ones (GAC, MIEX) described in the previous section. High gradient magnetic separators are used to sequester the nutrient laden magnetic particles from the solution. These systems have been shown to be 90% effective at phosphorous recovery and do not interfere with other biological processes. However, this technology is still in the developmental phase and there is limited published literature available to fill in the knowledge gap.

In summary, removal of nutrients from water has the potential to reduce the formation and proliferation of biofilms in anthropogenic water handling systems. However, this approach has to be continuous and implemented as part of a long-term strategy. While for terrestrial applications these nutrient removal technologies could have huge environmental impact, for spaceflight applications the generation of waste and the high use of consumables, including water might make these technologies an impractical approach for biofilm control.

3.7. Other strategies

During the space biofilm symposium, several additional biofilm-related ideas were proposed. Many of these proposals involve considerable engineering challenges and payload requirements and as a result would be more appropriate for planned bases on the Moon or Mars, rather than being employed in spacecraft. One suggestion includes incorporating microorganisms into ECLSS. This would facilitate nutrient removal during wastewater recycling. If photosynthetic organisms were involved in this process, it would also contribute to oxygen generation and possibly food production. An example from the biological perspective, touched on in the previous section, is to use organisms (e.g. photosynthetic algae or bacteria) for nutrient removal. At least one genus of algae, *Chlorella,* is being investigated as an easily cultivable food supplement [228–230]. This could have additional positive results, such as CO_2 removal, O_2 production, and generation of edible biomass. Large-scale experimentation with biological nutrient cycling (e.g. Biosphere 2 and bioregenerative life support tests) has been done (reviewed in Refs. [231,232]) on Earth, and at the least feasibility studies would need to be performed in a low gravity condition (i.e. Moon base) prior to consideration for a potential trip to Mars. Another strategy

Table 8
Nutrient accumulation technologies for P and N removal from water.

Nutrient Accumulation	Engineering Feasibility	Technology Maturity	Operability	Operating Cost	Safety Issues
Algae Accumulation	Med: complex technology	Med	High	Low	Low
EBPR Accumulation	High	High	Med	Low	Low
Chemical Accumulation	High	High	High	High: chemical required	Low
Adsorption/Ion-exchange	Med	Low	Med	Med: require adsorbent	Low
Membrane Filtration	High	Med	Med	High: membrane clogging and cleaning cost	Low
Magnetic Separation	Low	Low	Low	Low	Low

considered is 'embracing biofilm growth', i.e. not only allowing biofilm formation but planning for it by inoculating tanks and water systems with a known microbial species. The concept of embracing biofilms is analogous to biofiltration (reviewed in Refs. [208,219]), addressed previously. If inoculation with a specific species or microbial community were to be investigated, notable criteria for the selection would be safety concerns for space crew and the strains that would be non-pathogenic and not have a deleterious effect on equipment. However, biofilms in nature tend to be polymicrobial, so the initial biofilm community could serve as the anchor or stimulus for other strains to attach to the equipment surface. Additionally, the presence of biofilms, regardless of the organisms that form it, would still elicit engineering problems for equipment downstream – e.g. valve clogging, biofouling, potential corrosion, etc. – that would need to be addressed. Given the engineering concerns (fouling, corrosion, etc.), the most reasonable strategy for spaceflight is to contain biofilms rather than promote them.

Alternative engineering and operational approaches may be implemented to mitigate the risks derived from biofilm buildup in spacecraft, namely on water processor assemblies. One strategy to consider is to maintain the components most prone to biofilm formation, e.g. wastewater tank and valves immediately downstream, at temperatures as close to 4°C as possible. While temperatures below freezing could be achieved, there would be a major risk of equipment damage (notably leaks) due to ice formation and expansion. This approach could result in reduced microbial proliferation, although multiple psychrotolerant bacterial and fungal species can still grow, albeit slower, in these conditions. Nevertheless, this approach may reduce biofilm formation to a level where it is less complicated to handle. Another strategy revolves around the idea of having regenerable or at least readily exchangeable filtration systems installed between the wastewater tank and the first valve downstream. However, a viable approach may be to implement a solution in which the biofilm growth is properly monitored, managed, and contained, thus preventing the release of biomass that can impact the mechanical function of the system. Indeed, ongoing experience with the WRS in the ISS provides valuable data for longer term missions.

Specifically, to the case of the Mars transit vehicle architecture, where ECLSS will stay dormant for a long period of time, in the event that a biofilm-prone component can be identified, one concept would be to replace the item. Using the wastewater tank as one example a novel 'two-flexible bags' approach to the wastewater tank is proposed as described in Fig. 3. By the end of the journey to Mars, the original wastewater bag (Bag 1) would remain full, acknowledging that biofilm will grow. After the period of dormancy and prior to return to Earth, the contents of the original wastewater are diverted into a filter that until this point had not been exposed to the contents of the tank, and flushed into a new wastewater bag, having biofilm constituents collected in the new filter (steps B and C). The new wastewater bag (Bag 2) would now contain mostly bacteria-free water. With this approach, the added volume of an empty and a full bag would be similar to that of one hard-shell tank. Challenges that would need to be addressed to implement this approach include pressure rating of the bags to withstand potential biological gas formation and fully disable gas permeating through the bag's walls.

4. Next steps for research and development

Access to data enables informed decisions and, in the case of biofilm problems in NASA's water treatment systems. Ongoing experience with the ISS has resulted in the identification of wastewater components (Table 6) and cultivable microorganisms (Table 1). One thing that is not known at present is the changes likely to occur during dormancy. Questions that would need to be addressed are:

i) Would changes in microbial community composition and physiology during dormancy and associated stagnant wastewater [233] affect key ECLSS components either directly or by generating an altered chemical environment?

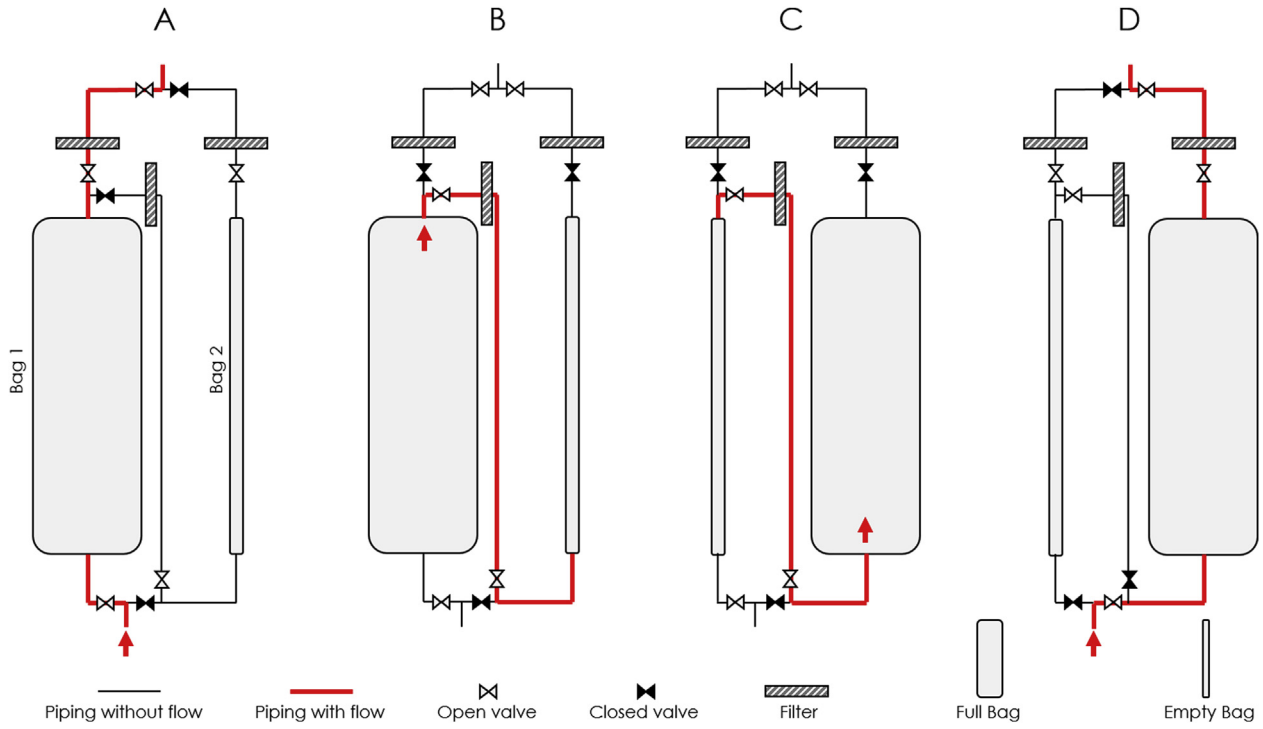

Fig. 3. Schematic and concept of operations of a proposed 'two-flexible bags' approach to the wastewater tank for future ECLSS. Instead of one hard-shell tank, this approach uses two flexible bags, where only one is completely full at any given time. Bag 1 is used for the Earth-to-Mars and Mars-orbit phases of mission (A) similarly to how the current wastewater tank is currently utilized. Before return to Earth, the contents of Bag 1 are transferred through a filter into an unused Bag 2 (B), resulting in the emptying of Bag 1 and the filling of Bag 2 (C), and the collection of biofilm in a filter that will no longer be used (vertical filter in schematic). The Mars-to-Earth mission phase uses the new Bag 2 as wastewater tank (D).

ii) Could the microbial issues be alleviated by enhancing biocide exposure either prior to or following dormancy; or alternatively draining susceptible valves and filter units prior to dormancy?
iii) Would changes in the microbiome and associated microbial physiology and metabolites occur in individual key components of the WPA prior to and during dormancy? This information would help identifying potential issues of concern.
iv) Would starvation induce biofilm detachment, and would this require additional filtration for released biomass during the ECLSS startup processes for a return flight to Earth?
v) Does prolonged growth in microgravity and anticipated increased radiation levels beyond low Earth orbit [234], affect biocide susceptibility of biofilm organisms?
vi) Would lowering cabin temperature during dormancy represent a potential strategy for decreasing microbial activity? Obviously, care would be needed to avoid damage due to freezing.
vii) Would the WRS return to an appropriate functioning level following dormancy, and would any specific measures (possibly equipment repair, filtration, chemical treatment) be needed to restore function (also mentioned in point iv, above)?

New engineering and operational approaches are recommended to be assessed for their effectiveness and impact on biofilm (a) detection, (b) formation inhibition, (c) detachment, and/or (d) filtration. We here describe them by posing key questions that need answers:

(a) **Detection.** How can we detect biofilms in tanks and other regions of the WPA as they are forming? How can we differentiate microbial presence in biofilms, from planktonic growth and can this data be used for assessing and adjusting biofouling control strategies and identification of potential problems?
(b) **Formation inhibition.** Which of the biocide options here described is most efficient and has the least impact on other WPA processes and in engineering requirements? What is the minimum biofilm inhibitory concentrations (MBIC) of these options in microgravity? Which surface coatings are the most efficient for this specific application, and for how long do they retain their functionality? Would an ionizing radiation approach be worth employing in addition to biocides for controlling microbial populations? Is cabin temperature reduction (e.g. to 4 C) during dormancy a viable option to slow biofouling? Finally, which method or combination of methods would be most effective in controlling biofouling, yet preserve the function and integrity of the WPA?
(c) **Detachment.** Would a programmed biofilm detachment strategy be appropriate? If so, would a signal-based approach or alternatively a combination physical (e.g. vibroacoustic, brushing or equivalent physical treatment) and biocide approach be warranted and if so, what parameters should be used?
(d) **Filtration.** Is filtration a viable option for controlling biomass including biofilms in the WPA? If so, what would be the mechanisms needed (e.g. filter location, filter pore size, monitoring and replacement schedule during outbound flight, dormancy, startup, and return flight to Earth)?
(e) **Equipment repair or replacement.** Is equipment replacement a viable option to address biofouling concerns during dormancy (example of wastewater tank is described in Fig. 3 and adjoining text)? Aside from the tank, are other components identified as "at risk" for biofouling damage, and if so, should replacements be carried?

5. Conclusions and future directions

Biofilms are certainly present on human-occupied spacecraft and have been associated with problems associated with life support and other equipment. Biofouling problems are not exclusive to systems operation on Earth, as the Russian Salyut 6 and 7, and Mir space stations, as well as the ISS had engineering challenges arise from biofilm buildup [8–10,21,22]. In the case of the ISS, the WPA has presented the most pressing challenges that need to be addressed before human exploration moves forward to Mars and beyond. In these extended duration missions, there are planned periods of ECLSS dormancy, where wastewater will stay stagnant in a tank for periods of months or years. While biofilms could certainly impact other aspects of a spacecraft, the primary focus of the NASA-Montana State University biofilm workshop and this review paper is on biofilm control strategies in the WPA during long-duration space flight.

The microorganisms associated with the WPA in the International Space Station is frequently monitored (addressed in Section 1 and Table 1) and there have also been some culture-independent studies performed as well (e.g. Ref. [11,12]). Previous biofilm studies performed in space, have shown differences in morphology, sensitivity to disinfectants, viability, biomass, and cell counts compared to matching Earth controls. With the exception of the Biofilm Surfing in Space (BOSS) studies Table 4 (reviewed in Cottin and Rettberg [130]), most spaceflight biofilm studies have been conducted over short periods of time with a small number of model microorganisms that have been typically been grown in monoculture. While these studies described in previous sections are beneficial, a key research requirement is understanding the changes that may occur in the WPA flora during planned dormancy and startup procedures. A recent report on the ISS by the National Academies proposed the development of a microbial observatory as a high-priority research item [235] and certainly long-term changes in biofilm communities related to space crew health, life support systems and spacecraft integrity in the unique spaceflight environment would be a key beneficial scientific objective. Given payload restrictions during a potential Earth-Mars flight, the use of biocides for controlling WPA biofilms appears to be the most relevant technology to be considered and details are presented in section 4, items i-vii. Related engineering and operation issues are also addressed in section 4.

While this article focuses on biofilm-related problems and control strategies that impact an Earth-Mars transit vehicle operating under microgravity conditions; biofilm-related issues would also be relevant to life support systems and other facilities during an extended stay on Mars or on the Moon. Microgravity analog devices (described in the introduction) greatly facilitate the number of investigations that can be done. Biofilm growth and biofouling would be anticipated in partial gravity conditions (Moon 0.17g; Mars 0.38 g) [27], but detailed experiments would need to be conducted in order to identify potential risk factors and mitigation strategies. Modeled partial gravity can now be modeled on Earth using RP device technology [27] and RWV (clinostat) technology [236–239], but longer-term tests would await *in situ* lunar testing. The rapid advancement of technology allows an increasing number of rigorous experimental protocols and equipment fabrication to be done during flights, which enhances the crew flexibility during prolonged mission. Two examples are gene sequencing [240] and three-dimensional printing (3D printing) [241]. We anticipate that ongoing technical and engineering developments along with biofilm research will enhance the success of future extended, crewed, space missions.

Acknowledgements

The authors thank all of the participants of the joint NASA-Montana State University Biofilm Workshop, and these two institutions which supported it. We do apologize to a number of outstanding investigators in space biology and biofilm research for omitting some references. We thank Misty Nosal from NASA's Marshall Space Flight Center (MSFC) for her insight into the mission architecture of the Lunar Gateway; Dr. Damon Landau from the Jet Propulsion Laboratory and Prof. Aaron Rosengren from the University of Arizona for their expert advice on Earth-Mars trajectories for crewed missions; and Shawn Reagan (MSFC)

for providing institutional support. Finally, we appreciate the helpful comments from the reviewers. LZ and RJCM also gratefully acknowledge research support from NASA (80NSSC17K0036 (LZ) and (NNX17AC79G) (RJCM)).

References

[1] McLean RJC, Beveridge TJ. Metal binding capacity of bacterial surfaces and their ability to form mineralized aggregates. In: Ehrlich HL, Brierley CL, editors. Microbial mineral recovery. New York: McGraw-Hill; 1990. p. 185–222.

[2] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010;8(9): 623–33. https://doi.org/10.1038/nrmicro2415.

[3] Purevdorj B, Costerton JW, Stoodley P. Influence of hydrodynamics and cell signaling on the structure and behavior of *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 2002;68(9):4457–64.

[4] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284:1318–22.

[5] Gu JD, Roman M, Esselman T, Mitchell R. The role of microbial biofilms in deterioration of space station candidate materials. Int Biodeterior Biodegrad 1998;41(1):25–33.

[6] McLean RJC, Cassanto JM, Barnes MB, Koo J. Bacterial biofilm formation under microgravity conditions. FEMS Microbiol Lett 2001;195(2):115–9.

[7] Pyle BH, McFeters GA, Broadaway SC, Johnsrud SC, Storga RT, Borkowski J. Bacterial Growth on surfaces and in suspensions. In: Biorack on spacehab. Biological experiments on Shuttle to Mir missions 03, 05, and 06. European Space Agency; 1999.

[8] Klintworth R, Reher HJ, Viktorov AN, Bohle D. Biological induced corrosion of materials II: new test methods and experiences from MIR station. Acta Astronaut 1999;44(7–12):569–78.

[9] Carter DL, Pruitt JM, Brown C, Bazley J, Gazda D, Schaezler R, Thomas F. *Status of ISS water Management and recovery* (2017-036). In: Presented at 47th international conference on environmental systems, Charleston, SC; 2017.

[10] Weir N, Wilson M, Yoets A, Molina TC, Bruce RJ, Sitler G, Carter DL. Microbiological characterization of the international space station water processor assembly external filter assembly S/N 01. In: Paper presented at the 42nd international conference on environmental systems (ICES), San Diego, CA; 2012.

[11] Lang JM, Coil DA, Neches RY, Brown WE, Cavalier D, Severance M, et al. A microbial survey of the international space station (ISS). Peer J 2017;5:e4029. https://doi.org/10.7717/peerj.4029.

[12] Singh NK, Wood JM, Karouia F, Venkateswaran K. Succession and persistence of microbial communities and antimicrobial resistance genes associated with International Space Station environmental surfaces. Microbiome 2018;6(1):204. https://doi.org/10.1186/s40168-018-0585-2.

[13] Doll S, Eckart P. Environmental control and life support systems (ECLSS). In: Larson WK, Pranke LK, editors. Human spaceflight: mission analysis and design. New York: McGraw-Hill; 2000.

[14] Perrin Y, Bouchon D, Delafont V, Moulin L, Héchard Y. Microbiome of drinking water: a full-scale spatio-temporal study to monitor water quality in the Paris distribution system. Water Res 2019;149:375–85. https://doi.org/10.1016/j.watres.2018.11.013.

[15] Garner E, Inyang M, Garvey E, Parks J, Glover C, Grimaldi A, et al. Impact of blending for direct potable reuse on premise plumbing microbial ecology and regrowth of opportunistic pathogens and antibiotic resistant bacteria. Water Res 2019;151:75–86. https://doi.org/10.1016/j.watres.2018.12.003.

[16] Yang HH, Urban PL. Dry ice fog extraction of volatile organic compounds. J Chromatogr A 2019;1585:196–201. https://doi.org/10.1016/j.chroma.2018.11.052.

[17] Zea L, Prasad N, Levy SE, Stodieck L, Jones A, Shrestha S, et al. A molecular genetic basis explaining altered bacterial behavior in space. PloS One 2016; 11(11). ARTN e0164359 10.1371/journal.pone.0164359.

[18] Zea L, Larsen M, Estante F, Qvortrup K, Moeller R, de Oliveira SD, et al. Phenotypic changes exhibited by E-coli cultured in space. Front Microbiol 2017;8. ARTN 1598 10.3389/fmicb.2017.01598.

[19] Hodges MP, Woodard D, Roberts MS. Utilization of aminosilane antimicrobial coatings in spacecraft potable and technical water systems. https://doi.org/10.4271/2007-01-3141; 2007.

[20] Kim W, Tengra FK, Young Z, Shong J, Marchand N, Chan HK, et al. Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa*. PloS One 2013;8(4): e62437.

[21] Yang J, Thornhill SG, Barrila J, Nickerson CA, Ott CM, McLean RJC. Microbiology of the built environment in spacecraft used for human flight. In: Gűrtler V, Trevors JT, editors. Microbiology of atypical environments. Elsevier; 2018. p. 3–26.

[22] Zea L, Nisar Z, Rubin P, Cortesao M, Luo J, McBride SA, et al. Design of a spaceflight biofilm experiment. Acta Astronaut 2018;148:294–300. https://doi.org/10.1016/j.actaastro.2018.04.039.

[23] van Loon JJWA. Some history and use of the random positioning machine, RPM, in gravity related research. Adv Space Res 2007;39(7):1161–5.

[24] Wuest SL, Richard S, Kopp S, Grimm D, Egli M. Simulated microgravity: critical review on the use of random positioning machines for mammalian cell culture. BioMed Res Int 2015;2015:971474. https://doi.org/10.1155/2015/971474.

[25] Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL. Microbial responses to microgravity and other low-shear environments. Microbiol Mol Biol Rev 2004;68(2):345–61. https://doi.org/10.1128/MMBR.68.2.345-361.2004.

[26] Castro SL, Niesel DW, Barrila J, Ott CM. Spaceflight and spaceflight analogue induced responses in Gram positive bacteria. In: Nickerson CA, Pellis NR, Ott CM, editors. Effect of spaceflight and spaceflight analogue culture on human and microbial cells. New York: Springer; 2016. p. 283–97.

[27] Manzano A, Herranz R, den Toom LA, Te Slaa S, Borst G, Visser M, et al. Novel, Moon and Mars, partial gravity simulation paradigms and their effects on the balance between cell growth and cell proliferation during early plant development. npj Microgravity 2018;4:9. https://doi.org/10.1038/s41526-018-0041-4.

[28] Boden DG, Hoffman SJ. Orbit selection and astrodynamics. In: Larson WJ, Pranke LK, editors. Human spaceflight: mission analysis and design. McGraw-Hill; 1999.

[29] Landau DF, Longuski JM. Trajectories for human missions to Mars, Part I: impulsive transfers. J Spacecraft Rockets 2006;43(5):1035–42. https://doi.org/10.2514/1.18995.

[30] Mattfeld B, Stromgren C, Shyface HR, Komar DR, Cirillo WM, Goodliff KE. Trades between opposition and conjunction class trajectories for early human missions to Mars. San Diego, CA: Paper presented at the American Institute of Aeronautics and Astronautics; 2014.

[31] Larson WJ, Pranke LK. Human Spaceflight: mission analysis and design. McGraw-Hill; 1999.

[32] Musk E. Making humans a multi-planetary species. New Space 2017;5(2):46–61. https://doi.org/10.1089/space.2017.29009.emu.

[33] Gazda DB, Lipert RJ, Fritz JS, Porter MD. Investigation of the iodine–poly(vinylpyrrolidone) interaction employed in the determination of biocidal iodine by colorimetric solid-phase extraction. Anal Chim Acta 2004; 510(2):241–7. https://doi.org/10.1016/j.aca.2004.01.010.

[34] Wong WC, Dudinsky LA, Garcia VM, Ott CM, Castro VA. Efficacy of various chemical disinfectants on biofilms formed in spacecraft potable water system components. Biofouling 2015;26(5):583–6.

[35] Shah S, Gaikwad S, Nagar S, Kulshrestha S, Vaidya V, Nawani N, et al. Biofilm inhibition and anti-quorum sensing activity of phytosynthesized silver nanoparticles against the nosocomial pathogen *Pseudomonas aeruginosa*. Biofouling 2019;35(1):34–49. https://doi.org/10.1080/08927014.2018.1563686.

[36] Königs AM, Flemming H-C, Wingender J. Nanosilver induces a non-culturable but metabolically active state in *Pseudomonas aeruginosa*. Front Microbiol 2015;6. https://doi.org/10.3389/fmicb.2015.00395. 395-395.

[37] Ninfa AJ, Ballou DP, Benore M. Fundamental laboratory approaches for biochemistry and biotechnology. 2 ed. Wiley; 2009.

[38] Willsey GG, Wargo MJ. Extracellular lipase and protease production from a model drinking water bacterial community is functionally robust to absence of individual members. PloS One 2015;10(11):e0143617. https://doi.org/10.1371/journal.pone.0143617.

[39] Finnegan S, Percival SL. EDTA: an antimicrobial and antibiofilm agent for use in wound care. Adv Wound Care 2015;4(7):415–21. https://doi.org/10.1089/wound.2014.0577.

[40] Arata, A. (2003). Silver dihydrogen citrate compositions comprising a second antimicrobial agent. USA Patent No. US20050202066A1.

[41] Pure Bioscience Inc. Silver dihydrogen citrate, technical data sheet. https://www.purebio.com/assets/001/5202.pdf; 2011.

[42] Gardner DE, Brady JV, Carlson GP, Faustman EM, Feigley CE, Gaulden ME, et al. Sources, treatment, and monitoring of spacecraft water contaminants. In: Methods for developing spacecraft water exposure guidelines. Washington DC: National Academy Press; 2000. p. 14–56.

[43] CDC. Peracetic acid sterilization. Atlanta, GA: CDC Retrieved from; 2008c. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/peracetic-acid.html.

[44] CDC. Hydrogen peroxide. Atlanta, GA: The National Institute for Occupational Safety and Health (NIOSH); 2019. CDC Retrieved from, https://www.cdc.gov/niosh/topics/hydrogen-peroxide/default.html.

[45] Mar Core Purification Inc. Minncare cold sterilant, technical data sheet. http://www.mcpur.com/main/library/12_brochures/3028402_(Minncare_App_Notes_Calculating).pdf; 2014.

[46] Sanderson, W. D. (2008). Solid biocide composition and sealed biocide article. US Patent No. US20080299161A1.

[47] CDC. Chemical disinfectants. Guideline for disinfection and sterilization in healthcare facilities. Atlanta, GA: CDC Retrieved from, https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html#Formaldehyde; 2008a.

[48] Fink J. Petroleum engineer's guide to oil field chemicals and fluids. 2 ed. Boston: Gulf Professional Publishing; 2015.

[49] Keasler V, De Paula RM, Nilsen G, Grunwald L, Tidwell TJ. Biocides overview and applications in petroleum microbiology. In: El-Sherik AM, editor. Trends in oil and gas corrosion research and technologies. Boston: Woodhead Publishing; 2017. p. 539–62.

[50] McKeen L. Introduction to food irradiation and medical sterilization. In: McKeen L, editor. The effect of sterilization on plastics and elastomers. third ed. Boston: William Andrew Publishing; 2012. p. 1–40.

[51] Pal K, Paulson AT, Rousseau D. Biopolymers in controlled-release delivery systems. In: Ebnesajjad S, editor. Handbook of biopolymers and biodegradable plastics. Boston: William Andrew Publishing; 2013. p. 329–63.

[52] Stuart MC, Kouimtzi M, Hill SR. World health organization (WHO) model formulary 2008. Geneva: World Health Organization; 2009.

[53] Karsa DR. F.2 - biocides. In: Johansson I, Somasundaran P, editors. Handbook for cleaning/decontamination of surfaces. Amsterdam: Elsevier Science B.V; 2007. p. 593–623.

[54] Taubert K, Kraus S, Schulze B. Isothiazol-3(2H)-Ones, Part I: synthesis, reactions and biological activity. Sulfur Rep 2002;23(1):79–121. https://doi.org/10.1080/01961770208047968.

[55] CDC. Frequently asked questions (FAQs) about sodium hypochlorite solution(SH). Atlanta, GA: CDC Retrieved from, https://www.cdc.gov/safewater/chlorination-faq.html; 2014.

[56] Borkow G, Gabbay J. Copper as a biocidal tool. Curr Med Chem 2005;12(18):2163–75. https://doi.org/10.2174/0929867054637617.

[57] CDC. Background D. Water. Guideline for disinfection and sterilization in healthcare facilities. Atlanta, GA: CDC Retrieved from, https://www.cdc.gov/infectioncontrol/guidelines/environmental/background/water.html; 2003.

[58] Gant VA, Wren MW, Rollins MS, Jeanes A, Hickok SS, Hall TJ. Three novel highly charged copper-based biocides: safety and efficacy against healthcare-associated organisms. J Antimicrob Chemother 2007;60(2):294–9. https://doi.org/10.1093/jac/dkm201.

[59] Hartshorn SR, Thind SS. 1.15 - heterocyclic compounds as additives. In: Katritzky AR, Rees CW, editors. Comprehensive heterocyclic chemistry. Oxford: Pergamon; 1984. p. 393–411.

[60] Karlsson HL, Toprak MS, Fadeel B. Chapter 4 - toxicity of metal and metal oxide nanoparticles. In: Nordberg GF, Fowler BA, Nordberg M, editors. Handbook on the toxicology of metals. fourth ed. San Diego: Academic Press; 2015. p. 75–112.

[61] Fu E, McCue K, Boesenberg D. Chemical disinfection of hard surfaces – household, industrial and institutional settings. In: Johansson I, Somasundaran P, editors. Handbook for cleaning/decontamination of surfaces. Amsterdam: Elsevier Science B.V; 2007. p. 573–92.

[62] Jia Z, shen D, Xu W. Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydr Res 2001;333(1):1–6. https://doi.org/10.1016/s0008-6215(01)00112-4.

[63] Rutala WA, Weber DJ. Disinfection, sterilization, and control of hospital waste. In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. eighth ed., 2. Philadelphia: Elsevier/Saunders; 2015. p. 3294–309.

[64] Scott VN, Walls I. Laboratory management | microbiological safety. In: Caballero B, editor. Encyclopedia of food sciences and nutrition. second ed. Oxford: Academic Press; 2003. p. 3443–9.

[65] Chang SL, Morris JC. Elemental iodine as a disinfectant for drinking water. Ind Eng Chem 1953;45(5):1009–12. https://doi.org/10.1021/ie50521a042.

[66] CDC. Iodophors. Guideline for disinfection and sterilization in healthcare facilities. Atlanta, GA: CDC Retrieved from, https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html#Iodophors; 2008b.

[67] Gershenfeld L. Povidone-iodine as a sporicide. Am J Pharm Sci Support Public Health 1962;134:78–81.

[68] Kawana R, Kitamura T, Nakagomi O, Matsumoto I, Arita M, Yoshihara N, et al. Inactivation of human viruses by povidone-iodine in comparison with other antiseptics. Dermatology 1997;195(suppl 2):29–35. https://doi.org/10.1159/000246027. Suppl. 2.

[69] Koerner JC, George MJ, Kissam EA, Rosco MG. Povidone-iodine concentration and in vitro killing time of bacterial corneal ulcer isolates. Digit J Ophthalmol 2018;24(4):24–6.

[70] Kondo S, Tabe Y, Yamada T, Misawa S, Oguri T, Ohsaka A, et al. Comparison of antifungal activities of gentian violet and povidone-iodine against clinical isolates of *Candida* species and other yeasts: a framework to establish topical disinfectant activities. Mycopathologia 2012;173(1):21–5. https://doi.org/10.1007/s11046-011-9458-y.

[71] Meireles A, Ferreira C, Melo L, Simões M. Comparative stability and efficacy of selected chlorine-based biocides against *Escherichia coli* in planktonic and biofilm states. Food Res Int 2017;102:511–8. https://doi.org/10.1016/j.foodres.2017.09.033.

[72] Soracco RJ, Wilde EW, Mayack LA, Pope DH. Comparative effectiveness of antifouling treatment regimes using chlorine or a slow-releasing bromine biocide. Water Res 1985;19(6):763–6. https://doi.org/10.1016/0043-1354(85)90124-1.

[73] Choudhury, P., Davis, R. L., Sanders, M. J., & Roark, D. N. (2000). Sulfamate stabilization of a bromine biocide in water. USA Patent No. US6110387A.

[74] Franklin MJ, Nivens DE, Vass AA, Mittelman MW, Jack RF, Dowling NJE, et al. Effect of chlorine and chlorine/bromine biocide treatments on the number and activity of biofilm bacteria and on carbon steel corrosion. Corrosion 1991;47(2):128–34. https://doi.org/10.5006/1.3585228.

[75] Moore, R. M. Jr, & Nalepa, C. J. (2001). Biocidal applications of concentrated aqueous bromine chloride solutions. US Patent No. US 6322822B1.

[76] Walker JT, Rogers J, Keevil CW. An investigation of the efficacy of a bromine containing biocide on an aquatic consortium of planktonic and biofilm micro-organisms including *Legionella pneumophila*. Biofouling 1994;8(1):47–54. https://doi.org/10.1080/08927019409378259.

[77] WHO. Alternative drinking-water disinfectants: bromine, iodine and silver. Geneva: World Health Organization; 2018.

[78] Yang, S., McCoy, W. F., Allain, E. J., Myers, E. R., & Dallmier, A. W. (2001). Stabilized bromine solutions, method of manufacture and uses thereof for biofouling control. USA Patent No. US6270722B1.

[79] Li W, Calle LM, Hanford AJ, Stambaugh I, Callahan MR. Investigation of silver biocide as a disinfection technology for spacecraft – an early literature review. In: Paper presented at the 48th international conference on environmental systems, Albuquerque, NM; 2018.

[80] Kanan A, Karanfil T. Formation of disinfection by-products in indoor swimming pool water: the contribution from filling water natural organic matter and swimmer body fluids. Water Res 2011;45(2):926–32. https://doi.org/10.1016/j.watres.2010.09.031.

[81] National Toxicology Program. Report on carcinogens monograph on haloacetic acids found as water disinfection by-products. Washington, DC: DHHS Retrieved from; 2018. https://ntp.niehs.nih.gov/ntp/roc/monographs/haafinal_508.pdf.

[82] Rodriguez B, Shindo D, Montgomery E. Electrochemical disinfection feasibility assessment materials evaluation for the international space station. In: Paper presented at the international conference on environmental systems, Vail, CO; 2013. https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20130011329.pdf.

[83] Gassie LW, Englehardt JD. Advanced oxidation and disinfection processes for onsite net-zero greywater reuse: a review. Water Res 2017;125:384–99. https://doi.org/10.1016/j.watres.2017.08.062.

[84] Dumas O, Wiley AS, Quinot C, Varraso R, Zock J-P, Henneberger PK, et al. Occupational exposure to disinfectants and asthma control in US nurses. Eur Respir J 2017;50(4):1700237. https://doi.org/10.1183/13993003.00237-2017.

[85] Akse JR, Holtsnider JT, Kliestik H, Pierson DL. Immobilized antimicrobials for the enhanced control of microbial contamination. https://doi.org/10.4271/2003-01-2405; 2003.

[86] Izenson MG, Jaeger MD, Steele J, Peyton B. Hydrophilic, biocidal coating for condensing heat exchangers. In: Paper presented at the 40th international conference on environmental systems, Barcelona, Spain; 2010.

[87] Lejars M, Margaillan A, Bressy C. Fouling release coatings: a nontoxic alternative to biocidal antifouling coatings. Chem Rev 2012;112(8):4347–90. https://doi.org/10.1021/cr200350v.

[88] Delgado-Ruiz R, Romanos G. Potential causes of titanium particle and ion release in implant dentistry: a systematic review. Int J Mol Sci 2018;19(11):3585. https://doi.org/10.3390/ijms19113585.

[89] Oliveira WF, Silva PMS, Silva RCS, Silva GMM, Machado G, Coelho LCBB, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* infections on implants. J Hosp Infect 2018;98(2):111–7. https://doi.org/10.1016/j.jhin.2017.11.008.

[90] Li Y, Xu Y, Fleischer CC, Huang J, Lin R, Yang L, et al. Impact of anti-biofouling surface coatings on the properties of nanomaterials and their biomedical applications. J Mater Chem B 2018;6(1):9–24. https://doi.org/10.1039/C7TB01695F.

[91] Lee K, Yu H, Zhang X, Choo K-H. Quorum sensing and quenching in membrane bioreactors: opportunities and challenges for biofouling control. Bioresour Technol 2018;270:656–68. https://doi.org/10.1016/j.biortech.2018.09.019.

[92] Yang L, Givskov M. Chemical biology strategies for biofilm control. Microbiol Spectr 2015;3(4). MB-0019-2015.

[93] Carniello V, Peterson BW, van der Mei HC, Busscher HJ. Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth. Adv Colloid Interface Sci 2018;261:1–14. https://doi.org/10.1016/j.cis.2018.10.005.

[94] Zouaghi S, Barry ME, Bellayer S, Lyskawa J, André C, Delaplace G, et al. Antifouling amphiphilic silicone coatings for dairy fouling mitigation on stainless steel. Biofouling 2018;34(7):769–83. https://doi.org/10.1080/08927014.2018.1502275.

[95] Galhenage TP, Hoffman D, Silbert SD, Stafslien SJ, Daniels J, Miljkovic T, et al. Fouling-release performance of silicone oil-modified siloxane-polyurethane coatings. ACS Appl Mater Interfaces 2016;8(42):29025–36. https://doi.org/10.1021/acsami.6b09484.

[96] Epstein AK, Wong TS, Belisle RA, Boggs EM, Aizenberg J. Liquid-infused structured surfaces with exceptional anti-biofouling performance. Proc Natl Acad Sci USA 2012;109:13182–7.

[97] Goodband SJ, Armstrong S, Kusumaatmaja H, Voitchovsky K. Effect of ageing on the structure and properties of model liquid-infused surfaces. Langmuir 2020;36(13):3461–70. https://doi.org/10.1021/acs.langmuir.0c00059.

[98] Chang Y-R, Weeks ER, Ducker WA. Surface topography hinders bacterial surface motility. ACS Appl Mater Interfaces 2018;10(11):9225–34. https://doi.org/10.1021/acsami.7b16715.

[99] Decker JT, Kirschner CM, Long CJ, Finlay JA, Callow ME, Callow JA, et al. Engineered antifouling microtopographies: an energetic model that predicts cell attachment. Langmuir 2013;29(42):13023–30. https://doi.org/10.1021/la402952u.

[100] Lin J, Jiang F, Wen J, Lv W, Porteous N, Deng Y, Sun Y. Fluorinated and un-fluorinated N-halamines as antimicrobial and biofilm-controlling additives for polymers. Polymer 2015;68:92–100. https://doi.org/10.1016/j.polymer.2015.05.014.

[101] Sun X, Cao Z, Porteous N, Sun Y. An N-halamine-based rechargeable antimicrobial and biofilm controlling polyurethane. Acta Biomater 2012;8(4):1498–506. https://doi.org/10.1016/j.actbio.2011.12.027.

[102] Dong C, Fu Y, Xie B, Wang M, Liu H. Element cycling and energy flux responses in ecosystem simulations conducted at the Chinese lunar palace-1. Astrobiology 2017;17(1):78–86. https://doi.org/10.1089/ast.2016.1466.

[103] Gupta A, Holoidovsky L, Thamaraiselvan C, Thakur AK, Singh SP, Meijler MM, et al. Silver-doped laser-induced graphene for potent surface antibacterial activity and anti-biofilm action. Chem Commun 2019;55(48):6890–3. https://doi.org/10.1039/C9CC02415H.

[104] Liu Z, Hu Y. Sustainable antibiofouling properties of thin film composite forward Osmosis membrane with rechargeable silver nanoparticles loading. ACS Appl Mater Interfaces 2016;8(33):21666–73. https://doi.org/10.1021/acsami.6b06727.

[105] Tribou M, Swain G. The effects of grooming on a copper ablative coating: a six year study. Biofouling 2017;33(6):494–504. https://doi.org/10.1080/08927014.2017.1328596.

[106] Menesses M, Belden J, Dickenson N, Bird J. Measuring a critical stress for continuous prevention of marine biofouling accumulation with aeration. Biofouling 2017;33(9):703–11. https://doi.org/10.1080/08927014.2017.1359574.

[107] Qian Z, Stoodley P, Pitt WG. Effect of low-intensity ultrasound upon biofilm structure from confocal scanning laser microscopy observation. Biomaterials 1996;17(20):1975–80.

[108] Sultana ST, Babauta JT, Beyenal H. Electrochemical biofilm control: a review. Biofouling 2015;31(9–10):745–58. https://doi.org/10.1080/08927014.2015.1105222.

[109] Keller N, Bruchmann J, Sollich T, Richter C, Thelen R, Kotz F, et al. Study of biofilm growth on slippery liquid-infused porous surfaces made from fluoropor. ACS Appl Mater Interfaces 2019;11(4):4480–7. https://doi.org/10.1021/acsami.8b12542.

[110] Subramanyam SB, Azimi G, Varanasi KK. Designing lubricant-impregnated textured surfaces to resist scale formation. Adv Mat Interfaces 2014;1(2):1300068. https://doi.org/10.1002/admi.201300068.

[111] Perrin E, Bacci G, Garrelly L, Canganella F, Bianconi G, Fani R, Mengoni A. Furnishing spaceship environment: evaluation of bacterial biofilms on different materials used inside International Space Station. Res Microbiol 2018;169(6):289–95. https://doi.org/10.1016/j.resmic.2018.04.001.

[112] Ichijo T, Yamaguchi N, Tanigaki F, Shirakawa M, Nasu M. Four-year bacterial monitoring in the international space station-Japanese experiment module "kibo" with culture-independent approach. npj Microgravity 2016;2:16007. https://doi.org/10.1038/npjmgrav.2016.7.

[113] Mora M, Perras A, Alekhova TA, Wink L, Krause R, Aleksandrova A, et al. Resilient microorganisms in dust samples of the International Space Station-survival of the adaptation specialists. Microbiome 2016;4. ARTN 65, 10.1186/s40168-016-0217-7.

[114] Venkateswaran K, Vaishampayan P, Cisneros J, Pierson DL, Rogers SO, et al. International Space Station environmental microbiome - microbial inventories of ISS filter debris. Appl Microbiol Biotechnol 2014;98(14):6453–66. https://doi.org/10.1007/s00253-014-5650-6.

[115] Francolini I, Vuotto C, Piozzi A, Donelli G. Antifouling and antimicrobial biomaterials: an overview. APMIS 2017;125(4):392–417. https://doi.org/10.1111/apm.12675.

[116] Majeed A, Sagar F, Latif A, Hassan H, Iftikhar A, Darouiche RO, et al. Does antimicrobial coating and impregnation of urinary catheters prevent catheter-associated urinary tract infection? A review of clinical and preclinical studies. Expert Rev Med Dev 2019;16(9):809–20. https://doi.org/10.1080/17434440.2019.1661774.

[117] Jakobsen TH, Tolker-Nielsen T, Givskov M. Bacterial biofilm control by perturbation of bacterial signaling processes. Int J Mol Sci 2017;18(9). https://doi.org/10.3390/ijms18091970.

[118] Gibson J, Drake J, Karney B. UV disinfection of wastewater and combined sewer overflows. In: Ahmad S, editor. Ultraviolet light in human health, diseases and environment, 996. Cham: Springer; 2017.

[119] Chahal C, van den Akker B, Young F, Franco C, Blackbeard J, Monis P. Pathogen and particle associations in wastewater: significance and implications for treatment and disinfection processes. Adv Appl Microbiol 2016;97:63–119. https://doi.org/10.1016/bs.aambs.2016.08.001.

[120] Lui GY, Roser D, Corkish R, Ashbolt NJ, Stuetz R. Point-of-use water disinfection using ultraviolet and visible light-emitting diodes. Sci Total Environ 2016;553:626–35. https://doi.org/10.1016/j.scitotenv.2016.02.039.

[121] Song K, Mohseni M, Taghipour F. Application of ultraviolet light-emitting diodes (UV-LEDs) for water disinfection: a review. Water Res 2016;94:341–9. https://doi.org/10.1016/j.watres.2016.03.003.

[122] Welch D, Buonanno M, Grilj V, Shuryak I, Crickmore C, Bigelow AW, et al. Far-UVC light: a new tool to control the spread of airborne-mediated microbial diseases. Sci Rep 2018;8(1):2752. https://doi.org/10.1038/s41598-018-21058-w.

[123] Häder DP, Sinha RP. Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact. Mutat Res 2005;571(1–2):221–33.

[124] Beukers R, Eker AP, Lohman PH. 50 years thymine dimer. DNA Repair 2008;7(3):530–43. https://doi.org/10.1016/j.dnarep.2007.11.010.

[125] Ikehata H, Ono T. The mechanisms of UV mutagenesis. J Radiat Res 2011;52(2):115–25. https://doi.org/10.1269/jrr.10175.

[126] Korczynski MS. Sterilization. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GB, editors. Manual of methods for general bacteriology. Washington, DC: American Society for Microbiology; 1981. p. 476–86.

[127] Shuryak I. Review of microbial resistance to chronic ionizing radiation exposure under environmental conditions. J Environ Radioact 2019;196:50–63. https://doi.org/10.1016/j.jenvrad.2018.10.012.

[128] Singh R, Singh D, Singh A. Radiation sterilization of tissue allografts: a review. World J Radiol 2016;8(4):355–69. https://doi.org/10.4329/wjr.v8.i4.355.

[129] Hu Q, Zhang XX, Jia S, Huang K, Tang J, Shi P, et al. Metagenomic insights into ultraviolet disinfection effects on antibiotic resistome in biologically treated wastewater. Water Res 2016;101:309–17. https://doi.org/10.1016/j.watres.2016.05.092.

[130] Cottin H, Rettberg P. EXPOSE-R2 on the international space station (2014-2016): results from the PSS and BOSS astrobiology experiments. Astrobiology 2019;19(8):975–8. https://doi.org/10.1089/ast.2019.0625.

[131] Billi D, Staibano C, Verseux C, Fagliarone C, Mosca C, Baque M, et al. Dried biofilms of desert strains of chroococcidiopsis survived prolonged exposure to space and mars-like conditions in low Earth orbit. Astrobiology 2019;19(8):1008–17. https://doi.org/10.1089/ast.2018.1900.

[132] Panitz C, Frosler J, Wingender J, Flemming HC, Rettberg P. Tolerances of *Deinococcus geothermalis* biofilms and planktonic cells exposed to space and simulated Martian conditions in low Earth orbit for almost two years. Astrobiology 2019;19(8):979–94. https://doi.org/10.1089/ast.2018.1913.

[133] Wadsworth J, Rettberg P, Cockell CS. Aggregated cell masses provide protection against space extremes and a microhabitat for hitchhiking co-inhabitants. Astrobiology 2019;19(8):995–1007. https://doi.org/10.1089/ast.2018.1924.

[134] Pezzoni M, Pizarro RA, Costa CS. Exposure to low doses of UVA increases biofilm formation in *Pseudomonas aeruginosa*. Biofouling 2018;34(6):673–84. https://doi.org/10.1080/08927014.2018.1480758.

[135] Petrova OE, Sauer K. Sticky situations: key components that control bacterial surface attachment. J Bacteriol 2012;194(10):2413–25.

[136] Petrova OE, Sauer K. Escaping the biofilm in more than one way: desorption, detachment or dispersion. Curr Opin Microbiol 2016;30:67–78.

[137] Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. Annu Rev Microbiol 2002;56:187–209.

[138] Bjarnsholt T, Jensen PO, Burmolle M, Hentzer M, Haagensen JA, Hougen HP, et al. *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. Microbiology 2005;151(Pt 2):373–83. https://doi.org/10.1099/mic.0.27463-0.

[139] Gupta K, Marques CN, Petrova OE, Sauer K. Antimicrobial tolerance of *Pseudomonas aeruginosa* biofilms is activated during an early developmental stage and requires the two-component hybrid SagS. J Bacteriol 2013;195(21):4975–87. https://doi.org/10.1128/JB.00732-13.

[140] Howlin RP, Cathie K, Hall-Stoodley L, Cornelius V, Duignan C, Allan RN, et al. Low-dose nitric oxide as targeted anti-biofilm adjunctive therapy to treat chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. Mol Ther 2017;25(9):2104–16. https://doi.org/10.1016/j.ymthe.2017.06.021.

[141] Marques CN, Davies DG, Sauer K. Control of biofilms with the fatty acid signaling molecule cis-2-decenoic acid. Pharmaceuticals 2015;8(4):816–35. https://doi.org/10.3390/ph8040816.

[142] Stoodley P, Wilson S, Hall-Stoodley L, Boyle JD, Lappin-Scott HM, Costerton JW. Growth and detachment of cell clusters from mature mixed-species biofilms. Appl Environ Microbiol 2001;67(12):5608–13.

[143] McDougald D, Rice SA, Barraud N, Steinberg PD, Kjelleberg S. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nat Rev Microbiol 2011;10(1):39–50. https://doi.org/10.1038/nrmicro2695.

[144] Dawe H, Berger E, Sihlbom C, Angus EM, Howlin RP, Laver JR, et al. D-methionine interferes with non-typeable *Haemophilus influenzae* peptidoglycan synthesis during growth and biofilm formation. Microbiology 2017;163(7):1093–104. https://doi.org/10.1099/mic.0.000491.

[145] Kim SK, Lee JH. Biofilm dispersion in *Pseudomonas aeruginosa*. J Microbiol 2016;54(2):71–85.

[146] Morgan R, Kohn S, Hwang SH, Hassett DJ, Sauer K. BdlA, a chemotaxis regulator essential for biofilm dispersion in *Pseudomonas aeruginosa*. J Bacteriol 2006;188(21):7335–43.

[147] Barraud N, Schleheck D, Klebensberger J, Webb JS, Hassett DJ, Rice SA, et al. Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. J Bacteriol 2009a;191(23):7333–42. https://doi.org/10.1128/JB.00975-09.

[148] Davies DG, Marques CNH. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol 2009;191(5):1393–403.

[149] Kolodin-Gai I, Romero D, Cao S, Clardy J, Kolter R, Losick R. D-amino acids trigger biofilm disassembly. Science 2010;328(5978):627–9.

[150] Sauer K, Cullen MC, Rickard AH, Zeef LA, Davies DG, Gilbert P. Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. J Bacteriol 2004;186(21):7312–26. https://doi.org/10.1128/JB.186.21.7312-7326.2004.

[151] Rybtke M, Hultqvist LD, Givskov M, Tolker-Nielsen T. *Pseudomonas aeruginosa* biofilm infections: community structure, antimicrobial tolerance and immune response. J Mol Biol 2015;427(23):3628–45.

[152] Hengge R. Principles of c-di-GMP signalling in bacteria. Nat Rev Microbiol 2009;7:263–73.

[153] Barraud N, Schleheck D, Klebensberger J, Webb JS, Hassett DJ, Rice SA, Kjelleberg S. Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic diguanosine-5'-monophosphate levels and enhanced dispersal. J Bacteriol 2009b;191(23):7333–42.

[154] Basu Roy A, Petrova OE, Sauer K. The phosphodiesterase DipA (PA5017) is essential for *Pseudomonas aeruginosa* biofilm dispersion. J Bacteriol 2012;194(11):2904–15.

[155] Mittelman MW, Jones ADG. A pure life: the microbial ecology of high purity industrial waters. Microb Ecol 2018;76(1):9–18. https://doi.org/10.1007/s00248-016-0736-6.

[156] Zlatanovic L, van der Hoek JP, Vreeburg JHG. An experimental study on the influence of water stagnation and temperature change on water quality in a full-scale domestic drinking water system. Water Res 2017;123:761–72. https://doi.org/10.1016/j.watres.2017.07.019.

[157] Biondi A, Zappala L, Stark JD, Desneux N. Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? PloS One 2013;8(9):e76548. https://doi.org/10.1371/journal.pone.0076548.

[158] Boulanger FX, Jandricic S, Bolckmans K, Wackers FL, Pekas A. Optimizing aphid biocontrol with the predator *Aphidoletes aphidimyza*, based on biology and ecology. Pest Manag Sci 2019;75(6):1479–93. https://doi.org/10.1002/ps.5270.

[159] Kadouri D, O'Toole GA. Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol 2005;71(7):4044–51.

[160] Velicer GJ, Mendes-Soares H. Bacterial predators. Curr Biol 2009;19(2):R55–6. https://doi.org/10.1016/j.cub.2008.10.043.

[161] Martin MO. Predatory prokaryotes: an emerging research opportunity. J Mol Microbiol Biotechnol 2002;4(5):467–77.

[162] Nair RR, Vasse M, Wielgoss S, Sun L, Yu YN, Velicer GJ. Bacterial predator-prey coevolution accelerates genome evolution and selects on virulence-associated prey defences. Nat Commun 2019;10(1):4301. https://doi.org/10.1038/s41467-019-12140-6.

[163] Findlay JS, Flick-Smith HC, Keyser E, Cooper IA, Williamson ED, Oyston PCF. Predatory bacteria can protect SKH-1 mice from a lethal plague challenge. Sci Rep 2019;9(1):7225. https://doi.org/10.1038/s41598-019-43467-1.

[164] Silva PHF, Oliveira LFF, Cardoso RS, Ricoldi MST, Figueiredo LC, Salvador SL, et al. The impact of predatory bacteria on experimental periodontitis. J Periodontol 2019;90(9):1053–63. https://doi.org/10.1002/jper.18-0485.

[165] Chan BK, Abedon ST. Bacteriophages and their enzymes in biofilm control. Curr Pharmaceut Des 2015;21(1):85–99. https://doi.org/10.2174/1381612820666140905112311.

[166] d'Herelle F. Bacteriophage as a treatment in acute medical and surgical infections. Bull NY Acad Med 1931;7(5):329–48.

[167] Chibani-Chennoufi S, Bruttin A, Dillmann ML, Brussow H. Phage-host interaction: an ecological perspective. J Bacteriol 2004;186(12):3677–86. https://doi.org/10.1128/JB.186.12.3677-3686.2004.

[168] Petter JG, Vimr ER. Complete nucleotide sequence of the bacteriophage K1F tail gene encoding endo-N-acylneruaminidase (Endo-N) and comparison to an endo-N homolog in bacteriophage PK1E. J Bacteriol 1993;175(14):4354–63.

[169] Sutherland IW, Hughes KA, Skillman LC, Tait K. The interaction of phage and biofilms. FEMS Microbiol Lett 2004;232(1):1–6.

[170] Furfaro LL, Payne MS, Chang BJ. Bacteriophage therapy: clinical trials and regulatory hurdles. Front Cell Infect Microbiol 2018;8. https://doi.org/10.3389/fcimb.2018.00376. 376-376.

[171] Kay MK, Erwin TC, McLean RJ, Aron GM. Bacteriophage ecology in *Escherichia coli* and *Pseudomonas aeruginosa* mixed-biofilm communities. Appl Environ Microbiol 2011;77(3):821–9. https://doi.org/10.1128/AEM.01797-10.

[172] Ackermann HW. Phage classification and characterization. Methods Mol Biol 2009;501:127–40.

[173] Górski A, Międzybrodzki R, Łobocka M, Głowacka-Rutkowska A, Bednarek A, Borysowski J, Scheres J. Phage therapy: what have we learned? Viruses 2018;10(6):288. https://doi.org/10.3390/v10060288.

[174] Simmons M, Drescher K, Nadell CD, Bucci V. Phage mobility is a core determinant of phage-bacteria coexistence in biofilms. ISME J 2018;12(2):531–43. https://doi.org/10.1038/ismej.2017.190.

[175] Lawrence JR, Scharf B, Packroff G, Neu TR. Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. Microb Ecol 2003;44(3):199–207.

[176] Xu G, Abdullah Al M, Sikder MNA, Warren A, Xu H. Identifying indicator redundancy of biofilm-dwelling protozoa for bioassessment in marine ecosystems. Environ Sci Poll Res 2018;25(30):30441–50. https://doi.org/10.1007/s11356-018-3063-2.

[177] Denet E, Vasselon V, Burdin B, Nazaret S, Favre-Bonte S. Survival and growth of Stenotrophomonas maltophilia in free-living amoebae (FLA) and bacterial virulence properties. PloS One 2018;13(2):e0192308. https://doi.org/10.1371/journal.pone.0192308.

[178] Shaheen M, Scott C, Ashbolt NJ. Long-term persistence of infectious *Legionella* with free-living amoebae in drinking water biofilms. Int J Hyg Environ Health 2019;222(4):678–86. https://doi.org/10.1016/j.ijheh.2019.04.007.

[179] Mattison RG, Taki H, Harayama S. The bacterivorous soil flagellate, *Heteromita globosa*, reduces bacterial clogging under denitrifying conditions in sand-filled aquifer columns. Appl Environ Microbiol 2002;68(9):4539–45. https://doi.org/10.1128/aem.68.9.4539-4545.2002.

[180] Matz C, Bergfeld T, Rice SA, Kjelleberg S. Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing. Environ Microbiol 2004;6(3):218–26.

[181] Sibille I, Sime-Ngando T, Mathieu L, Block JC. Protozoan bacterivory and *Escherichia coli* survival in drinking water distribution systems. Appl Environ Microbiol 1998;64(1):197–202.

[182] Weronika E, Łukasz K. Tardigrades in space research - past and future. Orig Life Evol Biosph 2017;47(4):545–53. https://doi.org/10.1007/s11084-016-9522-1.

[183] Rebecchi L, Altiero T, Guidetti R, Cesari M, Bertolani R, Negroni M, et al. Tardigrade resistance to space effects: first results of experiments on the LIFE-TARSE mission on FOTON-M3 (September 2007). Astrobiology 2009;9(6):581–91. https://doi.org/10.1089/ast.2008.0305.

[184] Neves RC, Hvidepil LKB, Sørensen-Hygum TL, Stuart RM, Møbjerg N. Thermotolerance experiments on active and desiccated states of *Ramazzottius varieornatus* emphasize that tardigrades are sensitive to high temperatures. Sci Rep 2020;10:94. https://doi.org/10.1038/s41598-019-56965-z.

[185] Engevik MA, Versalovic J. Biochemical features of beneficial microbes: foundations for therapeutic microbiology. Microbiol Spectr 2017;5(5). https://doi.org/10.1128/microbiolspec.BAD-0012-2016. 10.1128/microbiolspec.BAD-0012-2016.

[186] Saulnier DMA, Spinler JK, Gibson GR, Versalovic J. Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. Curr Opin Biotechnol 2009;20(2):135–41. https://doi.org/10.1016/j.copbio.2009.01.002.

[187] Vuotto C, Longo F, Donelli G. Probiotics to counteract biofilm-associated infections: promising and conflicting data. Int J Oral Sci 2014;6(4):189–94. https://doi.org/10.1038/ijos.2014.52.

[188] Visick KL, Fuqua C. Decoding microbial chatter: cell-cell communication in bacteria. J Bacteriol 2005;187(16):5507–19.

[189] Federle MJ, Bassler BL. Interspecies communication in bacteria. J Clin Invest 2003;112(9):1291–9.

[190] Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. Annu Rev Genet 2009;43:197–222.

[191] Nutman AP, Bennett VC, Friend CRL, Van Kranendonk MJ, Chivas AR. Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. Nature 2016;537(7621):535–8. https://doi.org/10.1038/nature19355.

[192] Rutten MG. The geological aspects of the origin of life on earth. Amsterdam; New York: Elsevier Pub. Co; 1962.

[193] Bailey JE, Ollis DF. Biochemical engineering fundamentals. McGraw-Hill; 1986.

[194] Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol 1995;49:711–45.

[195] Boltz JP, Smets BF, Rittmann BE, van Loosdrecht MCM, Morgenroth E, Daigger GT. From biofilm ecology to reactors: a focused review. Water Sci Technol 2017;75(7–8):1753–60. https://doi.org/10.2166/wst.2017.061.

[196] Daigger GT, Littleton HX. Simultaneous biological nutrient removal: a state-of-the-art review. Water Environ Res 2014;86(3):245–57. https://doi.org/10.2175/106143013x13736496908555.

[197] Goldman JC, Caron DA, Dennett MR. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C: N ratio1. Limnol Oceanogr 1987;32(6):1239–52. https://doi.org/10.4319/lo.1987.32.6.1239.

[198] Norland S, Fagerbakke KM, Heldal M. Light element analysis of individual bacteria by X-ray microanalysis. Appl Environ Microbiol 1995;61:1357–62.

[199] Payne SM. Iron and virulence in the family *Enterobacteriaceae*. Crit Rev Microbiol 1988;16:81–111.

[200] Duboc P, Schill N, Menoud L, van Gulik W, von Stockar U. Measurements of sulfur, phosphorus and other ions in microbial biomass: influence on correct determination of elemental composition and degree of reduction. J Biotechnol 1995;43(2):145–58. https://doi.org/10.1016/0168-1656(95)00135-0.

[201] Chandy JP, Angles ML. Determination of nutrients limiting biofilm formation and the subsequent impact on disinfectant decay. Water Res 2001;35(11):2677–82. https://doi.org/10.1016/S0043-1354(00)00572-8.

[202] Volk CJ, LeChevallier MW. Impacts of the reduction of nutrient levels on bacterial water quality in distribution systems. Appl Environ Microbiol 1999;65(11):4957–66.

[203] Hudson JJ, Taylor WD, Schindler DW. Phosphate concentrations in lakes. Nature 2000;406(6791):54–6. https://doi.org/10.1038/35017531.

[204] Gora S, Chaulk M. Study on the characteristics and removal of natural organic matter in drinking water systems in Newfoundland and Labrador, Final Report. St. John's NF, Canada: CBCL Ltd; 2011.

[205] EPA. Enhanced coagulation and enhanced precipitative softening guidance manual. EPA; 1999. EPA 815-R-99-012.

[206] Eslami H, Ehrampoush MH, Esmaeili A, Salmani MH, Ebrahimi AA, Ghaneian MT, et al. Enhanced coagulation process by Fe-Mn bimetal nano-oxides in combination with inorganic polymer coagulants for improving As(V) removal from contaminated water. J Clean Prod 2019;208:384–92. https://doi.org/10.1016/j.jclepro.2018.10.142.

[207] Gregory R, Edzwald JK. Sedimentation and flotation. In: Edzwald JK, editor. Water quality and treatment - a handbook on drinking water. 6 ed. Denver, CO: American Water Works Association, McGraw-Hill; 2011.

[208] Kirisits MJ, Emelko MB, Pinto AJ. Applying biotechnology for drinking water biofiltration: advancing science and practice. Curr Opin Biotechnol 2019;57:197–204. https://doi.org/10.1016/j.copbio.2019.05.009.

[209] Greenwalt CJ, Hunter JB, Lin S, McKenzie S, Denvir A. Ozonation and alkaline-peroxide pretreatment of wheat straw for *Cryptococcus curvatus* fermentation. Life Support Biosph Sci 2000;7(3):243–9.

[210] Newcombe G. Charge vs. porosity — some influences on the adsorption of natural organic matter (NOM) by activated carbon. Water Sci Technol 1999;40(9):191–8. https://doi.org/10.1016/S0273-1223(99)00656-3.

[211] Boyer TH, Singer PC. A pilot-scale evaluation of magnetic ion exchange treatment for removal of natural organic material and inorganic anions. Water Res 2006;40(15):2865–76. https://doi.org/10.1016/j.watres.2006.05.022.

[212] Ashok V, Hait S. Remediation of nitrate-contaminated water by solid-phase denitrification process-a review. Environ Sci Pollut Res Int 2015;22(11):8075–93. https://doi.org/10.1007/s11356-015-4334-9.

[213] Mehta CM, Khunjar WO, Nguyen V, Tait S, Batstone DJ. Technologies to recover nutrients from waste streams: a critical review. Crit Rev Environ Sci Technol 2015;45(4):385–427. https://doi.org/10.1080/10643389.2013.866621.

[214] Washington State Department of Health. Nitrate treatment and remediation for small water systems. Olympia, WA: Washington State Department of Health; 2018. 331-309 (Revised).

[215] Fux I, Birnhack L, Tang SCN, Lahav O. Removal of nitrate from drinking water by ion-exchange followed by nZVI-based reduction and electrooxidation of the ammonia product to N2(g). Chem Eng 2017;1(1):2.

[216] Matatov-Meytal Y, Barelko V, Yuranov I, Sheintuch M. Cloth catalysts in water denitrification: I. Pd on glass fibers. Appl Catal B Environ 2000;27(2):127–35. https://doi.org/10.1016/S0926-3373(00)00141-7.

[217] Ramavandi B, Mortazavi SB, Moussavi G, Khoshgard A, Jahangiri M. Experimental investigation of chemical reduction of nitrate ion in aqueous solution by Mg/Cu

bimetallic particles. React Kinet Mech Catal 2011;102(2):313–29. https://doi.org/10.1007/s11144-010-0274-z.
[218] Zhu I, Getting T. A review of nitrate reduction using inorganic materials. Environ Technol Rev 2012;1(1):46–58. https://doi.org/10.1080/09593330.2012.706646.
[219] Terry LG, Summers RS. Biodegradable organic matter and rapid-rate biofilter performance: a review. Water Res 2018;128:234–45. https://doi.org/10.1016/j.watres.2017.09.048.
[220] Basu OD, Dhawan S, Black K. Applications of biofiltration in drinking water treatment – a review. J Chem Technol Biotechnol 2016;91(3):585–95. https://doi.org/10.1002/jctb.4860.
[221] Lovley DR. Electromicrobiology. Annu. Rev. Microbiol 2012;66:391–409.
[222] Nancharaiah YV, Venkata Mohan S, Lens PNL. Recent advances in nutrient removal and recovery in biological and bioelectrochemical systems. Bioresour Technol 2016;215:173–85. https://doi.org/10.1016/j.biortech.2016.03.129.
[223] Zhao Z, Zhang Y, Woodard TL, Nevin KP, Lovley DR. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Bioresour Technol 2015;191:140–5.
[224] Kornberg A, Rao NN, Ault-Riché D. Inorganic polyphosphate: a molecule of many functions. Annu Rev Biochem 1999;68:89–125.
[225] Bunce JT, Ndam E, Ofiteru ID, Moore A, Graham DW. A review of phosphorus removal technologies and their applicability to small-scale domestic wastewater treatment systems. Front Environ Sci 2018;6(8). https://doi.org/10.3389/fenvs.2018.00008.
[226] Fields MW, Hise A, Lohman EJ, Bell T, Gardner RD, Corredor L, et al. Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Appl Microbiol Biotechnol 2014;98(11):4805–16. https://doi.org/10.1007/s00253-014-5694-7.
[227] Morse GK, Brett SW, Guy JA, Lester JN. Review: phosphorus removal and recovery technologies. Sci Total Environ 1998;212(1):69–81. https://doi.org/10.1016/S0048-9697(97)00332-X.
[228] Liu J, Chen F. Biology and industrial applications of *Chlorella*: advances and prospects. In: Posten C, Feng Chen S, editors. Microalgae biotechnology. Geneva: Springer; 2014 [Cham].
[229] Niederwieser T, Kociolek P, Klaus D. A review of algal research in space. Acta Astronaut 2018a;146:359–67. https://doi.org/10.1016/j.actaastro.2018.03.026.
[230] Niederwieser T, Kociolek P, Klaus D. Spacecraft cabin environment effects on the growth and behavior of *Chlorella vulgaris* for life support applications. Life Sci Space Res 2018b;16:8–17. https://doi.org/10.1016/j.lssr.2017.10.002.
[231] Allen JP, Nelson M, Alling A. The legacy of Biosphere 2 for the study of biospherics and closed ecological systems. Adv Space Res 2003;31(7):1629–39. https://doi.org/10.1016/s0273-1177(03)00103-0.
[232] Dong F, Mohd Zaidi NF, Valsami-Jones E, Kreft J-U. Time-resolved toxicity study reveals the dynamic interactions between uncoated silver nanoparticles and bacteria. Nanotoxicology 2017;11(5):637–46. https://doi.org/10.1080/17435390.2017.1342010.
[233] Tsagkari E, Sloan WT. Turbulence accelerates the growth of drinking water biofilms. Bioproc Biosyst Eng 2018;41(6):757–70. https://doi.org/10.1007/s00449-018-1909-0.
[234] Cucinotta FA. Review of NASA approach to space radiation risk assessments for Mars exploration. Health Phys 2015;108(2):131–42. https://doi.org/10.1097/hp.0000000000000255.
[235] National Research Council. Recapturing a future for space exploration: life and physical sciences research for a new era. Washington, DC: The National Academies Press; 2011.
[236] Allen LA, Forward T, Stodieck L, Klaus D, Zea L. The effects of simulated Lunar and Martian gravities on the growth and morphology of Escherichia coli, Shewanella oneidensis, methicillin-resistant Staphylococcus aureus, and Pseudomonas aeruginosa. In: Paper presented at the 35th annual meeting of the American society for gravitational and space research, denver CO; 2019.
[237] Forward T, Allen L, Klaus D, Zea L. Growth dynamics of bacteria under simulated Lunar and Martian gravities. Washington DC: Paper presented at the 70th International Astronautical Congress; 2019.
[238] Leidich, Thomas EA, Klaus DM. A novel testing protocol for evaluating particle behavior in fluid flow under simulated reduced gravity conditions. https://doi.org/10.4271/2009-01-2359; 2009.
[239] Zea L, Stodieck L, Klaus DM. Bacterial growth and susceptibility to antibiotics in simulated reduced levels of gravity. Orlando, FL: Paper presented at the American Society for Gravitational and Space Research; 2013.
[240] Castro-Wallace SL, Chiu CY, John KK, Stahl SE, Rubins KH, McIntyre ABR, et al. Nanopore DNA sequencing and genome assembly on the international space station. Sci Rep 2017;7(1):18022. https://doi.org/10.1038/s41598-017-18364-0.
[241] Prater TJ, Bean QA, Beshears RD, Rolin TD, Werkheiser N, Ordonez EA, et al. Summary report on phase I results from the 3D Printing in Zero G technology demonstration mission. 2016. NASA Technical Report 2016-219101.