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Biofilm reactors for the treatment of used water in space:potential, challenges, and future perspectives

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A B S T R A C T

Water is not only essential to sustain life on Earth, but also is a crucial resource for long-duration deep space exploration and habitation. Current systems in space rely on the resupply of water from Earth, however, as missions get longer and move farther away from Earth, resupply will no longer be a sustainable option. Thus, the development of regenerative reclamation water systems through which useable water can be recovered from "waste streams" (*i.e.,* used waters) is sorely needed to further close the loop in space life support systems. This review presents the origin and characteristics of different used waters generated in space and discusses the intrinsic challenges of developing suitable technologies to treat such streams given the unique constrains of space exploration and habitation (*e.g.*, different gravity conditions, size and weight limitations, compatibility with other systems, *etc.*). In this review, we discuss the potential use of biological systems, particularly biofilms, as possible alternatives or additions to current technologies for water reclamation and waste treatment in space. The fundamentals of biofilm reactors, their advantages and disadvantages, as well as different reactor configurations and their potential for use and challenges to be incorporated in self-sustaining and regenerative life support systems in long-duration space missions are also discussed. Furthermore, we discuss the possibility to recover value-added products (*e.g.*, biomass, nutrients, water) from used waters and the opportunity to recycle and reuse such products as resources in other life support subsystems (*e.g.,* habitation, waste, air, *etc*.).

1. Introduction

For a long time, humankind has shared the desire to explore space; there are currently different efforts for space missions to the Moon, Mars, and beyond [1,2]. Water is a crucial resource for crewed long-duration deep space exploration and habitation. As the best-known example of extended space missions, the International Space Station (ISS) recycles most of its water via a suite of physical and chemical treatment processes. However, to meet its water demands, the ISS still relies on water resupply from Earth. As future missions will travel further from Earth with longer duration, the need for self-sufficient systems that reliably provide water without resupply from Earth will be essential. Water of different purity will be needed for diverse purposes, including consumption by the crew, sanitation, laundry, urinal flushing and food preparation, as well as for oxygen (O_2) generation, potential food production (*e.g.*, plant cultivation), and different research activities. It has been estimated that for space missions longer than 30 days, about 2.4 kg of water per crewmember per day would be required to cover human consumption needs (*i.e.,* considering only drinking water) [3]. A suitable option to fulfill water demands would be to recover and recycle water from waste streams such as urine and hygiene wastewater. For instance, the total wastewater generation rates (kg of water per crewmember per day) for various missions have been estimated to be 3.7 on the ISS, 4.1 on a transit vehicle, 11.4 for the Early Planetary Base (EPB), and 29.3 for a mature planetary base [4]. Thus, through regenerative water reclamation systems, it would be possible to convert the "wastewater" or "used water" into useable water for different purposes while potentially recovering value-added products (VAPs). Designing more reliable, robust, self-sustaining and regenerative water subsystems would further close the water loop in the Environmental Control and Life Support System (ECLSS). Given the potential to convert waste into resources, this review will use the term "used water" to refer to wastewater. This review will provide an overview of the different used waters generated in space, including their origin and characteristics, as well as the intrinsic challenges for treatment of such streams during space exploration and habitation.

Current systems used in space missions rely solely on physicochemical methods for the treatment of used water streams [5]. Organic wastes are generally not treated but are gathered and either returned to

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List of abbreviations

APH	Advanced Plant Habitat
BLSS	Bioregenerative Life Support Systems
BOD	Biochemical Oxygen Demand
BPA	Brine Processor Assembly
CELSS	Closed/Controlled Ecological Life Support Systems
COD	Chemical Oxygen Demand
EBR	Expanded-Bed Reactors
ECLSS	Environmental Control and Life Support System
EPB	Early Planetary Base
EPS	Extracellular Polymeric Substances
FBR	Fixed-Bed Reactor
HC	Humidity Condensate
HRT	Hydraulic Retention Time
ID	Inner Diameter
ISS	International Space Station
MABR	Membrane Aerated Biofilm Reactor
MBfR	Membrane Biofilm Reactor
OD	Outer Diameter
OGA	Oxygen Generating Assembly
PHAs	Polyhydroxyalkanoates
PBR	Packed-Bed Reactor
SSA	Specific Surface Area
TBR	Trickling filter Biofilm Reactor
TN	Total Nitrogen
TOC	Total Organic Carbon
TSS	Total suspended solids
UPA	Urine Processor Assembly
VAPs	Value-added products
WPA	Water Processor Assembly
WRM	Water Recovery and Management

Earth for analysis or burnt in the atmosphere. In this review, we discuss the potential use of biological systems, particularly biofilm-based systems, as a possible alternative or addition to current technologies for water reclamation and waste treatment in space. In general, biological systems do not require addition of potentially harmful chemicals (*e.g.,* urine is treated at the ISS using chromium trioxide, a highly toxic substance, in a solution of phosphoric acid [8]), offer the possibility of self-regeneration and need minimal maintenance or energy for operations. Furthermore, the products of the biological degradation of pollutants typically results in biomass, water, and inorganic species depending on the available energy source for microbial growth (*e.g.,* production of carbon dioxide and nitrogen gas). Some of these products can be considered VAPs, given that they could be recovered, recycled and reused as resources in other life support subsystems (*e.g.,* habitation, waste, air, etc.). This review will discuss the advantages, disadvantages, and limitations of biological systems for water treatment, particularly under space conditions (*e.g.*, microgravity, partial gravity). Biological systems are typically classified as suspended growth, attached growth, or combined systems. While biological pollutant degradation metabolisms in these systems are similar, there are clear distinctions between the reactor types. As indicated by the name, in suspended growth systems, microorganisms in the form of single cells, aggregates or flocs are maintained in suspension along with the water to be treated. In attached growth (also known as fixed film or biofilm) systems, microbial growth occurs on a surface; this can be achieved in different ways including attaching the microbial film to the reactor walls, fixed plates in the fluid flow, hollow tubes, or different carriers and packing media. There are systems in which the fixed film is attached to carriers suspended within the water to be treated; these types of systems are commonly referred to as moving bed biofilm bioreactors. This review will focus on attached growth systems, herein referred to as biofilm systems, due to their potential to treat used water in space and their potential use to recover VAPs for their integration in ECLSS. This review will discuss the fundamentals of biofilm systems, their potential applications for waste stream treatment in space, as well as the opportunities and challenges to incorporating these kinds of systems in long-duration missions.

2. Used water in space: characteristics, challenges and opportunities

2.1. Origin and characteristics

Used water aboard crewed spacecraft or space stations is typically the result of a combination of primary waste stream sources including humidity condensate, hygiene water, urine/urine flush, and the product from the Sabatier process [6]. The humidity condensate is the water condensed and collected from heat exchangers that control cabin humidity levels; this effluent contains small amounts of carbonaceous and nitrogenous compounds mainly comprised of low molecular weight carboxylic acids and alcohols [7]. Hygiene water is typical grey wastewater originating from washing, showering, and other general hygiene practices; hygiene water is characterized by the presence of soaps, particulates, and dissolved organics. Urine and urine flush commonly contain high concentrations of urea (CH_4N_2O) as well as dissolved organic compounds and inorganic salts [6]. Currently, solid human waste (*e.g.*, feces) generated during spaceflight is collected and disposed of separately. For instance, in the ISS, fecal matter is stored in an aluminum can that, when full, is jettisoned off the ISS to burn up on reentry. However, NASA's Exploration Capabilities Program is looking into the possibility of reclaiming resources from solid organic wastes, *e.g.*, water content from feces, to further close the water loop in ECLSS. Beyond water, solid organic wastes also contain nutrients that can potentially be recovered as VAPs to sustain plant growth or other activities. While discussion of the treatment of solid organic waste is beyond the scope of this review, the integration of solid organic waste treatment with used water treatment for ECLSS will be discussed throughout this review, as the two streams intertwine (or could intertwine) in several instances.

The ISS produces wastewater at an approximate rate of 3.7 kg/d per crewmember; this waste stream is mainly a mixture of pretreated urine, flush, and humidity condensate [4]. The ISS wastewater is typically combined with the Sabatier water product (water produced from the CO_2 reduction system using the Sabatier process to convert CO_2 and H_2 to water and CH_4) and treated in the Water Recovery and Management (WRM) system [8,9]. The WRM system can recover about 70% of the waste stream [10], through chemical pretreatment combined with physical desalination and post-treatment to drinking water standards [8, 11]. The WRM system is comprised of three units (Fig. 1): the Urine Processor Assembly (UPA), the Brine Processor Assembly (BPA), and the Water Processor Assembly (WPA). In the UPA, crew urine is stored in the wastewater storage tank assembly, where the waste stream is chemically stabilized with H_3PO_4 and Cr^{6+} to prevent microbial growth. Afterwards, the waste stream is recirculated through the distillation assembly, where the distillate stream is evaporated in vacuum and then condensed before it is sent to the WPA for further treatment [11,12]. In the BPA, residual brine from the UPA is further processed to increase water recovery to 98% [13]. The WPA uses a series of different physicochemical technologies including filtration, ion exchange, adsorption, catalytic oxidation, and iodination to treat the humidity condensate, grey wastewater, the Sabatier product water, and the water treated by the UPA [11,14]. The WPA produces potable water for crew consumption, science experiments, and for the oxygen generating assembly

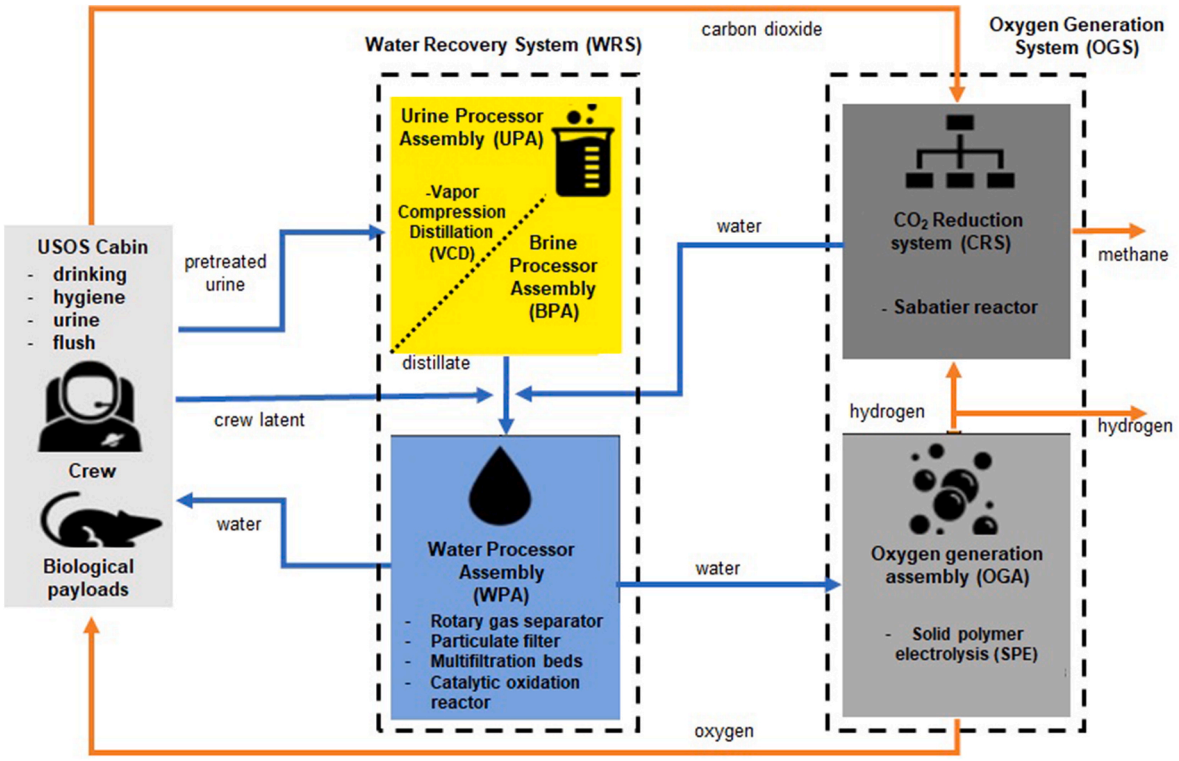

Fig. 1. Water Recovery and Management system treating used water generatd at the United States On-Orbit Segment (USOS) cabin at the International Space Station.

(OGA) [15]. The OGA produces O_2 that is used in the cabin, and hydrogen that is either vented overboard or used in the Sabatier system.

Compared to ISS wastewater, the wastewater generated in planetary missions (*e.g.,* Mars) is expected to include other waste streams in addition to those currently considered in the ISS (*e.g.*, wastewater generated from laundry) [4]. An Early Planetary Base (EPB) has been estimated to produce wastewater at a rate of approximately 14 L/d per crewmember [16]. Wastewater formulations, known as *"Ersatz"* (German for *substitute*), have been developed to mimic the composition of different waste streams in space [6]. Ersatz formulations contain different fractions of the primary stream sources and simulate the characteristics of used water generated on a transit mission, on an EPB, as well as EPB wastewater that has undergone various physicochemical and biological treatments (*e.g.*, effluent from the bioreactor, effluent from the reverse osmosis system, etc.) [6]. Waste streams from ISS and EPB can be considered high strength based on high concentrations of carbon and nitrogen (Table 1) [6], with total carbon and nitrogen concentrations much higher than typical values for municipal wastewater (Total Organic Carbon [TOC]: 80–250 mg/L; Total Nitrogen [TN]: 20–60 mg/L) [17]. Most waste streams in space are also characterized by having low carbon-to-nitrogen ratios (C:N $<$ 1), high total dissolved solids concentrations (1000–3000 mg/L), and nitrogen mostly present as urea or ammonia/ammonium ion (NH_3/NH_4^+) [6]. Having C:N $<$ 1 is a challenge for the traditional denitrification process in wastewater treatment, in which carbon acts as the electron donor to reduce nitrate (NO_3^-) to N_2. In traditional activated sludge systems used for wastewater treatment on Earth, it has been reported that C:N $<$ 2 results in decreased nitrogen removal efficiencies [18]. Typically, on Earth, an external carbon source would be added to compensate for the lack of carbon; however, in space this same strategy would be challenging since the goal is to minimize the use of consumables. The use of excess hydrogen from the OGA would be an opportunity to provide (non-carbon) reduction equivalents though. Other challenges include the high NH_3 concentration, which could limit nitrification, and low alkalinities, which can result in fluctuating pH values [19].

Table 1
Characteristics of different wastewater produced in space vs. municipal wastewater on Earth.

	Transit Wastewater[a]	EPB Wastewater[a]	ISS Wastewater[a]	Municipal Wastewater on Earth[b]
Total C (mg/L)	2209	631	1500	80–250 mg/L
Total N (mg/L)	221	852	2000	20–60 mg/L
C:N ratio	10.00	0.74	0.75	3.00–4.00
pH	2.7	8.9	7.0	6.0–10.0

[a] [6].
[b] [17].

Composition and volume of wastewater generated in space may fluctuate based on the number of crewmembers onboard, the crew urine composition, and the amount of product water generated from the Sabatier system [20]. The composition and quantity of urine produced by crewmembers is significant for the treatment process, as urine is a key component in space wastewater. Ref. [21] indicated that urine would make up about 18% of the wastewater in partial gravity habitats, however urine composition and volume generated varies for each individual depending on their sex, metabolic rates, nutrition, hydration, health status, and place of origin [22]. Furthermore, it has been demonstrated that microgravity can have effects on the human body that impact the composition and quantity of urine. For instance, there is increased calcium excretion in urine due to bone loss experienced by crewmembers during spaceflight [23]. It has also been suggested that under microgravity conditions, crewmembers experience less sensation of thirst, which can result in decreased water intake and thus decreased urine production [24]. Whereas urine of crewmembers has been collected and analyzed on multiple occasions, the data collected have

been mostly for medical purposes [22,25,26]; hence, there is a lack of information regarding relevant parameters for wastewater treatment, particularly regarding the organic content in urine (*e.g.,* Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) concentrations and loads) as well as total suspended solids (TSS). Furthermore, there are no data for partial gravity habitation wastewater systems, so there remains a technology gap between terrestrial (1 *g*) and ISS (microgravity) conditions. This information would be valuable to estimate, for example, the amount of oxygen or other oxidants required for biological treatment of this waste stream. Ref. [27] compiled information on the various urine generation rates reported from space studies; on average the total urine wastewater load is in a range of 1.5–2 kg per crewmember per day. Knowing the urine composition and volume generated is crucial to design adequate treatment systems.

2.2. Challenges for treatment of used water in space

There are unique challenges associated with the treatment of used waters for long-duration space missions, including: *(i)* designing treatment systems compatible with space conditions and limitations (*e.g.,* microgravity, self-sustaining, compact and confined unit treatments), and *(ii)* integrating treatment technologies as part of ECLSS.

With the current plans to return to the Lunar surface and eventually the Martian surface, water treatment technologies for space will need to operate under gravity conditions different from terrestrial gravity (1 *g*), including microgravity (10^{-4} to 10^{-6} *g*) and partial gravity (10^{-4} to < 1 *g*). In space, objects are subjected to weightlessness (zero-gravity) or almost weightlessness (microgravity) conditions.

Microgravity occurs in most low-Earth-orbit spaceflight platforms such as space shuttles and the ISS, where "the Earth's gravitational force is almost entirely balanced by the inertia force (proportional to the change in time of the velocity vector)" [28]; microgravity represents a fraction of the gravitational acceleration experienced at sea level on Earth, with values typically in the range of 10^{-4} to 10^{-6} *g* [29,30]. On Earth, gravity plays a key role in the way fluids behave and how they flow; on Earth, wastewater systems have to account for or rely on gravity to move waste streams through the treatment process and separate solids, liquid, and gases based on density. In the absence of gravity, fluid dynamics change, and flow is driven by capillary forces and surface tension [31]. Various phenomena are experienced under microgravity, including loss of the hydrostatic pressure gradient, buoyancy, natural convection (thermal/concentration), and sedimentation [28,32]. All of this has consequences in the behavior of fluid phases in space. For instance, separation of gases and liquids occur uniquely in space; gas bubbles coalesce into larger bubbles and typically move to the center of vessels as the liquid moves to the surface due to differences in density. Thus, differences in the behavior of gases and liquids becomes highly relevant in designing suitable wastewater treatment systems for use in space. During its first years of operation, the ISS water recovery system experienced issues related to the lack of gravity, for example, two-phase fluid dynamics were altered, affecting the operation of the different unit treatments in the system [11]. Current treatment technologies require the presence of pressurized systems to achieve the flow of waste streams and to separate gas and liquid phases [33].

Partial gravity is experienced in environments like the surface of the Moon (0.17 *g*) or Mars (0.38 *g*), and density driven processes in these partial gravity environments display characteristics similar to those encountered in terrestrial systems; in gravity or density driven systems, this would be contrary to behavior in low-Earth-orbit spaceflight conditions. Water treatment technologies designed for the EPB systems will experience partial gravity and can then make use of gravity-dependent phenomena, such as particle settling. However, the rate and extent of settling or flotation will differ from terrestrial 1 *g* systems. Proposed treatment technologies should be tested under different gravity scenarios and periods of time to assure their functionality and robustness, *e.g.*, testing under short duration (<90 days) microgravity, long duration (>90 days) microgravity, periods of dormancy (>90 days), and planetary surface conditions with partial gravity [34].

Given the physical constraints in spacecraft and extraplanetary bases, technologies designed for space have unique technical considerations. Reactor size and mass are top considerations for any space system due to constraints imposed by launch vehicles for mass delivery, as well as the cost of payload to reach low-Earth orbit (*i.e.*, the cost of payload can range between $1200 per pound with companies like SpaceX, to $30,000 per pound for the now retired NASA space shuttles used until 2011) [35]. Furthermore, if a system is too large for a single launch vehicle, the system must be modular and will require in-space assembly; thus, any technology proposed should be designed to minimize the volume, footprint, and overall mass [33]. The proposed treatment systems in space must be self-contained (closed environment), reliable, durable, robust, lightweight and compact. Additionally, it is necessary to minimize the amount of supplemental chemicals used for wastewater treatment, particularly dangerous (*e.g.,* hazardous, toxic, or explosive) chemicals, to avoid potential risks to crewmembers since these systems will be housed inside. While biological systems are promising technologies for wastewater treatment, their use as standalone technologies in space is, at this point, unlikely; biological treatment systems specifically must be capable of producing a high-quality treated effluent with similar recovery efficiencies compared to what would be expected with physicochemical treatments (> 90%, *e.g.*, chemical treatment and physical desalination at the WRM system at the ISS) [11–13]. Thus, it would be expected that biological treatments would be used in combination with other physicochemical treatment units in space. It will also be crucial to determine optimal storage and recovery conditions of bioreactors after dormancy (>90 days). Further, due to the cost and feasibility of resupplying resources in space, all systems proposed for space applications must optimize and minimize the use of spares and consumables such as filters, disposable membranes, pretreat solutions, etc. Thus, there is a need to design ***self-sustaining systems*** that do not depend on re-supplying resources from Earth; these systems should ideally also require minimal maintenance and handling by crewmembers so that they do not have to spend significant periods of time attending these systems, which would take them away from performing other tasks.

When designing future water treatment technologies, a holistic approach should be taken, considering not only the needs for water treatment (*e.g.*, desired water quality) and recovery of VAPs from waste streams, but also the limited conditions for the transport of the proposed technologies and their compatibility and integration with already existing systems in space. Future wastewater treatment units could be modular to minimize space and overall mass and facilitate their transport, as well as to allow for easy and rapid exchange when necessary. Furthermore, it is crucial to have a comprehensive understanding of the already existing systems in space (*e.g.*, materials, fluxes, operation, etc.), so that the proposed technologies can be compatible and operate cohesively with what is already in use [33].

2.3. Resource recovery from used water in space

Different factors such as length of mission, distance, cost of propulsion, and resupply dictate the requirements for waste management in space [36]. While short-duration missions focus on collection, compaction and storage or disposal of waste, long-duration missions additionally require stabilization and disinfection of wastes, and resource recovery [36]. Thus, a paradigm shift from "waste removal" to "resource recovery" is necessary to advance waste management in space and reduce payload [33]. Converting waste into resources would reduce waste liabilities and increase sustainability of the treatment technologies for long-duration exploration or permanent habitation in ECLSS. However, current views are that human habitats will have to be resupplied with many critical resources until beyond 2030. Ultimately, ECLSS are envisioned to be regenerative and sustainable, which means that waste processing needs to be optimized so the loss of resources is

minimized to produce and/or recover water, nutrients, food and oxygen repeatedly and independently from resupplying these materials from Earth. Such systems are known as Bioregenerative Life Support Systems (BLSS) or Closed/Controlled Ecological Life Support Systems (CELSS) and their use would significantly reduce launch mass and therefore the costs of future missions.

On Earth, different biotechnologies and/or their combination with physicochemical treatments (*e.g.*, micro- & ultrafiltration membranes, osmosis systems, bioelectrochemical systems, etc.) have been integrated to concentrate, transform and recover resources from used waters, including municipal and industrial wastewater [37,38]. Under this premise, used waters in space are not a waste, but rather a resource; thus, the design of technologies for water treatment should also consider the recovery of VAPs, including clean water, fertilizers, nutrients, energy, metals, and secondary byproducts (*e.g.*, polymers) (Fig. 2).

Reusable water recovery. Efforts have been made to recover water from waste streams in space. Besides the recovery of water from the ISS WRM system [8,10], NASA has also explored the potential use of different technologies to recover water from brines, including aerosol dryers [39,40], wick evaporation [41,42], membrane systems [43,44], and bulk/surface drying [45–47].

Nutrient recovery. Currently, there are no technologies ready for space deployment to recover or reuse nutrients (*e.g.,* nitrogen and phosphorus) from waste streams; transformation of wastes for food production (edible biomass) will be essential to further close the loop in ECLSS [48]. Given its high concentration of nutrients, recovery of VAPs from urine would be a very attractive alternative in space. Through treatment of source-separated urine (separated from other waste streams), nutrients such as urea, ammonia, nitrate, phosphate and different mineral precipitates like struvite ($MgNH_4PO_4*6H_2O$) could be recovered [49] and potentially be used to amend food production compartments (*e.g.*, as fertilizers for plants). The precipitation of struvite using ureolytic microorganisms (*i.e.,* microbes that produce enzymes that can split urea, releasing NH_4^+ and increasing the pH) has been proposed as an alternative to recover phosphorus from wastewater in terrestrial systems. Due to its slow-releasing properties [50], struvite has been suggested as an alternative to P-rich fertilizers [51,200]; the recovered struvite in space could then be used as fertilizer for food production or could also be used as an alternative source of nutrients for cultivating algae [52]. Different forms of nitrogen can also be used as nutrients; for instance, photoautotrophic microbes (*e.g.*, microalgae and cyanobacteria) can assimilate nitrate (NO_3^-), urea or ammonium (NH_4^+), and could be used in space to produce biomass. Current nitrogen recovery processes on Earth include ammonia stripping, struvite precipitation, and membrane technology [53].

Biogas recovery. Depending on the treatment technology used (*e.g.,* bioelectrochemical systems, anaerobic digestion, etc.), different gas streams such as methane, hydrogen, and carbon dioxide can be produced, all of which could be used to produce energy [54]. Another gas stream that could be recovered is $N_{2(g)}$, which is used for balancing the atmosphere in the air revitalization system; crew air needs to be balanced and topped off with makeup air against incidental losses. O_2 can be produced from water, CO_2, and other sources; however, $N_{2(g)}$ is more difficult to obtain, and it would have to be hauled from Earth. Timmer et al. [201] estimated that a long-duration (1000-day) deep space exploration (*e.g.,* to Mars) mission with 4 crewmembers would require 8–74 kg of $N_{2(g)}$. Thus, recovery of N as $N_{2(g)}$ from used water and/or urine (*e.g.,* through nitrification/denitrification processes) would add great value to long-duration and habitation missions.

Other product recovery. On Earth, there are different emerging VAPs including bioplastics and microbial protein, that have been recovered from industrial and municipal wastewater. Bioplastics such as polyhydroxyalkanoates (PHAs) are plastics made from bio-based polymers that can be recovered from biomass, produced by different microorganisms (*e.g.*, algae, bacteria, fungi), or synthesized by bio-derivatives [37]. On Earth, PHAs have been successfully produced from pure and mixed cultures (*i.e.*, activated sludge) [55]. Protein-rich biomass, a.k.a microbial protein, is a VAP that could be generated from nutrients and CO_2 recovered from used waters. On Earth, microorganisms including algae, fungi and bacteria have been used to produce microbial protein that could be used as feedstock for animals [56,57]. To satisfy microbial growth for microbial protein production, a C:N ratio >5 in wastewater should be required (considering $C_5H_7O_2N$ as the formula for biomass [58]); given the low C:N ratios in used water in space, added sources of carbon would be a consideration in space. The production of microbial protein via electrochemical ammonia recovery from source-separated urine is one example; here, the co-production of hydrogen allowed for the direct production of microbial protein from gaseous phase hydrogen using hydrogen oxidizing bacteria [59]. This approach could potentially be used in long-duration or habitation missions in space. Currently, there are no technologies available in space for the recovery of other products such as PHAs or microbial protein, which would be crucial for habitation systems.

Metals recovery. The use of microorganisms to remove and recover metals and metalloids from different metals-laden wastewaters (*e.g.*, acid mine drainage, industrial wastewater) has shown promise on Earth.

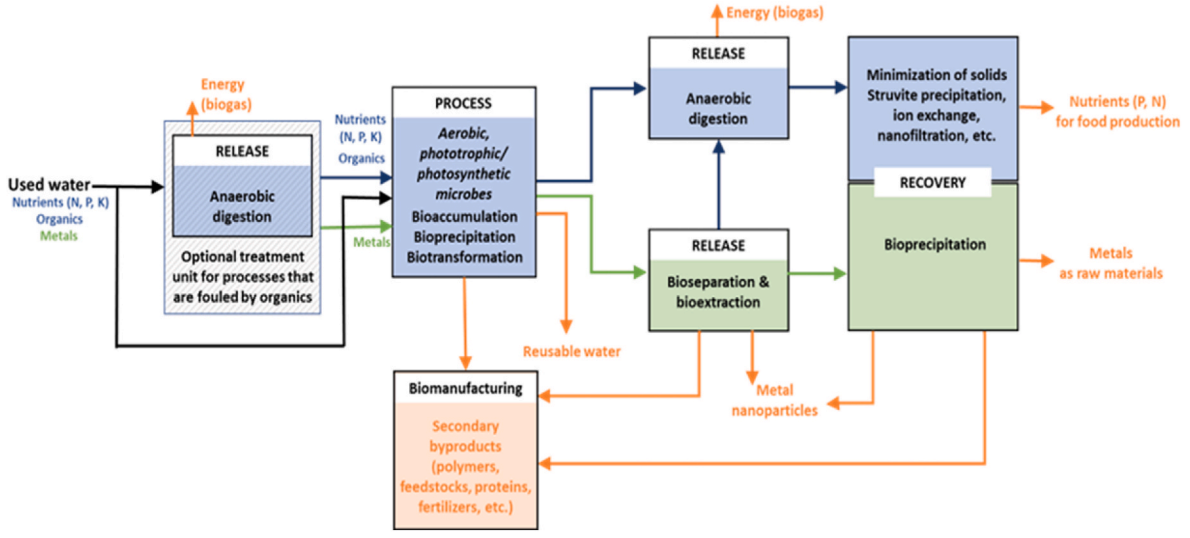

Fig. 2. Fig. 2 Process (Partition)-Release-Recovery concept for wastewater treatment and potential recovery of value-added products (*e.g.,* nutrients, feedstocks, metals, etc.) in space. Based on proposed systems used on Earth for municipal wastewater [198,199].

For instance, metals/metalloids can be recovered as nanoparticles synthesized by microbes; metal nanoparticles have unique characteristics (different from bulk materials) and have been widely used in different applications, including antimicrobials, biocatalysts, sensors, etc. [60]. While the recovery of metals and metalloids from used waters in space would be of interest, this would depend on the concentrations of these inorganic pollutants. Eventually, this could be of interest in habitation missions.

3. Biofilms in wastewater treatment: principles and applications

On Earth, the use of attached-growth microbial systems to remove organic carbon and nutrients (*e.g.*, nitrogen and phosphorus) from various waste streams dates from over 100 years ago [61,62]. These types of systems rely on the formation of biofilms, in which microbial cells, responsible for the conversion of organic material and nutrients, grow attached to the surface of support media and are embedded in a self-produced matrix of extracellular polymeric substances (EPS) [63,64]. Most knowledge on biofilms has been elicited from research on different medical, ecological, biotechnological and environmental engineering fields [65–68]. Biofilms develop through different stages, often starting with a pre-conditioning step in which macromolecules and nutrients sorb to a surface, followed by cell transport adhesion and then irreversible attachment of cells; next, microbial microcolonies develop and EPS are produced, followed by maturation of the biofilm and finally partial biofilm detachment [64,69,70]. Due to the social and physical interactions among microorganisms and the unique properties of the EPS matrix, biofilm communities exhibit "emergent properties" that differ from the known properties of individual (free-living) microbial components of the biofilm [71]. For instance, biofilms exhibit social cooperation and enhanced resistance to antimicrobials and other stress conditions. This is typically attributed to different factors including the presence of the EPS matrix, the inherent physiological heterogeneity of biofilms, adaptive microbial strategies and presence of persister cells [71–73].

Biofilm systems or biofilm reactors (terms used interchangeably) consist of four main components: the biofilm, the surface on which the biofilm grows (*i.e.*, substratum or carrier), the bulk fluid (*e.g.,* waste effluent in wastewater treatment), and the gas phase if the system requires or contains one (*e.g.*, air or oxygen, hydrogen, methane, nitrogen, etc.) [67], (Fig. 2). In wastewater treatment applications, substrata used to retain and grow microorganisms traditionally consist of inert materials such as rocks, stones, sand, wood, rubber, plastic and ceramics. There are new materials used in biofilm reactors known as "active substrata" that provide not only a support for biofilm growth, but also other functionalities; for example hollow gas transfer membranes allow for the delivery of electron donors (*i.e.*, chemicals that donate electrons to another compound in redox reactions during microbial growth), whereas the anode of an electrochemical cell can act as an electron acceptor (chemicals that accept electrons transferred to it from other compounds) in biofilm reactors [74]. The spatial-temporal characteristics (*e.g.*, architecture) and physicochemical heterogeneity of biofilms in wastewater treatment reactors will depend on different factors, including the type of carrier, microbial community (metabolism, physiology and interactions), physicochemical characteristics of the wastewater to be treated, and operational conditions in the reactor such as shear stress, retention times, etc. [71,75].

Differently from suspended-growth systems, such as activated sludge systems, biofilm reactors mostly retain microorganisms within the biofilm, although microbial cells will periodically detach from the biofilm and potentially exit the reactor along with the treated effluent. In biofilm reactors, microbial cells can be either attached to fixed or free moving carriers [67]. In fixed-media systems, the biofilm forms on a static medium in the reactor, examples of this type of reactor include the membrane biofilm reactor and fixed-bed biofilm reactors (*e.g.*, trickling filters). In moving-media systems, the biofilm develops attached to carriers that are constantly or intermittently being moved mechanically or by the fluids themselves [76]. Examples of these reactors include the rotating biological contactor, moving bed biofilm reactor, and the fluidized bed reactor. The different types of bioreactors used in wastewater treatment will be discussed in the next section. The fact that microorganisms are (for the most part) attached to carriers creates a clear distinction between the biofilm biomass and the bulk liquid in the reactor, resulting in Refs. [67,77]:

i. Separation of the biomass from the treated effluent. If biomass is retained effectively, downstream biosolids separations from biofilm reactors can represent an advantage over suspended growth systems in which a separation process (*e.g.,* sedimentation or membrane separations) is needed to remove biomass from the bulk liquid. Even in biofilm systems, biomass removal will be necessary, albeit, typically to lower extents than in suspended systems. While not reviewed here, processes for liquid–solids separation can include gravity dependent processes of dissolved air flotation, sedimentation basins, and flocculation/settling, as well as other processes that may be less sensitive to microgravity and partial gravity including membrane (ultra- and micro-) filtration, granular media filtration, and porous-disc filtration.
ii. Diffusional substrate concentration gradients. Transport of substrates into and through the biofilm can occur via diffusion which slows desired reaction rates. How deep substrates penetrate the biofilm depends on the porosity and/or diffusivity of the biofilms, concentration of the substrates in the bulk liquid, as well as mass transfer and reaction rates inside the biofilm.
iii. Stratification of biofilms. In multi-species biofilms, the formation of diffusional substrate concentration gradients can promote microbial growth rate gradients as well; fast growing microorganisms will typically be found on the outer layer of the biofilm in areas of greater exposure to the growth limiting substrate in the bulk fluid, whereas slow growing (and often more efficient) microorganisms are typically found on the inside of the biofilm where they are more protected from removal. This stratification protects slower-growing microorganisms from external shear forces, biocides, detachment and wash-out. The stratification can also enable the establishment of multiple seemingly exclusive metabolisms inside a very thin (10s–100s of micrometers) biofilm, such as aerobic ammonium oxidizers on one side of the biofilm while nitrate reduction occurs in the absence of oxygen only a few micrometers away on the other side of the biofilm.

Due to biofilms' unique structures, functions and emergent properties [71,78], biofilms can have increased robustness and resistance to toxic and recalcitrant compounds, leading to more stable communities and more effective transformation of substrates. Thus, on Earth, biofilm reactors have been widely used in wastewater treatment applications for the removal of organic carbon (measured as COD and BOD) and nutrients (*e.g.,* nitrogen and phosphorus).

The removal of total nitrogen (TN) from wastewater is one of the major goals in wastewater treatment plants. Biological nitrogen removal in wastewater starts with the nitrification process, in which the oxidation of inorganic nitrogen as ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) is carried out by a group of autotrophic aerobic microorganisms (utilize inorganic carbon and O_2) known as nitrifiers. In a secondary step known as denitrification, microorganisms in anoxic environments (devoid of O_2) perform the reduction of nitrite and nitrate to nitrogen gas (N_2) [79]. Denitrifiers may be heterotrophic (utilizing organic carbon as electron donor) or autotrophic (utilizing electron donors such as sulfur or H_2). It should be noted that in some systems anaerobic ammonium oxidation (anammox) is being established as an alternative to denitrification [80,81]; the anammox process is attractive because of its high efficiency and low energy consumption. Furthermore, anammox bacteria do not require an organic carbon source, the

production of sludge is minimized compared to aerobic processes, and there is a reduction in CO_2 emissions. However, slow growth of anammox bacteria and retention of bacteria in the reactor remain challenges of anammox processes [202].

Given that nitrification and denitrification are performed by diverse groups of microorganisms that thrive under different environments, as outlined above, these processes typically are carried out individually in sequential bioreactors or in different steps as part of the same system [82]. However, nitrification and denitrification processes, either individually or simultaneously, have been successful in biofilm reactors [83], and simultaneous nitrification and denitrification occur naturally in biofilm systems due to the inherent O_2 gradients across a biofilm, where nitrifiers are active in the oxic zones of the biofilm, while denitrifiers thrive under the anoxic zones of the biofilm (Fig. 3). Both groups of microorganisms, nitrifiers and denitrifiers, utilize different dissolved organic and inorganic carbon sources present in the bulk fluid, which results in a simultaneous COD removal. The successful removal of TN by heterotrophic biological systems depends on having an appropriate carbon-to-nitrogen ratio (C:N), if there is not enough carbon in the system (*e.g.,* COD), the removal of nitrogen, particularly in the denitrification step, becomes limited [84]. A common practice to achieve proper C:N ratio for the denitrification process is to add external organic carbon sources (*e.g.,* acetic acid, methanol, etc.) to the reactor to ensure sufficient electron donors [85].

4. Biofilm reactors as technologies to treat used water in space

4.1. Influence of space conditions on biofilms

The microgravity environment experienced during space travel (10^{-3} to 10^{-6} times the terrestrial gravity) impacts microbial life [29,30]. The lack of buoyant convection under microgravity conditions results in low-shear fluid environments for microbial growth. Reduced convection creates substrate and metabolic product concentration gradients around microbial cells, which has been proposed to be one of the main factors behind altered microbial behavior in space [86]. Microgravity seems to influence growth and cell physiology [87–91], biofilm formation [92–98], stress resistance [87,99] and virulence [89,100,101].

To consider implementation of biofilm reactors in space, it is crucial to have a fundamental understanding of biofilm formation and development under space conditions. Biofilm formation in space has been demonstrated; various studies have reported fungal and bacterial biofilm formation on different surfaces and water systems in space shuttles, the ISS, and the MIR space station [102–106]. Moreover, several microorganisms with the ability to form biofilms have been isolated from spacecraft environments, including water and waste lines in the ISS [107–109]. Although research on biofilms in space is still in its infancy, in general it has been observed that biofilm formation increases in some microorganisms (*e.g.*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli*) in both spaceflight and ground-based simulated microgravity studies [95,96,101]. Under simulated microgravity conditions, *E. coli* biofilms were thicker and showed increased resistance to stress (*e.g.*, salt, antibiotics) compared to biofilms of the same species grown under normal gravity conditions [93]. Moreover, production and composition of EPS can also be affected under reduced gravity conditions; Mauclaire and Egli [203] observed increased EPS production under microgravity conditions in *Micrococcus luteus* strains isolated from the ISS, as compared to the Earth reference strain under Earth gravity conditions. EPS contained less colloidal carbohydrates and proteins, depending on the isolate, under microgravity conditions [203]. While most biofilm studies under microgravity conditions have focused on bacteria, a few studies have been performed on fungi. Experiments with yeast (*i.e.*, unicellular fungi including *Candida albicans* and *Saccharomyces cerevisiae*) under simulated microgravity showed that low-shear environments result in differential expression of genes, increased filamentation, increased or decreased biofilm formation (depending on the system) and randomness in the budding (asexual reproduction) pattern [110–112]. More extended discussion on biofilms, their impacts on human health, strategies to prevent their growth and relevance to space travel can be found elsewhere [113–118].

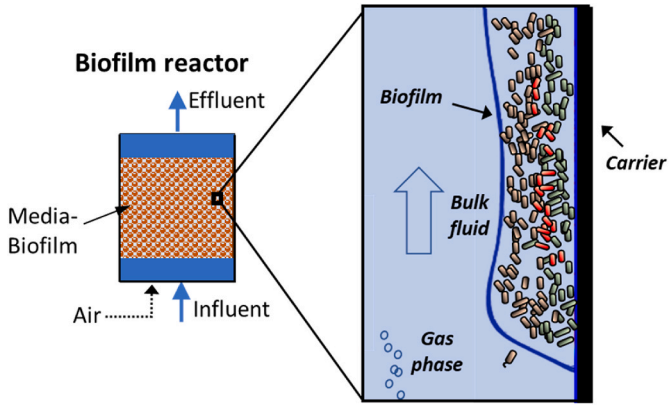

Fig. 3. Elements of biofilm reactors: Biofilm, comprised of microorganisms embedded in a matrix of extracellular polymeric substances; Carrier, fixed or free-moving surface to which the microorganisms attach; Bulk fluid, which is the liquid that contains nutrients and/or compounds to be removed; and the gas phase (*i.e.*, air bubbles), which can be optional depending on the system.

Most information regarding biofilms in space is focused on the impact on human health. However, if bioprocesses, specifically biofilm-based technologies, are to be used for water and waste treatment, along with recovery of resources, further studies should focus on environmentally relevant (*e.g.,* pollutant-degrading) microorganisms and resource-producing (including food and other high-VAPs) microorganisms and their behavior in space. It is also important to gain more knowledge on the different microbial mechanisms involved in biofilm formation and the performance of biofilm reactors including the following: microbial growth, cell attachment and detachment, colony formation, cell aggregation, biofilm thickness, EPS production rate and composition on Earth and in space.

4.2. Advantages and disadvantages of biofilm reactors

For applications in space, traditional biological suspended-growth systems are not ideal for use in wastewater treatment under microgravity conditions due to their reliance on air/gas buoyancy in the reactor and their significantly large amounts of biomass (sludge) generated [16]. However, suspended-growth systems tend to have higher unit process rates compared to immobilized biomass systems; these systems could be potentially used in partial gravity environments (*e.g.,* Mars or the Moon). There are several advantages to biofilm reactor systems for the treatment of waste streams compared to conventional suspended-growth systems. Biofilm reactors typically have lower energy demands, with simple operation and maintenance, increased operational stability, and reduced hydraulic retention time [76,119]. Biofilm reactors often allow for high volumetric loadings to be treated without the need for liquid-solid separation or solids recirculation (biomass) to the reactor [120]. Furthermore, biofilm reactors exhibit lower sludge production, with minimal issues with sludge bulking and better sludge thickening properties [76]. Biofilms offer high specific surface area, which allows for an increased utilization of substrates (*i.e.*, nutrients and contaminants) from the bulk liquid in a smaller space; this lowers the volume requirements for biofilm reactors [76]. Biofilms have a very heterogeneous nature fostering increased availability of different ecological niches within the biofilm, which allows the co-existence of microorganisms with different growth requirements (*e.g.,* aerobic and anoxic conditions near each other). Increased biofilm microbial

diversity may allow systems to degrade a wide range of complex substrates (*e.g.*, organic pollutants) [71,78]. Moreover, as described above, the EPS matrix confers protection to the cells within the biofilm, which provides biofilm reactors with a higher tolerance to variation in waste stream characteristics (*e.g.,* temperature, pH, toxic compounds) and shock loads [120]. Biofilm reactors also allow for easy separation of the biomass and the treated effluent, which reduces the need for post-treatment to separate the liquid phase from the microbial cells (solid phase). Lastly, on Earth, biofilm reactors have also been used to produce and/or recover high VAPs, including treated water, nutrients, biomass and various microbial products [45,121,122]. This is relevant for technologies to treat used water in space. Converting waste into resources would reduce the liabilities of wastes, increase sustainability of the systems, allow for integration of the treatment technology to further close the loop of life support systems, and recover valuable resources that would otherwise have to be transported into space (Fig. 5).

Whereas biofilm reactors can have advantages over traditional suspended-growth systems for wastewater treatment, there are some inherent limitations. Biofilm reactors generally have longer start-up times since these systems require time for the formation of active biofilms on the carriers [77,123,124]; this is a bigger issue for systems that use anaerobic biofilms, in which the start-up period can take long periods of time, especially compared to aerobic processes [123,124].

One of the main challenges in co-diffusional biofilm systems is the diffusional limitation within the biofilm due to poor mass transfer, which results in reduced reaction rates. Biofilm thickness is key in the successful operation of biofilm reactors, and it is also one of the most difficult parameters to control. Biofilm thickness can impact biofilm structure, activity and biodiversity [125]. Depending on the system (*e.g.,* microorganisms and environmental conditions), there is a maximum biofilm thickness that allows for optimal transfer of O_2, nutrients and removal of pollutants (*e.g.,* COD) in the biofilm. Thin biofilms might not provide sufficient active biomass to reach the desired conversion. Increased biofilm thickness can limit the diffusive transport of O_2 and substrates into the biofilm [77], leading to decreased biofilm efficiencies [126,127]. Furthermore, excess biomass accumulation in biofilm reactors and carrier materials can lead to clogging of the reactors or carriers, which results in decreased reactor working volume and thus reduced treatment capacity of the system [123,124]. Optimal biofilm thicknesses can be predicted based on well-determined reaction and diffusion kinetics [126,127].

4.3. Biofilm reactor configurations

Biofilm reactors can be classified based on the number of phases involved (*i.e.*, air, bulk water, biofilm-loaded carrier), how electron donors and acceptors are applied, and whether the biofilm is attached to fixed- or moving-media [67,128]. In this review, biofilm reactors (Fig. 6) will be classified into fixed-bed (media), expanded-bed (moving media), and hybrid reactors. The next sections will discuss the types of reactors that have been proposed for potential treatment of used water in space and will discuss the feasibility of such systems under different gravity conditions, relevant to different types of missions.

4.3.1. Fixed-bed reactors

Fixed-bed reactors (FBRs) are characterized by the attachment and development of the biofilm on static media; this type of reactors can be non-submerged such as the trickling filter (Fig. 6A), partially submerged like the rotating biological contactor (a.k.a. Rotary disc) (Fig. 4B), or fully submerged such as packed-bed reactors (Fig. 6C) and membrane biofilm reactors (Fig. 6D) [67,128]; Sørensen 2020). Except for a trickling filter reactor, FBRs do not rely on buoyancy, and thus these systems could be used under microgravity conditions. The FBRs that have been proposed to treat space-based wastewater are discussed below.

4.3.1.1. Trickling filter biofilm reactor. The trickling filter biofilm reactor (TBR), also known as biofilter, is a type of non-submerged FBR. In this three-phase biofilm reactor, the liquid is flowing through a packed bed with biofilm covered media (*e.g.,* plastic or inert mineral carriers); the bed is never fully saturated and contains a gas (*e.g.*, air) and a liquid (*e.g.*, water) phase (Fig. 6A) [130]. Air can be supplied either by mechanical means such as air distribution piping and fans, or by natural ventilation [130,131]. The treated effluent exits the reactor but is often recirculated a number of times to achieve the desired water quality (*e.g.,* removal of pollutants). Like other biological systems, TBRs require a liquid-solid separation unit, which most commonly consists of a secondary clarifier [130]. Different factors affect the efficiency of TBRs, including the type of filter media (*e.g.,* plastic rings, polystyrene, rubber, gravel, zeolite, sponge, etc.) [131,132] and its characteristics (*e.g.,* specific surface area, weight), as well as the void ratio in the packed bed (*i.e.,* fraction of the total volume in the reactor filled with air after the media has been filled into the filter) [133,134]. On Earth, TBRs have been used for the treatment of different waste streams, including municipal and industrial wastewater, to oxidize organic carbon, perform

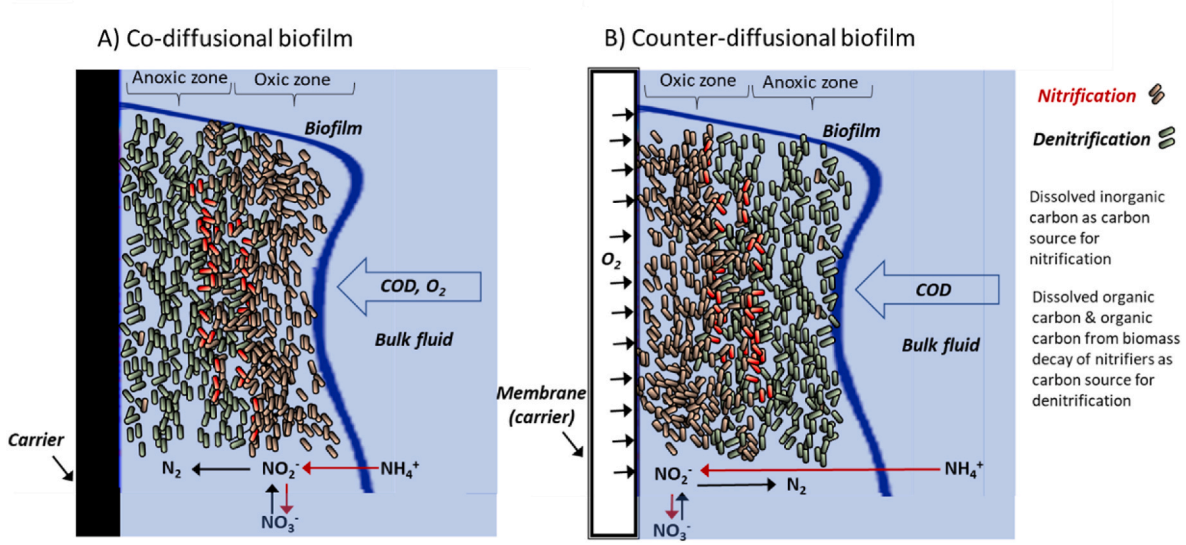

Fig. 4. Substrate conversion and simultaneous aerobic nitrification and heterotrophic denitrification in a (A) co-diffusional biofilm, and (B) counter-diffusional biofilm. Modified from Ref. [83].

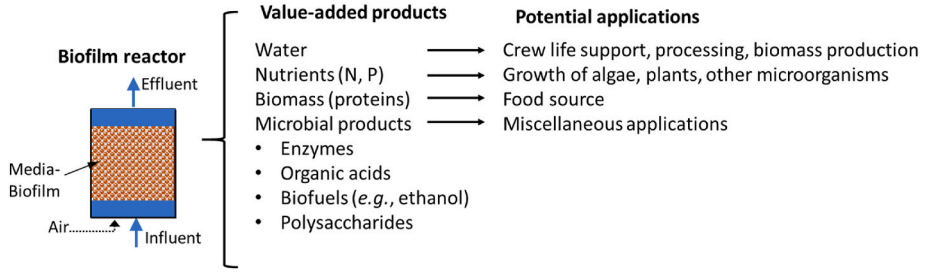

Fig. 5. Value-added products obtained during water treatment and potential applications.

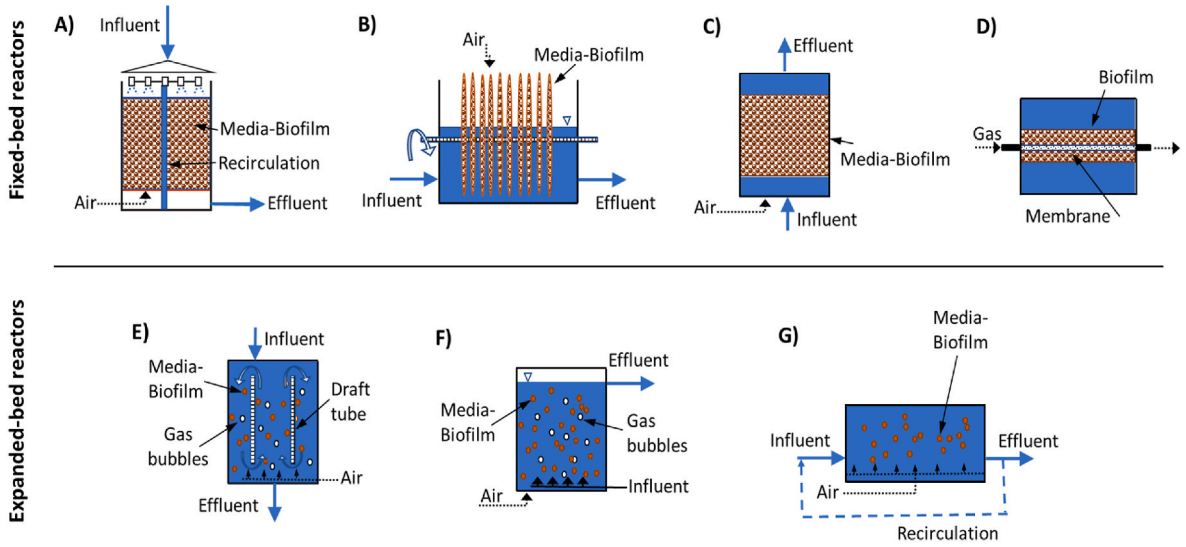

Fig. 6. Biofilm reactor configurations. Fixed-bed reactors: (A) Trickling filter, (B) Rotating biological reactor, (C) Packed-bed reactor (up-flow), (D) Membrane biofilm reactor. Expanded-bed reactors: (E) Suspended reactor (airlift), (F) Fluidized bed reactor, and (G) Moving bed biofilm reactor. Modified from Ref. [129].

nitrification or denitrification [134–136], and even remove toxic heavy metals such as Cr^{6+} [137]. TBRs have been used in combination with other biological systems to enhance the removal of pollutants from waste effluents; for instance, TBRs have been used as a pre-treatment unit for wastewater with high organic loads [138] before activated sludge treatment, and as post-treatment after up-flow anaerobic sludge blanket reactors [139]. TBRs are characterized by high concentrations of cells and microbial activity, high tolerance for treating effluents with varying hydraulic and organic loads, low energy requirements, low cost, simplicity and ease of operation and maintenance; furthermore, compared to suspended processes such as activated sludge, TBRs typically have shorter treatment times [130,140]. Some disadvantages of TBRs include the possibility of clogging, poor nutrient removal efficiency (specifically for denitrification, as this process requires the absence of oxygen and preventing the natural convection of air in the TBF is not feasible) and limited oxygen transfer efficiency in the reactor, [140,141].

Few TBRs have been proposed to treat synthetic waters with compositions similar to used waters in space [142,143], (Table 3); however, it should be noted that these studies have been performed on Earth with synthetic wastewater and have not been tested under microgravity conditions. TBRs rely on gravity and on density differences between air and water to maintain unsaturated water flow. Thus, TBRs are the least likely to be used for space operations under microgravity conditions; operations under microgravity would likely cause improper air and water flow, resulting in poor performance. Partial gravity TBR's may operate similar to here on Earth, but fluid dynamics of these biofilm reactors should be explored more thoroughly before application on the Moon or Mars.

Table 2
Compounds transformed in MBfRs using different gas-phases.

Gas	Pollutant removed	References
Oxygen/Air	Carbonaceous and nitrogenous compounds.	[144–148]
Methane (CH_4)	SeO_4^{2-}, BrO_3^-, ClO_4^-, CrO_4^{2-}, NO_3^-/NO_2^-	[97,98, 149–153]
Hydrogen (H_2)	SO_4^{2-}, CrO_4^{2-}, AsO_3^-, TCE, ClO_4^-, BrO_3^-, SeO_4^{2-}	[154–158]

4.3.1.2. Packed-bed reactor. The packed-bed biofilm reactor (PBR) is a reactor packed with solid support media engineered to provide high surface area to promote biofilm formation while also allowing wastewater to flow through the system. These reactors, as defined here, are completely saturated and, on Earth, are usually fed from the bottom and operated in up-flow mode (Fig. 6C), although downflow configurations also exist (Sørensen 2020). In PBRs, the concentration of substrates decreases axially from the inlet to the outlet of the reactor. This type of reactor can be run under aerobic or anoxic conditions (*e.g.*, when denitrification is desired). Most commonly, this type of reactor is used as a three-phase system for aerobic processes, in which air or O_2 is supplied as gas bubbles [67]. When low levels of organic carbon are present in the wastewater, the electron donors for microbial processes may be insufficient for denitrification, such that addition of an external electron donor (*e.g.*, methanol, sugar or other non-defined C sources) is needed; the most common carbon-free electron donors used in PBRs include elemental sulfur, hydrogen, hydrogen sulfide and thiosulfate [167,168].

The type of support material used is key in PBR performance, as the carrier characteristics (*e.g.,* porosity and particle size) will determine biomass retention and overall reactor efficiency. Common support

Table 3
Summary of published studies on the potential use of biofilm reactors for wastewater treatment in space and extraterrestrial environments.

Reactor	System configuration & Operational conditions	Application and removal efficiency	References
Trickling filter reactor	**System configuration** *Influent*: simulated advanced life support graywater (TOC: 250 mg/L; COD: 880 mg/L; TN: 35 mg/L) *Reactor*: 60 cm long acrylic tube with top and bottom acrylic fitting; TV 5L. *Packing material*: Tri-packs SA: 281 m^2/m^3, OD: 2.5 cm; Bee-cell packing material, SA: 653 m^2/m^3, ID: 1.3 cm, Biobale SA: 825 m^2/m^3, ID: 0.4 cm. **System operation** Feed flow rate 5 L/d; recirculation flow rate 100 L/d; T: 20 °C; Gas supplied: air. Startup period: 40 d. Experiment 1: packing material: Tri-packs; operation: 65 d; 6 replicates. Experiment 2: packing material: Bee-cell (3 replicates) & Biobale (3 replicates); operation: 145 d.	Experiment 1: under steady-state performance (after 40 days), TOC removal: 65%; nitrification did not occur. Experiment 2: under steady-state performance (after 40 days), TOC removal: 50–65%.	[142]
Trickling filter reactor	**System configuration** *Influent*: simulated advanced life support graywater (COD 16.1 mM, 14.3 mM DOC, 6.9 mM TN, 0.4 mM $N–NH_4^+$) Operated to treat graywater along with gas phase contaminants (H_2S, NH_3). *Reactor*: 91 cm long acrylic tube with top and bottom acrylic fitting; ID: 10.2 cm; TV: 4.9 L. *Packing material*: 2.54 cm Jaeger rings **System operation** *Phase 1* → Fed-batch inoculation for 10 d (25 g activated sludge); flow rate 300 L/d for 24 h periods. Gas-phase flowrate (20% O_2) constant throughout inoculation at 2 L/min; no $H_2S_{(g)}$, $CO_{2(g)}$ or $NH_{3(g)}$ were supplied. *Phase 2* → Gas-phase loading experiments. Grey wastewater flow rate 5 L/d; recirculation flow rate 375 L/d; HLR 1.9 m/h; T: 20 °C; Total gas (H_2S, NH_3, CO_2) flowrate 1.24 L/min, air (20% O2) flowrate 0.52 L/min. Different concentrations of gases were used at different periods of time. CO_2 0–2660 ppm; $HN_{3(g)}$ 0–145 ppm; $H_2S_{(g)}$ 0–18 ppm. Total operation time: 300 d.	>90% removal efficiency of parent surfactant and waste gas constituents.	[143]
MABR	**System configuration** *Influent*: modified EPB wastewater Counter-diffusion Membrane Aerated Nitrifying Denitrifying Reactor. The system can be coupled to the osmosis secondary treatment system. Liquid-up flow configuration. *Reactor*: Column, shell: 158 L, OD: 45.7 cm, ID: 40.6 cm; Liquid: 104 L. *Membranes*: #membranes = 1775, SA = 27.6 m^2, SSA = 265 m^2/m^3. **System operation** Inoculation & start up period: 2 months. Continuous flow operation. Five testing periods were performed with various operational conditions: loading rates = 15–40 L/d; effluent as flow = 200–600 mL/min; Gas pressure = 7.5–9.5 psi; liquid pressure = 8–10.5 psi; operation time = 15–32 d Gas supplied: O_2 or air.	Removal C 90%, nitrification 70%, denitrification 50%.	[159]
MABR	**System configuration** *Influent:* ISS (DOC 780 g/m^3; TN 740 g/m^3), EPB Ersatz (DOC 2300 g/m^3; TN 2200 g/m^3) *Reactor:* rectangular configuration with cross flow; dimensions: L = 90 cm, W = 52 cm, H = 50 cm; TV = 0.23 m^3; Liquid volume: 0.095 $m^{3.}$ *Membrane:* OD = 0.55 cm; #membranes = 1755. SSA = 100 m^2/m^3. **System operation** Continuous flow operation for EPB and ISS; pulses for ISS. *Continuous EPB*: 15 L/crew·d; operation time = 15 d; V treated = 451 L; *Gas supplied*: air (30 L/d). *Continuous ISS*: 4 L/crew·d; operation time = 42 d; V treated = 340 L. *Pulse ISS*: 4 L/crew·d; operation time = 28 d; V treated = 227 L. *Pulse ISS + pulse hygiene*: 11.5 L/crew·d;	Continuous EPB: C removal 87%; 45% nitrification. Continuous ISS: C removal 94%; 49% nitrification. Pulse ISS: C removal 93%; 47% nitrification. Pulse ISS + pulse hygiene: C removal 80%, 54% nitrification.	[160]

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Table 3 (*continued*)

Reactor	System configuration & Operational conditions	Application and removal efficiency	References
	operation time = 14 d; V treated = 316 L.		
MABR	**System configuration** *Influent:* EPB (DOC 410–790 mg/L; TN 540–840 mg/L), HC (DOC 200–270 mg/L; TN 30–42 mg/L), ISS (DOC 1800–2600 mg/L; TN 1900–3300 mg/L), urine (DOC 200–4500 mg/L; TN 3200–5100 mg/L) *Reactor:* Seven (A1,2; B1-5) rectangular MABR were used. *A1*: L = 82 cm, W = 53 cm, H = 40 cm; TV = 173 L; LV: 104 L; #membranes = 1755; SSA = 117 m^2/m^3; treated EPB, HC. *A2*: L = 81 cm, W = 44 cm, H = 40 cm; TV = 143 L; LV: 110 L; #membranes = 1552; SSA = 90 m^2/m^3; treated EPB, ISS. B1: L = 66 cm, W = 30 cm, H = 58 cm; TV = 115 L; LV: 90 L; #membranes = 1228; SSA = 105 m^2/m^3; treated EPB, urine. B2, B3: L = 69 cm, W = 31 cm, H = 50 cm; TV = 107 L; LV: 48 L; #membranes = 1228; SSA = 119 m^2/m^3; urine. B2, B3: L = 30 cm, W = 15 cm, H = 30 cm; TV = 13 L; LV: 11 L; #membranes = 307; SSA = 104 m^2/m^3; urine. **System operation** MABR treating EPB, ISS waste streams: continuous and On-production modes. MABR treating HC and urine waste streams: continuous mode. Operation time = 1.5–5 years. HRT: EPB, 3.9–7.7 d; ISS, 16–32 d; urine, 20–100 d; HC, 6–28 d. *Gas supplied*: Air; different DO concentrations tested: fully aerobic (>2 mg/L) and low DO (<2 mg/L).	ISS: OC removal 84–98%; ON oxidation efficiency 56–70%. EPB: OC removal 80–98%; ON oxidation efficiency 35–70%. HC: OC removal 80–94%; ON oxidation efficiency 36–81%. Urine: OC removal 90–99%; ON oxidation efficiency 30–70%.	[16]
MABR	**System configuration** *Influent:* EPB (DOC, 540–780 g/m^3; TN, 644–880 g/m^3), Transit (DOC, 950–1000 g/m^3; TN, 590–1500 g/m^3), ISS (DOC, 1900–2700 g/m^3; TN, 2100–3100 g/m^3)	EPB wastewater:	[161]

Table 3 (*continued*)

Reactor	System configuration & Operational conditions	Application and removal efficiency	References
	Reactor: L = 89 cm, W = 51 cm, and H = 51 cm; TV = 0.23 m^3	Aerobic mode: DOC removal 85–95%; ON oxidation efficiency 50–60%; N removal 10–20%. Anoxic mode: DOC removal 62%; ON oxidation efficiency 50%; N removal 26%.	
	Membrane: siloxane; OD = 0.55 cm, #membranes = 1600.	Transit wastewater: Aerobic & anoxic mode: DOC removal 85–86%; N removal 60%.	
	System operation Continuous and on-production modes; aerobic (DO > 3 mg/L) and anoxic (DO ~0.5–2 mg/L). *Gas supplied*: 100% air to 100% O_2 (0.6 L/d).	ISS wastewater: Aerobic mode: DOC removal 90–95%; ON oxidation efficiency 60–65%; N removal 13% Anoxic mode: DOC removal 90–95%; ON oxidation efficiency 70%; N removal 50–55%.	
Modified-MABR	**System configuration** *Influent:* EPB Ersatz (COD:N = 1.8; NH_4^+-N = 650 g/m^3) *Reactor dimensions:* ID = 15.2 cm, L = 35.5 cm; TV: 6.4 L. *Gas headspace:* 0.54 L *Membrane:* silicone; ID-0.1 cm, OD-0.21 cm; L = 2.4 × reactor L; # membranes = 100. *Gas supplied:* Air. *Modification:* additional surface area was provided by adding polyethylene biospheres (106 cm^2, 6.3×10^{-3} L) connected to the membranes. Total SSA = 291 m^2/m^3. **System operation** *Operation time:* 1 year start-up period, 3 years continuous operation. Different conditions were tested: Number of days (44–133); P_{air} (1.4–11 kPa); influent flow rate (0.22–0.86 L/d); HRT (5.9–23.4 d); RR (10–250); OLR (19.2–94 g DOC/m^3·d); NLR (46–123 g TN/m^3·d).	Nitrification, denitrification and carbon removal. Removal: TC: 75.3 g/(m^3·d) NH_4^+-N: 0.22 g-N/(m^3·d)	[19]
MABR	**System configuration** *Influent*: EPB Ersatz (DOC 436–593 mg/L; NH_4^+-N 565-1030 mg-N/L; C:N = 0.59–0.79). *Reactor:* acrylic; dimensions: ID = 10.2 cm; L = 25.4 cm; TV: 2.08 L; WV = 1.56 L. *Gas supplied:* O_2 *Membrane*: silicone; ID-0.1 cm, OD-0.21 cm; L = 3.6 × reactor L; #membranes = 150.	Nitrification: 65%, Denitrification: 35%. DOC removal 0.33 g-C/(m^2·d), nitrification 0.32 g-N/(m^2·d); TN removal efficiency: 36.5%	[162]

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Table 3 (*continued*)

Reactor	System configuration & Operational conditions	Application and removal efficiency	References
	Total SSA = 597 m^2/m^3. **System operation** *Operation time*: 555 d; HRT: 2.7–10.8 d; liquid velocity: 0.07–0.2 cm/min; P_{O2} (22.8–27 kPa); number of days (11–123); RR (50–250); OLR (44–207 g TOC/m^3·d); NLR (70–304 g TN/m^3·d); pH (6.2–7.9).		
Hybrid systems			
Packed-Bed reactor & MABR	**System configuration** *Influent:* Transit mission waste stream *Hybrid system:* the anaerobic packed-bed reactor was located upstream of the MABR. *Packed-bed (Up-flow)* *Reactor dimensions:* TV = 1.6 L; WV = 1.1 L *Packing material*: lava rock (SA = 0.2 m^2) *MABR Reactor:* cylindrical polyvinyl chloride column; WV = 3.56 L. *Membrane:* silicon; ID-0.08 cm, OD-0.17 cm; L = 2.3 × reactor L; #membranes = 150; Total SA = 1.09 m^2; Useable SA = 0.825 m^2. *Gas supplied:* Air (41 kPa). **System operation** Different conditions were tested: Number of days (30–85); influent flow rate (0.86–2.3 L/d); HRT (2.02–5.39 d).	Packed-bed reactor: carbon removal MABR: Nitrification, denitrification Removal: DOC removal 80–90% (400-600 g-C/[m^3·d]). TN removal < 0% (∼ 400 g-C/[m^3·d]).	[163]
Packed-Bed reactor & Trickling filter	**System configuration** *Influent:* wastewater generated by JSC crew (hygiene water, urine) confined in a test chamber for 91 d mixed with simulated humidity condensate. (TOC 247 mg/L; NH_4^+-N 150 mg/L). *Hybrid system:* the packed-bed reactor was located upstream of the TBR, followed by physicochemical treatment. *Packed-bed (Up-flow)* *Reactor:* acrylic cylinder; dimensions: ID = 25.4 cm, L = 218 cm; TV = 0.11 m^3. *Packing material*: 60 acrylic plates covered by a porous polymer support. *Gas supplied*: air (1.5 L/min); air was injected at 16.5 cm from the bottom of the reactor.	Overall removal efficiencies (considering TBR effluent quality): TOC removal 95%; NH_4^+-N removal 60%.	[164]

Table 3 (*continued*)

Reactor	System configuration & Operational conditions	Application and removal efficiency	References
	TBR Reactor: acrylic cylinder; dimensions: ID = 25.4 cm, L = 145 cm; TV = 0.07 m^3. *Packing material:* alternating layers of polypropylene Pall rings (2.5 cm) and ceramic saddles (6 mm). Gas supplied: air (2 L/min). **System operation** Influent flow rate: 75 mL/min of wastewater for both PBR and TBR; RR:150 mL/min of recycled water from the TBR for the PBR; 10,000 mL/min of recycled water from the TBR for the TBR. HRT: 1 d for PBR, 0.66 d for TBR.		
Pack-Bed reactor & MABR	**System configuration** *Influent:* EPB Ersatz (DOC 239–272 mg/L; NH_4^+-N 150–294 mg/L; TN 243–413 mg/L; C:N = 0.74) *Hybrid system:* the packed-bed reactor was located upstream of the MABR. *Packed-bed (Up-flow)* *Reactor:* polyvinyl chloride column; dimensions: ID = 7.6 cm, L = 43.8 cm; TV = 2.5 L; WV = 1.1 L; packing ratio = 0.69. *Packing material*: lava rock (5 cm). *MABR Reactor:* acrylic column; dimensions: ID = 10.2 cm, L = 54.7 cm; TV = 4.3 L; WV = 3.78 L. *Membrane:* silicon; ID = 0.08 cm, OD = 0.17 cm; L = 2.3 × reactor L; #membranes = 150; SSA = 186 m^2/m^3; Total SA = 0.825 m^2. *Gas supplied:* Air (20.7 kPa). **System operation** Different conditions were tested: Number of days (41–138); influent flow rate (0.72–5.04 L/d); HRT (0.98–6.8 d). RR: 10:1. Operation time: 417 d.	PBR: denitrification; MABR: nitrification. Removal: PBR: DOC removal (Avg 87%); TN removal (55%). MABR: nitrification efficiency 60–80%, low values associated with decreasing retention times.	[165]
Membrane-aerated, membrane-coupled bioreactor (M2BR)	**System configuration** Wastewater: modified EPB Ersatz, 4:1 COD to N ratio, COD 800 mg/L, TN 700 mg/L. *Hybrid system:* the effluent of the MABR was fed to an external filtration membrane module and returned to the bioreactor. *MABR Reactor:* polycarbonate column	COD & N removal: 90% Experiment 1: COD removal efficiency 87%; NH_3 removal efficiency neglectable. Experiment 2:Phase 1: NH_3 removal	[166]

(*continued on next page*)

Table 3 (*continued*)

Reactor	System configuration & Operational conditions	Application and removal efficiency	References
	(1.5 L) *Membrane:* polyolefin; OD = 0.3 cm; SA = 0.118 m^2; *Gas supplied:* Air or O_2 (200 mL/min @ 1 atm).	efficiency 90%. Phase 2: COD removal efficiency 90%. Phase 3: 90% removal efficiency COD and TN. Phase 4: COD 90% removal efficiency.	
	Filtration membrane module 0.2 μm pore size polysulfone microfiltration membrane cartridge, SA 0.042 m^2. **System operation** Flow rate to the MABR was adjusted to maintain 1.5 L in the reactor. Different conditions were tested: Experiment 1. Full-strength EPB Ersatz; HRT: 24 h; operation time: 66 d; no biosolids removal. Experiment 2. Four phases: Phase 1: 29 d; COD 0 mg/L; NH_3 200 mg N/L; Phase 2: 23 d, COD 220 mg/L, NH_3 170 mg N/L; Phase 3: 33 d, COD 480 mg/L, NH_3 180 mg N/L; Phase 4: 20 d, COD 780 mg/L, NH_3 700 mg N/L		
	Experiment 3. Phase 1: 46 d; COD 0 mg/L; NH_3 200 mg N/L; Phase 2: 51 d, COD 480 mg/L, NH_3 180 mg N/L; Phase 3: 12 d, COD 480 mg/L, NH_3 180 mg N/L; Phase 4: 17 d, COD 780 mg/L, NH_3 700 mg N/L.	Experiment 3: Phase 1: NH_3 removal efficiency 95%. Phase 2: NH_3 removal efficiency 90%. Phase 3: NH_3 removal efficiency 60–82%. Phase 4: NH_3 removal efficiency 40%.	

ID= Inner diameter; OD = outer diameter; L = Length; TC = Total carbon; TN = Total nitrogen; TV = Total volume; LV=Liquid volume; SSA= Specific surface area; SA = surface area; HRT= Hydraulic retention time; RR= Recycle ratio; OLR= Organic loading rate; NLR= Nitrogen loading rate; WV= Wetted volume; T = temperature; EPB = Early planetary base; HC=Humidity condensate. Continuous mode = waste streams were mixed in an influent tank daily and pumped into the reactor continuously over 24 h; On-production mode = each separate waste component was pumped into the reactor as produced during a nominal crew day.

materials used in PBRs include plastic rings, sand, gravel, rocks, ceramics, clay, and granular carbon, while alternative materials such as bamboo [169] have also been used in wastewater treatment. While high biomass concentrations in and on the carriers allow for high treatment capacities, clogging issues and channeling in the packed bed can arise, causing a decrease in reactor efficiency. To address this pitfall, periodic backwashing is performed. The use of carriers with high void fraction (*e.g.,* corrugated plastic media with void fraction higher than 90%) has been proposed as an alternative to decrease clogging issues in PBRs; these types of carriers provide enough surface for biofilm accumulation while allowing enough void space for the flow of fluids (air and water) [170].

Due to their operational flexibility and capability to provide large volumetric productivity, on Earth, PBRs have been used for the treatment of a variety of different waste streams including the dairy, food and beverage industry [171,172], petroleum refineries [170,173] and municipal wastewater [174]. Different PBRs have been proposed as part of hybrid systems (combination of one or more different types of reactors) to treat synthetic waters with similar compositions to those obtained from various used waters in space (see Table 3); these systems are discussed in Section 4.3.3. It should be noted that all the studies targeting treatment of used water in space with PBRs have been performed on Earth, with synthetic waters and have not been tested under microgravity conditions. In partial gravity, PBR's will likely operate similar to how they operate on Earth, but these biofilm reactors should be studied in additional detail before use on Mars or a lunar base as subtle changes in the fluid dynamics may impact operations.

4.3.1.3. Membrane biofilm reactor. Membrane biofilm reactors (MBfRs) use gas-permeable, typically hollow-fiber, membranes for both biofilm immobilization on the membranes' exterior and gas transfer [175,176] (Fig. 6D). In a MBfR, typically a gaseous electron donor or acceptor (*e.g.,* O_2, H_2, CH_4) diffuse through the membrane to the biofilm growing on the membrane; complementary substrates (electron donor or acceptor), typically in the form of waste products when used for water treatment, diffuse from the bulk liquid into the biofilm (Fig. 4B, [176]. As pressurized gas enters the biofilm through the membrane from the opposite direction as substrates from the bulk liquid, a counter-diffusional system develops (Fig. 4B, [176]. Having a counter-diffusional biofilm allows the diffusion of two substrates (*i.e.,* gas-substrate and substrate delivered with the bulk fluid) from opposite sides of the biofilm simultaneously, often making the inner (center) part of the biofilm the most metabolically active zone [176]. This is different from conventional biofilm systems in which the most active zone is often the outer layer of the biofilm (cf. Fig. 4A). Compared to traditional biofilms, counter-diffusional biofilms possess unique characteristics and behavior, including *(i)* stratification of the microorganisms within the biofilms, which promotes the existence and interrelation of microorganisms with different growth needs (*e.g.,* nitrifiers and denitrifiers) and *(ii)* low liquid diffusion layer resistance [175,176].

On Earth, MBfRs have been used in laboratory or pilot studies for the treatment of various waste streams, including municipal wastewater [177–181], digester influent and source separated urine [182,183]. Either on their own or in combination with other treatment units (hybrid systems), MBfRs have also been used for the treatment of high strength wastewaters [144–146], the removal of COD and nutrients (*e.g.,* nitrogen) via simultaneous nitrification and denitrification processes [147,148,162], as well as the removal of micropollutants such as pharmaceuticals [184]. As mentioned above, MBfRs can have different gas-phases. When air or O_2 are used as the gas phase, the system is often referred to as a membrane aerated biofilm reactor (MABR). In MABRs, O_2 is used as the electron acceptor to oxidize compounds (often organic and nitrogenous). In hydrogen (H_2)-based MBfRs, H_2 is delivered as the electron donor to be oxidized by microorganisms that reduce oxidized contaminants such as perchlorate (ClO_4^-), selenate (SeO_4^{2-}) and trichloroethylene (TCE) [154–156,185]. Similarly, methane (CH_4)-based MBfRs have been used for the bioreduction of selenate (SeO_4^-) [97,98,150], bromate (BrO_3^-) [151], perchlorate [152], chromate [153], and NO_3^-/NO_2^- [149]. Table 2 provides a summary of the compounds transformed in MBfRs using oxygen, hydrogen and methane as gas-phases.

Similar to other biofilm reactors, MBfRs provide advantages like supporting the growth of slow-growing microorganisms, decreased amounts of sludge production compared to suspended-growth systems, no requirement for aeration tanks and sludge storage, as well as the capability to maintain high biomass concentrations in the reactor [175,176]. Compared to conventional activated sludge systems, MBfRs have been suggested to have higher gas transfer efficiency, which results in lower energy consumption and thus costs [186]. Further, MBfRs require

small reactor volumes and allow for the development of mixed microbial communities with different growth requirements [175,176]; these types of reactors also display high functional stability against shock loads and toxic inhibitors [175,176]. Some of the challenges of MBfRs include biomass control and membrane fouling; as the thickness of the biofilm increases there is greater mass transfer resistance which can decrease biofilm activity. Further, membrane capital costs can be high and defects on the membranes can affect biofilm formation and activity. Moreover, the challenge with counter-diffusion systems such as MBfRs, for simultaneous nitrification and denitrification is to identify the optimum rate of gas transfer to ensure that enough gas is transferred to the biofilm to promote complete NH_4^+ oxidation, but not so much that the biofilm is fully penetrated with O_2 [175,176].

When it comes to applications in space, MBfRs possess unique characteristics that might make them good candidates for space-based water treatment. The compact size and modular nature of these reactors makes them suitable to be installed and/or integrated into existing space habitation water recycling systems. The bubbleless gas transfer in MBfRs is applicable in microgravity and partial gravity environments, and minimizes foaming [16] and transfer of volatiles (*e.g.,* odors, organics). Accumulation of biomass on the membrane not only facilitates the separation of the biomass from the treated effluent, but it also minimizes the release of microorganisms with the effluent, which could pose a threat to human health [166]. For the past couple of decades, the use and incorporation of MBfRs into space habitation water recycling systems has been investigated. NASA has funded research on the application of MBfRs mostly for the removal of nitrogen species from water, either as coupled nitrification/denitrification systems [187,188], or as stand-alone nitrification systems [189]. On-ground testing of various laboratory scale systems has been performed (from bench top reactors to stand alone systems) using MBfRs, specifically MABRs, for the treatment of different space-based used waters (*e.g.*, ISS, transit, EPB) (Table 3). Investigations have focused on testing different configurations, operational conditions, reactor designs and membrane characteristics to achieve enhanced C and N removal efficiencies. For instance, different reactor geometries and sizes (*e.g.*, cylindrical, rectangular) have been tested to optimize and facilitate the integration of MABRs into flight hardware. The effect of varying membrane surface area (specific surface area - SSA) was tested in these systems; for example, [19]; added polyethylene biospheres connected to the membranes to provide additional surface area. Early studies by Ref. [159] demonstrated the successful use of two full size MABRs (cylindrical geometry, SSA 265 m^2/m^3, parallel flow) to treat EPB used water. The MABRs achieved C removal of 90%, nitrification ~ 70%, and denitrification~ 50% [160]. used a MABR (rectangular geometry, low SSA, cross flow regime) to treat various space habitation used waters (*e.g.*, ISS, EPB), achieving overall dissolved organic carbon removal of ~ 90% and nitrification of~ 50%. After treating 1400 L, the system had not shown any maintenance issues; furthermore, flow lines of the system were not clogged by microbial growth and no buildup of biofilm in the reactor was observed compared to similar systems with parallel flow cylinder configurations [159]. [162] used a MABR that was capable of performing simultaneous nitrification and denitrification for an EPB Ersatz formulation dominated by nitrogen species (C:N < 1); the system showed a removal efficiency of 36% for total nitrogen species (removal rate 0.24 g-N/[m^2·d]). In a different study [16], investigated the performance and optimal loading capacities of multiple MABRs with various habitation waste streams (*e.g.*, EPB, HC, ISS, urine). The MABRs stably operated for up to 5 years with limited maintenance (less than 0.5% of the membranes failed or were plugged), minimal consumables (O_2 and recirculation pumps) and no solids processing. A wide range of organic N and C loading rates were tested, 2–220 g-C/(m^3·d) and 7–200 g-N/(m^3·d) across all types of used waters; the MABRs were able to oxidize organic C (>80%) proportionally to loading rates for all types of used waters, whereas N oxidation varied between 30% and 80% independently of the loading rates. These results showed the potential of MABRs to stabilize and pretreat used waters in space to reduce downstream growth and prevent pH increase, which could result in the volatilization of N species (*e.g.,* NH_3) and precipitation of mineral species such as $CaSO_4$ or $NH_4MgPO_4{\bullet}6H_2O$ (struvite).

It should be noted that all the studies to treat space-based used water with MBfRs have been performed on Earth and have not been tested under microgravity conditions. Traditional MABRs, like the ones used in the discussed studies, are not very effective for the removal of nitrogen compounds for carbon limited wastewater such as waste streams in space with low C:N; however, an alternative could be the use of MBfRs with alternative electron donors including H_2 to promote autohydrogenotrophic denitrification [190], or methanol to achieved enhanced denitrification.

4.3.2. *Expanded-bed reactors*

Expanded-bed reactors (EBR) include systems in which biofilms grow attached to free-moving carriers that are maintained in continuous suspension by air, high liquid velocity or mechanical stirring. These reactors are typically completely submerged and include systems like suspended reactors (*e.g.,* airlift reactor) (Fig. 6E), fluidized bed reactors (Fig. 6F) and moving-bed biofilm reactors (Fig. 6G) [67,128,129]. Among the different types of biofilm reactors, generally EBR are not suitable for applications in space under microgravity conditions given that the beds in these systems are typically expanded by the upward flow of liquid and gas bubbles, which makes them dependent on buoyancy. However, these types of systems could be potentially used in partial gravity environments. If mechanical stirring is used, moving-bed biofilm reactors could potentially be used under microgravity conditions (Fig. 7).

4.3.3. *Hybrid systems*

Hybrid systems involve the combination of two or more different treatment systems to increase the removal efficiency of pollutants from a waste effluent. In this case, hybrid systems refer to the combination of a biofilm system with any other type of system, *e.g.*, suspended-growth reactors, to enhance the quality of the treated effluent. The different treatment technologies can either be integrated in the same reactor, or they can be separated into different reactors operating in a sequential mode [191]. Some of the reasons for choosing a hybrid system may include improving biomass separation, enhancing nitrification (or any other process that involves the use of slow-growing microorganism), or the overall performance of the system.

Examples of hybrid systems include combinations of packed-bed reactors with other bioreactors such as the trickling filter or MABRs. Ref. [163] used a hybrid system to treat a transit mission waste stream (comprised of urine, flush water, humidity condensate), using an anaerobic packed-bed reactor for denitrification coupled to a MABR for nitrification (see Table 3 for details of the experimental set-up and operational conditions). The packed-bed reactor was located upstream of the MABR to reduce the organic carbon load entering the nitrifying reactor, and through recirculation, nitrate (NO_3^-) and nitrite (NO_2^-) produced during nitrification in the MABR were used in the packed-bed reactor for denitrification, which required the high organic load [164]. developed a hybrid system to treat and recycle wastewater (hygiene water, urine, humidity condensate; TOC 247 mg/L; NH_4^+-N 150 mg/L) generated by crewmembers confined in a test chamber for 91 days as part of the Lunar-Mars Life Support Test Project – Phase III at Johnson Space Center. The system consisted of a PBR for organic carbon removal coupled with a TBR for ammonia removal, followed by physicochemical treatment for final consumption by the crew. The system treated ~ 110 kg wastewater per day, with removal efficiencies of 95% and 60% for TOC and NH_4^+-N, respectively. Both biological reactors were inoculated with commercially available microbial consortia; microbial populations in the reactors were characterized by the presence of unique heterotrophic and denitrifying bacteria (*i.e.*, *ϵ-Proteobacteria, Cytophagales, Planctomycetales*) and ammonia-oxidizing bacteria that were closely

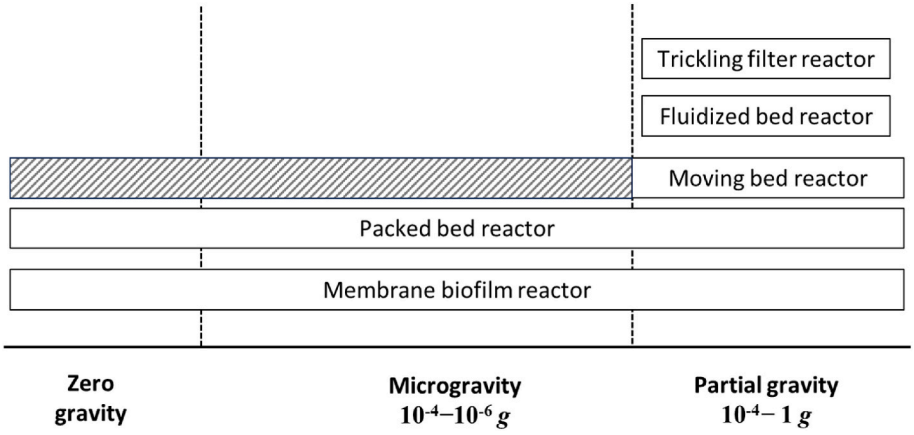

Fig. 7. Potential usability of biofilm reactors under different gravity environments. Note: if operated using mechanical stirring, moving bed biofilm reactors could be used in a broader range of gravity environments.

related to common denitrifiers (*Pseudomonas-Paracoccus-Achromobacter* group) found in other wastewater treatment systems. Ref. [165] evaluated the use of a hybrid system consisting of a PBR for denitrification and a MABR for nitrification, operated in series to treat EPB Ersatz (DOC 239–272 mg/L; NH_4^+-N 150–294 mg/L; TN 243–413 mg/L; C:N = 0.74). The system was operated with a 10:1 recycle ratio and various loading rates and hydraulic retention times (HRTs) (1–6.8 d). This nitrification-denitrification system showed to be robust enough, reaching on average 87% and 55% removal efficiency of organic carbon and total nitrogen, respectively, in the PBR, and 60–80% nitrification efficiency in the MABR, with lower nitrification efficiencies with decreasing HRT. According to the authors, the system performance was hindered by kinetic and stoichiometric limitations. The pH of the effluent (controlled by the rate of nitrification) was inversely related to the HRT; at long HRTs the MABR showed alkalinity limitations as the pH of the system got close to 6.0. At short HRTs, the MABR presented kinetic limitations. The PBR suffered from DOC limitations. Ref. [166] developed and tested a membrane-aerated, membrane-coupled bioreactor (M2BR) to treat a modified EPB used water (Ersatz formulation). In this coupled system, the effluent of the MABR (cylindrical geometry, SA 0.118 m^2) was fed to an external filtration membrane module and returned to the bioreactor. Four different experiments, each with different phases, were performed using various strengths of the EPB Ersatz, operation times, nutrient loading, and oxygen content of the aeration gas, to assess the performance of the system. The first experiment used full-strength EPB Ersatz and the system was run for 66 d using air as the aeration gas; while COD removal of 87% was achieved, no ammonia oxidation was observed during this experiment. In the second experiment, the authors aimed to establish an actively nitrifying biofilm in the system before feeding it with the EPB Ersatz; thus, initially, the system was fed with an ammonia-only solution for 29 d (NH_3 removal efficiency >90%), and later fed a dilute version of the EPB Ersatz to also establish a heterotrophic bacterial community in the M2BR. After achieving ~ 90% removal of both COD and NH_3 (and 35% of the nitrate), the system was fed a more concentrated EPB Ersatz with a C:N ratio that would support simultaneous nitrification/denitrification in the M2BR, achieving high removal efficiencies of both COD and total nitrogen species (> 90%). Although most of the microbial activity was assumed to occur in the biofilm, a large amount of suspended biomass was observed in the system. While removal of COD and TN was observed in this second experiment, a decrease in the nitrification/denitrification efficiency was observed when the system was fed the full strength EPB Ersatz. This was attributed to the O_2 demand exceeding the O_2 transfer rate in the MABR, thus, a third experiment aimed to address this issue. The third experiment had four phases, all of which were similar to the ones in the second experiment, except that pure O_2 was used instead of air. While NH_3 removal efficiency and simultaneous increase of nitrate in the effluent were observed in the presence of O_2, once the full-strength EPB Ersatz was used, decreased NH_3 removal efficiency and nitrate concentrations were again observed. While the M2BR system showed the potential to simultaneously remove both C and N compounds, the system did not perform effectively when using full strength EPB Ersatz. This highlights the challenge of having low C:N ratio in most space-based used waters, which leads to the lack of electron donor to achieve complete denitrification.

4.4. Bioreactors for treatment of used water in space: overall lessons learned

In this review we have discussed the potential use of biofilm reactor systems as alternatives or additions to current technologies for water reclamation and waste treatment in space. Biofilm systems can align well with the unique constraints of treating used water in space, including the need to assure and maximize effluent quality and recover or produce VAPs (*e.g.*, N_2, nutrients for food production, feedstocks for polymers, etc.) while avoiding the use of potentially harmful chemicals. Most biofilm reactor systems discussed in this review have shown potential to treat various types of used water generated in space (*e.g.*, ISS, transit, EPB), either in the form of simulated/synthetic water (*e.g.*, Ersatz formulations) or collected used water, with various removal efficiencies depending on the composition of the water, the configuration of the system, and the operational conditions (Table 3). Most systems have been designed to remove organic carbon and nitrogen compounds, either independently or concomitantly via simultaneous nitrification and denitrification, using either a single reactor or a combination of different reactors (*i.e.*, hybrid systems). While most systems have shown the ability to remove both C and N compounds, the treatment of full-strength space-based used waters remains a challenge due to the low C:N ratios, which makes it stoichiometrically difficult to achieve complete denitrification. All reviewed systems, ranging from bench top reactors to stand alone systems, have been tested on Earth with some promising results; however, the performance under different gravity conditions needs further investigation. Fig. 7 summarizes options for biofilm reactor selection under different gravity conditions. It is not clear what the lower limits of partial gravity are for efficient operation of density driven reactors (*e.g.*, trickling filter or fluidized bed reactors) such that additional research and testing will be necessary under various partial gravity conditions. Clearly, not all discussed systems would be appropriate under microgravity conditions, but all might be feasible options for partial gravity environments (*e.g.*, lunar/planetary surface).

5. Limitations, challenges, opportunities, and scope for future research

This review has identified various opportunities and challenges related to the use of biofilm reactors for the management of used water and the recovery of VAPs in space. The main challenges, limitations, opportunities, and scope for further research for different space missions will be discussed in the following sections.

5.1. Limitations and challenges

There are several limitations and challenges for the use of biofilm-based reactors in space. Basic requirements for space bioreactors are limited by weight, size, durability and reliability, compatibility with other systems, crew time and biosafety (see Section 2.2.1). Additional unique challenges are related to the space environment (*e.g.*, gravity conditions), the type (*e.g.*, exploration vs. colonization) and extent of mission (short vs. long duration). There are several limitations and challenges for the use of biofilm-based reactors in space. *Gravity* is one of the main factors that limits the use of bioreactors in space. Different biofilm reactors used on Earth were designed to operate with "normal" (*i.e.*, 1 *g*) gravitational forces; however, the lack of Earth-like gravitational forces in space can result in changes in fluid dynamics, including bubble formation and movement, which complicates or makes almost impossible the operation of certain reactors in space in the manner they are run on Earth (*e.g.*, trickling filters). Density difference-dependent processes will not occur in microgravity and in partial gravity will not behave in the same manner as in Earth's gravitational field. Hence, microgravity compatible bioreactor systems are limited to a few options (*e.g.*, MBfRs) and must be chosen carefully and/or specifically designed to fulfill mission-critical services without requiring density driven flows. Furthermore, microgravity and partial gravity might also have effects on stability of metabolically active biological systems. As stated before, biofilm reactors proposed to treat space-based wastewater have been tested on Earth; however, the knowledge of the impact of microgravity or partial gravity conditions on microbial systems, specifically on biofilms and their stability and functionality in bioreactors, is currently limited.

The *duration of prospective missions* represents another challenge for the use of bioreactors in space. For instance, there might be potentially different performance expectations of these systems during short duration (<90 days) and long duration (>90 days), microgravity missions vs. the operation on the lunar or a planetary surface with at least partial gravity. These reactors should be robust and resilient enough to ensure the stability of the system even for missions with the longest duration (>10–20 months). Questions, however, remain regarding the start-up and *resiliency of bioreactors*, including the recovery of bioreactors from culture crashes, potentially detrimental contamination of the desired microbial communities, as well as large sloughing events, which could very quickly remove a large part of the biologically active community and thus may cause temporary loss of function. It is important to understand that operational bioreactors for treatment of used water in microgravity and partial gravity will likely be undefined mixed prokaryotic communities enriched for a specific purpose (*e.g.,* carbon removal), and will be subject to a number of issues that can impact process operations. Overall, it is critical that any biological system used on planetary bases or in space have a backup system or contingency and be tested as thoroughly as possible since bioreactor failures are well documented but can be managed. Operational wastewater failures in space will likely be similar to those observed on earth, which have been shown to result from bacteriophage [192], toxic upsets [193], predation [194], foaming, scaling, biofouling [195], poor biomass settling, operator error, physical damage, and design problems, among others. We expect that the optimal source of inoculum for bioreactor start-up will be identified during future research and development activities. Based on experience in the water and wastewater treatment industry, stable consortia performing the desired functions establish themselves during the optimization of the treatment process. Hence, the microbial consortia establishing themselves in pilot studies are anticipated to become the source of start-up cultures for these reactors. While inoculation with individual strains or defined mixed cultures of microbes might be considered a strategy, operation of wastewater treatment reactors on Earth indicates that undefined mixed prokaryotic communities will establish themselves for specific purposes. Cultures are expected to adapt to changing conditions in time and location as they do on Earth in response to changes in the environment. Furthermore, while it has been shown on Earth that stable biofilm communities establish themselves and often recover quickly, backup seed cultures may be necessary in space, and it should be considered how to best supply such seed cultures and decrease necessary start-up times at the beginning of the mission, after possible failure or after dormancy. For instance, these systems could be designed using modules with pre-seeded microbes, which can be exchanged quickly if necessary and can ideally be regenerated on board or on planet (*e.g.*, through off-line seeding). A remaining concern for these bioreactor systems will always be the necessary start-up or recovery time after perturbation (*e.g.*, an upset through toxic materials or adverse conditions) or after extended dormancy. However, biofilm systems are generally characterized as resilient when exposed to environmental stresses, including the lack of nutrient supply, desiccation, etc., which would potentially occur during extended dormancy. While we expect biofilm systems to perform stably and reliably in the face of changing operating conditions and after longer-term dormancy, these processes likely have to be simulated, as will the start-up phases. Studies on Earth might or might not be able to provide the long-term microgravity conditions necessary to perform these inoculum & viability studies easily. *Maintenance* of these bioreactors is also a challenge; especially in events where biomass is detaching, either slowly or through sudden biofilm sloughing events, biomass would have to be removed reliably from the effluent of the reactors. One of the most common ways of removing biomass is filtration. Membrane filtration systems are already being applied in used water treatment systems, but other filtration systems might also be possible and desirable to provide proper quality water for downstream use. While removal of biomass might be necessary for the safe operation of the system, it also provides an opportunity to recover biomass for other purposes, *e.g.*, seeding of reactors or as raw material for other processes, that might become part of ECLSS, such as use of biomass as fertilizer for plant or fungal growth or as feedstock for the generation of bioplastics or similar products.

Scalability and integration of the envisioned biofilm-based used water and VAP systems is another area that will require increased attention. While modular systems can be expanded upon if designed properly, size restrictions in planetary developments or in spacecraft could pose limitations. Furthermore, integration of biofilm systems with current physicochemical processes for water treatment (*e.g.,* the WRM in the ISS) needs to be considered. On Earth, some approaches enabling the potable reuse of water include filtration (incl. Micro-, ultra- and nanofiltration), reverse osmosis, ion exchange, activated carbon treatment, disinfection and oxidation processes (*e.g.*, UV, chlorine, ozone) as well as thermal treatments (*e.g.,* distillation, sterilization or pasteurization) [196]. However, as summarized in Refs. [117,118]; the types of processes and chemicals to be used might be limited in ECLSSs; for instance chlorine and bromine (commonly use on Earth for water treatment) are currently not the most favored biocides to be used in space due to their ability to form hazardous byproducts; however, more energy-intensive processes such as thermal treatment, radiation treatments (such as UV), and the *in situ* generation of oxidants such as ozone might be feasible approaches in space because energy efficiency is usually not a strong consideration in the process design in space. The exact level of integration of biofilm systems with other physicochemical treatment units in space remains at this point unclear. Hence, future work will have to address questions and concerns regarding the performance and reliability of biologically based systems because physicochemical treatment

processes are currently the state of the art in space travel [8,9]. As indicated throughout this review, one of the main drivers for the integration of these more ecosystem-like treatment and processing systems could be their potential to *be self-sustaining*, requiring no or only minor inputs of raw-materials and energy, and to produce VAPs. Microorganisms reproduce while removing undesirable components from water and air, potentially producing desirable, higher value products, which could become an integral part of a more efficient resource utilization and recycling scheme that seems to be necessary for long-duration space missions. Utilizing biological processes for life support systems could be achievable through proper design and might significantly reduce the need to supply parts and feedstocks (necessary chemicals) through resupply missions.

Composition of the space-based used water is also a major challenge in the use of biological reactors. While many wastewater treatment systems on Earth have C:N $>$ 1 ratios, thus generally supplying sufficient electron donor to support substantial denitrification, used water in space often has C:N $<$ 1 ratios. Thus, while many of the bioreactor systems discussed were able to remove C and N compounds, complete denitrification was often not achieved due to the lack of electron donor in the system. This is something that could be addressed by using autohydrogenotrophic denitrification systems [190] or other electron donors, *e.g.,* elemental sulfur. Similar challenges are foreseen for phosphorus management.

Minor challenges might result from considerations related to the transport of the reactors to their point of use. This will not be discussed in detail here because the challenges are similar -if not identical-to the challenges faced when establishing physicochemical treatment reactor systems.

One major concern that has been raised is the potential unsafe and non-aesthetic nature of biological processes. While this concern is understandable due to the concerns of microbial infections, and while biofilms have been shown to harbor pathogens for longer periods than other systems, biological and especially biofilm-life support systems offer significant opportunities as well. Research and development activities in this area should integrate the study of concerns that exist and likely develop educational and informational materials and activities, which will allow stakeholders to learn more about the strengths and risks of biological and other life support systems along with their potential in space.

5.2. Opportunities and scope for future research

While much progress has been made in the testing and operation of space compatible bioreactors on Earth, there is a significant amount of research still needed to build and successfully operate bioreactors in partial gravity and microgravity. It is anticipated that bioreactor development in space will progress in a manner similar to plant growth in space. Plants were first grown in space in a micro-greenhouse by Soviet astronauts in 1981, NASA astronauts first ate lettuce grown in space in 2015, and in 2018 the Advanced Plant Habitat (APH) was added to the ISS to accelerate learning to grow plants in space.

Most microbial experiments in space have been performed in batch reactors, yet future research is needed to develop and test continuous flow bioreactors in partial and microgravity that can effectively treat used water to demanding standards for drinking water and lower requirements for other water needs. Like the APH, a module should be developed for advanced bioreactor studies in space, which can be continuously operated with used water to determine treatment effectiveness and reliability under actual conditions. The bioreactor module could be paired with the APH and other modules to perform studies where effluent treated by biofilm systems could be used to provide nutrients (*e.g.*, N, P) for plant cultivation for food production, or cultivation of algae for O_2 production. Additional research is needed in the areas of harvesting microbial products (*e.g.*, edible fungi), biomass recovery for composting or use as a fertilizer, use of liquids containing N and P for plant growth as well as in other areas.

Additional studies are needed to understand biofilm formation, development, and control in continuous systems in space. Potential process streams for water recovery include the following: urine, fecal or food waste, hygiene water, condensate, and Sabatier [33]. In a dedicated bioreactor module, these streams could be carefully tested in space to determine treatment efficiency, reliability, and observe and correct potential operational issues. Further, the effects of radiation, microgravity, mutations, shifting microbial communities, and changes in environmental compositions in the bioreactors should be studied carefully. It is not clear what the lower limits of partial gravity are for efficient operation of density driven reactors (*e.g.*, trickling filter or fluidized bed) such that additional research and testing would be necessary under specific partial gravity conditions. These studies would allow a critical perceptual change of bioprocesses from currently unused in space, to limited use for waste treatment and removal, and eventually to resource recovery and recycling with integration into all other life support systems.

Microgravity and partial gravity design considerations need to be tested on Earth and in space to determine the range of operational parameters and assess potential need for bioreactor redundancy to allow necessary maintenance and ensure dependable operations. Development of bioreactor technology for integration into critical regenerative life support systems should include small- and large-scale experimental studies along with real-time monitoring and computer modeling at the subsystem and the water recovery system level [197] to predict and correct potential problems before they prevent proper bioreactor operations.

The studies described above would provide critical insights into continuous flow biofilm reactor operation in space, pave the way for these reactors to be implemented for used water treatment systems, and ultimately allow these reactors to be operated as integral parts of life support system infrastructure in space. With additional development these bioreactor systems would provide services well beyond used water treatment and could include a variety of VAP generation services. While the scope of this review was the potential use of biofilm-based systems for wastewater treatment in space, it should be highlighted that other types of biological systems, *e.g.*, suspended-growth systems, of course also have potential roles in space, particularly under partial gravity conditions. Hence, suspended-growth systems and other bioreactors for potential use in space should be further explored, separately and in combination with biofilm-based systems.

6. Conclusions

As future missions will travel further from Earth and for longer duration, the need for self-sufficient systems that can reliably provide water without relying on resupply from Earth will be essential. Through regenerative water reclamation systems, it would be possible to convert ‘wastewater’ and ‘used water’ into useable water for different purposes while recovering value added products (VAPs). Designing more reliable, robust, self-sustaining and regenerative water subsystems would further close the loop in ECLSS. Biological reactor systems are promising alternatives or additions to existing technologies for the treatment of space-based used water (*e.g.*, ISS, transit, EPB); these systems can align well with the unique constraints of treating water in space (*e.g.*, microgravity, limited size, compatibility with other systems) while assuring and optimizing effluent quality and recovery of VAPs (*e.g.*, clean drinking water, N and P nutrients). A variety of biofilm reactors, from benchtop to larger systems, have successfully removed organic carbon and nitrogen compounds from space-based used waters or their analogs on Earth. Treatment efficiencies vary depending on the water composition, the configuration of the system, and the operational conditions. System performance under different gravity conditions needs significant additional investigation, as only packed bed or membrane biofilm reactors would perform well under microgravity conditions but many other reactor types could be feasible options for partial gravity

environments.

We make the case that numerous opportunities exist in integrating biological (and specifically biofilm) systems into ECLSS:

1. Biological components and processes (*e.g.*, plant growth) will be necessary to provide food on extended missions. Biological systems provide an opportunity to contribute to this need by growing algal or fungal biomass directly for consumption or to efficiently scavenge nutrients, such as N and P, from used or process waters, which can then be used as fertilizer for plant growth.
2. Under Earth gravity conditions, biological reactors, including biofilm systems, have been proven to be viable alternatives to resource and energy intensive physicochemical water treatment approaches. Most domestic and many industrial water treatments rely on biological processes to provide clean water.
3. Biological processes for water and air treatments can generally be characterized as low energy, low maintenance and little resource-intensive processes that provide opportunities to recycle and reuse resources in other life support subsystems (*e.g.,* habitation, waste, air).
4. Because spacecraft and future planetary or lunar bases will be resource-limited, resource recovery and recycling will have to become an essential component in the design, planning and maintenance of these settlements.

Because the health and safety of crewmembers must remain the foremost concern, the risks and opportunities outlined here will have to be balanced. Thus, more research, development and testing will be necessary under zero-, micro-, and partial gravity conditions, either on Earth or as part of future space missions. Active treatment systems and backup systems will have to be designed, built and operated to ensure the safety of crew and spacecraft. We look forward to being part of the community that will provide the necessary insights and technologies to safely implement, establish and improve biological components into NASA's and other space agencies' life support systems.

CRediT authorship contribution statement

Erika J. Espinosa-Ortiz: Conceptualization, lead, Visualization, lead, Writing – original draft, lead. **Robin Gerlach:** Conceptualization, supporting, Writing – original draft, supporting, Writing – review & editing, equal. **Brent M. Peyton:** Writing – original draft, supporting, Writing – review & editing, equal. **Luke Roberson:** Writing – original draft, supporting, Writing – review & editing, equal. **Daniel H. Yeh:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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