

Review

Potential and Challenges of Microalgae in Wastewater Treatment for Bioregenerative Life Support Systems During Long-Term Space Missions

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Abstract

The engineering, resource, and financial constraints in space and spacecraft so far have not allowed the incorporation of biological components into a closed-loop bioregenerative life support system (BLSS), despite decades of research. The expected increase in deep-space exploration and planetary bases with limited access to Earth-based resources necessitates the development of self-sustaining hybrid BLSS technology. The created physico-chemical systems, together with photosynthetic organisms and bacteria, aim to revitalize the air, produce food, and recycle nutrients and water in mutually beneficial mini-ecosystems. While plants are best in the function of food production and bacteria in waste recycling, the incorporation of microalgae would add immense benefits in optimizing the life support system (LSS) and increasing the degree of closure. Microalgal photobioreactors (PBRs) could perform wastewater treatment (WWT), removing the nitrogen (N) and phosphorus (P) in the human-derived wastewater (WW), and couple it with converting carbon dioxide (CO₂) from the cabin to oxygen (O₂) and food production. As microalgal WWT on Earth is an emerging field with engineering hurdles, power, mass, volume, microgravity fluid dynamics, and other constraints have also prevented their operations in space. However, in space vehicles, there is no need for large upscaling of a laboratory prototype system, and the WW effluent is easier to predict, facilitating microalgal extraplanetary use in comparison to Earth treatment plants. These factors, combined with the qualities of microalgae such as surface-to-volume efficiency, fast growth rate, high yield, and tolerability to WW, etc., have led to many preliminary testbeds, prototypes, and ground demonstrations from space agencies, space centers, and academia, which show promising results. Microalgal participation in space WWT is beyond current operational practice; however, PBRs are on the space agenda, and the scientific community is elaborating the technologies that would allow their successful implementation.

Keywords: space; microalgae; cyanobacteria; wastewater treatment; photobioreactor; space station; spacecraft; life support system; bioregenerative life support system

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1. Introduction

The life support system (LSS) that continuously sustains the human crew in space vehicles so far has only utilized physicochemical processes [1–3]. Incorporating living or-

ganisms for extraplanetary closed-loop recycling of gas, water, and matter into a bioregenerative LSS (BLSS) would have very high additional power requirements in the conditions of limited volume and mass that have not allowed for BLSS implementation so far. In addition, the limited technology handling live organisms and the limited knowledge of bioprocesses under microgravity are other obstacles [4].

Abiotic LSSes have operated reliably so far, but they are dependent on easy replenishment from Earth, and therefore can only be applied for low-Earth orbit missions [5]. With the expected increase of deep-space expeditions and the expected colonization of the Moon, Mars, and other celestial bodies, it has become clear that biological components in the LSS are mandatory in the new space era [4,6–8]. The hybrid LSS combining abiotic and bioregenerative processes possibly has the most potential [1]. Photosynthesizing autotrophic organisms are obligatory for the future LSS, and their main functions would be air revitalization and food production [1]. Plants are indispensable in these assignments, mostly because they provide the most optimal nutritional ratio, but also for psychological support [6,9].

Similarly, microbial bioreactors participating in wastewater treatment (WWT) in space would require time and a large reactor volume, so up until now, abiotic technology has only been used. However, with the expected future increasing number of astronauts on missions, thus increasing hygiene wastewater (WW), biological treatment technologies show potential to be one key solution [5]. Bacteria are highly recommended for waste and WW recycling, and most projects of space agencies and academia develop them as the first line of WW treatment [3,10–12].

In addition, coupling WWT with oxygen (O₂) and food production is an elegant and even obligatory solution to increase the degree of closure of a space LSS. That can be achieved through the use of microalgae [13,14], as they start to be implemented in WWT on Earth, mainly in consortia with bacteria [15]. Microalgae have the advantage over plants of surface-to-volume efficiency, short harvest cycle, higher yield [11], and better, although not limitless, tolerability to WWT [1,11,16]; so, when cultured in engineered photobioreactors (PBRs), they could successfully add benefits to an LSS through making it faster, more compact, and reliable [1,8,17] and decrease the need for soil, which is a heavy resource [18]. Their contribution to utilizing and/or recycling WW would be very valuable because water is an expensive resource, especially in long-term space missions. It must be noted that the price of water on a space station is about \$10,000 per pound [5].

Microalgae have flown in space since the 1960s, in petri dishes, tubes, or flasks for basic biology experiments [19], and more recently for early-phase bioreactor validation [2,20], and neither of these experiments included WWT because of the financial and engineering constraints. The *Euglena* and Combined Regenerative Organic-Food Production in Space (Eu:CROPIS) mission of the German Aerospace Center is the only flight where microalgae were planned to take part in waste recycling (synthetic urine initially); however, irrigation could not be initiated, and the *Euglena* remained dormant [21,22].

Nevertheless, extraterrestrial WW recycling and further water utilization by microalgae still constitute a major focus of active current experimental and theoretical work. International or national space agencies, space centers and facilities, as well as academia, have developed several prototypes, conceptual designs, ground demonstrations, and testbeds including PBR as a unit operation in the system of WWT.

Examples include the PhotoMembrane BioReactor (PMBR) of The Advanced Exploration Systems (AES) program of the National Aeronautics and Space Administration (NASA) [11], the Space-Based Biochemical Conversion System (BIOSYS) of the University of Louisiana [3], NASA's Established Program to Stimulate Competitive Research (EPSCoR) [18], the Micro Ecological Life Support System Alternative (MELiSSA) project of

the European Space Agency (ESA) [23], the Sustainable AI-automated Bioreactor for Environment Remediation (S.A.B.E.R) [24], etc.

Some of these projects formulate that the urine and other waste streams would first pass through a bacterial bioreactor, and then through a PBR. Instead of bacteria mineralizing organics in the same reactor as microalgae, so that the latter could process them, this practice of bacterial “pre-purification” of the WW is separated in space and time. First, bacterial pretreatment would break down organics, reducing the high nitrate (NO_3^-) and phosphate (PO_4^{3-}) content in WW. Then, microalgae would indirectly perform WWT and simultaneously utilize the carbon dioxide (CO_2) exhaled by the crew and produce O_2 and edible biomass. Other projects envision direct contact of the WW with axenic microalgae or with microalgal–bacterial consortia.

This paper aims to provide an overview of the research related to microalgal and cyanobacterial participation in WW treatment and utilization and to present in an organized way the projects and programs of space agencies, centers, and academia that develop such future bioremediation.

2. Methodology

The literature in this narrative review was identified through searches in Scopus and Google Scholar. Scopus was used as the primary database, with a search of the terms “microalgae” or “cyanobacteria” with “wastewater” or “wastewater treatment”, together with the space-related keywords “space”, “spaceflight”, “microgravity”, “gravity”, “spacecraft”, “cosmos”, and “astronaut”. Google Scholar was additionally consulted to identify relevant publications using alternative terminology (e.g., “effluent” or “sewage”) that may not be indexed in Scopus. The time window included sources from the year 2000, focusing on the latest research, but if previous key sources were mentioned in the found literature, these sources were also included. The date of the last search was 10 February 2026 for Scopus and 20 March 2026 for Google Scholar, and no language restrictions were applied. Conference proceedings and agency reports were included, whereas patents were not. The retrieved literature was selected based on relevance to the topic of this review. The sources were selected to provide a comprehensive overview of WWT approaches involving microalgae in space applications rather than through a formal systematic review protocol. Studies involving only bacterial, physicochemical, or other non-microalgal WWT approaches in space were excluded unless they provided essential context.

3. History of BLSS

The LSS on space missions—in space stations, spacecraft, rockets, or hypothetical future colonies on the Moon, planets, or asteroids—has the function of providing the crew or the future settlements with O_2 , water, and food.

So far, the space LSSes on the International Space Station (ISS) and all other manned spacecraft have relied on physicochemical processes without including live organisms, such as bacteria, microalgae, plants, or animals [1–3]. These abiotic systems have been named first-generation [7]. For example, the ISS utilizes electrolysis of water to O_2 and the Sabatier reaction for the generation of water and methane by reacting hydrogen with CO_2 over a catalyst at high temperatures and pressure [18].

Until the mid-20th century, ecologists theorized that a small-scale closed ecosystem could not be created because of the intricacy of controlling bioregenerative systems, especially consisting of microbial monocultures and humans [25]. This was corroborated in pioneering studies in the 1950s, when the idea of using plants as both food and a regenerating tool first emerged, and logically led to the inclusion of microalgae for LSS in the research of the US Air Force [9,25,26]. Mice, rats, or monkeys were used as test animals,

and in some cases, their wastes were used to cultivate *Chlorella* microalgae, although water was not recycled. Because the knowledge about microalgal cultivation was scarce and special devices for a PBR did not exist, gas imbalances appeared, and the steady state in the closed systems did not last long. Therefore, pessimistic scientific arguments—reasonable to some extent—that unicellular cultures would require complex and energy-intensive control systems and that cultures could genetically degenerate with time led to the general conclusion that research on simple ecosystems for LSSes should cease [25].

In addition, around 1970, about 10 years after the formation of NASA, the first US-tested bioregenerative approaches were in two directions: photosynthesis by microalgae and chemosynthesis by “hydrogen bacteria”. The latter system suggested that water be electrolyzed to H₂ and O₂ (a.k.a. “artificial photosynthesis”), and then the H₂ would be oxidized by the hydrogen bacteria *Cupriavidus necator* and *Acidovorax facilis*. The former was given as food to human test subjects without extensive knowledge about the bacteria, a trial that astonished Gitelson et al. [25], and, without surprise, it caused severe negative consequences to human health, which was widely noted. If *A. facilis* was given, the reactions would be likely milder. However, notably, that disappointment, in addition to the early unsuccessful algal research, and the fact that any microbial culture could not fulfil human nutritional requirements, stifled NASA’s bioregenerative research to a few contracts dealing with microalgae [25].

On the other side of the Iron Curtain, views on LSSes and algal inclusion within were more optimistic. The Soviet Union in 1961 conducted a ground 30-day experiment with a 4.5 m³ sealed room, which revealed that about 30 L of *Chlorella* culture was enough to revitalize the air for one man [27]. However, *Chlorella* biomass was found to contain too little carbohydrate and to be difficult for digestion by humans unless processed. Consequently, later Soviet projects incorporated more traditional sources of food—land plants—alongside microalgae to enable complementary O₂ and food production [10].

Therefore, microalgal processes have been studied in the LSS context since the beginning of the space age, which was marked by the launch of the Sputnik 1 satellite in 1957 [28], and even earlier, from the start of BLSS development [29].

The Hybrid Biosphere System (BIOS) was a pioneer program in the development of BLSSes [10]. It was a large-scale confined analogue environment manned by humans. It used microalgae in large PBRs and land plants for air revitalization (BIOS-1 in the early 1960s and BIOS-2 in the 1970s) and later also included plant-based food production (BIOS-3) used for tests until at least 1984 in Siberia [4,10,30]. BIOS-3 achieved degrees of closure of 100, 85, 40, and 20% for O₂, water, nitrogen (N), and minerals, respectively [25]. In the programs, unprocessed urine and other WW were more often mixed with nutrient media to successfully cultivate plants hydroponically than fed to the PBRs [4,10,30]. Nevertheless, BIOS indirectly indicated the possibility and the feasibility of using microalgae for WWT in space [30]. Primarily, the more thorough research on microalgae in the program showed the feasibility and stability of a closed BLSS with naturally established microalgal–bacterial consortia. With them, the system becomes controllable and stable long-term [25].

This fact and the advance of American research likely led to the “new start” of NASA, which was not until the mid-1970s, when the agency began to consider the bioregenerative concept again as reasonable and promising and strategized to include it in a new or second-generation LSS [7]; however, crop plants took research dominance over microalgae. More engineering approaches and other organisms were to be examined [25]. The controlled ecological life support system (CELSS) program was started. The basis of its work was that the BLSS would indeed be an ecosystem but mostly resembling a terrestrial farm, and unlike a natural ecosystem, it would have a purpose—the sustenance of a human crew [31]. Around that time, in 1975, Eugeni Shepelev introduced the concept

of a closed ecological LSS with a closed cycle of substances [4]. Around the 1990s, in addition to land plant studies, e.g., research named “Salad Machine”, the CELSS goals included experiments of microalgal and cyanobacterial growth and purification. The diminishing Cold War likely contributed to openness for collaboration with the BIOS-3 program and Japanese academia [31].

Another closed mini-ecosystem environment LSS experiment is the Biosphere 2 project of NASA, which consisted of a 1.3 ha structure of different biomes (marshland, rainforest, savannah, etc.) in Arizona. In the 1990s, it was used to study artificial ecosystems with a high degree of closure and a human crew [32,33].

Similar projects and systems, with the goal of ecopoiesis—the creation of an ecosystem capable of supporting life [34]—are the Advanced Life Support System Test Bed (ALSSTB) (the NASA Johnson Space Research Center, under the CELSS program), the Closed Ecological Experiment Facility (CEEF) of the Japan Aerospace Exploration Agency, and the Lunar Palace program of the Beijing University of Aeronautics and Astronautics. According to Sachdeva et al. [35] and Kibe et al. [36], the possibility of including the microalgae *Chlamydomonas* for waste recycling, besides the production of food and O₂, is researched in CEEF, although plants are mainly studied. The other facilities did not include algae and focused on land plants, and/or animals for future Lunar or Martian bases [10,37]. For example, ALSSTB researched closed-system vascular plant cultivation, maximizing its productivity, oxidizing materials that could not be further utilized, etc. [25]. The manned mission in the Lunar Palace in 2018, in fact, holds the record of the longest stay in a self-contained laboratory with the highest system closure—for 370 days [10,38].

4. Feasibility of BLSS

Regardless of whether microalgae, bacteria, or abiotic agents performed the water recycling and WWT, high energy consumption was always one of the main problems of such closed-loop test (eco)systems [39].

Thus, despite the obvious benefits of mimicking nature and despite the active research and development, a bioregenerative LSS has not yet been achieved in practice in spaceflight. So far, algae and plants have not been used even to produce O₂ and scrub the CO₂ exhaled by the astronauts in a closed system. Even a closed-loop abiotic LSS does not exist yet. That is due to engineering hurdles—the fact that the operation of biological systems requires higher power consumption than the physicochemical ones is the main closed-system constraint [4]. The tests in space are also very expensive, so we do not know sufficiently about extraterrestrial product conversion and kinetics of bioprocesses, such as waste degradation, water purification, or fermentation in space conditions [29]. Even as early as the beginning of the CELSS program, the working assumptions predicted that a Bioregenerative Life Support System (BLSS) would require relatively large amounts of power, and suggested that it would be either from the Sun or from nuclear sources [31]. BLSSes would also require large areas, volumes, and initial weight (especially for systems with land plants), and technology handling living organisms in the conditions of zero or microgravity [4,40].

Nevertheless, experts suggest that a biological system would outperform abiotic systems, especially for prolonged missions [3,41–43]. The conclusions of the research so far point out that the main disadvantage of abiotic LSSes is that they do not manufacture food [44], but recycling waste into food is essential for distant missions [14], but also the most challenging and difficult part in closing the mass loop [16]. In addition, abiotic LSSes are long-term more energy-intensive, and inclined to malfunction after prolonged use. They recycle only parts of the water and the CO₂, use a lot of consumables, and produce a lot of waste; thus, they are not self-sufficient enough in future extended missions lasting months

or years, e.g., deep-space flights or habitation on the Moon and Mars, where resupplies from Earth would be expensive and infrequent or even impossible [1–3,11,29,45].

For example, among all extraterrestrial vehicles, the ISS is the largest that humans have lived in for long periods of time [1]. It contains the most advanced regenerative LSS in space, the Environmental Control and Life Support System (ECLSS) [46]. Its Ionomer-membrane Water Processor recovers only 85% of the water from urine, flush water, and condensate, while the desired recovery rate is over 90% [3,5,47]. The other WW streams are jettisoned (discarded overboard) [3].

Apart from the problem with the inability to be fully recycled, in some cases, the waste also uses precious vehicle space, e.g., the urine nutrients are concentrated in brine with toxic compounds required for storage before being disposed of. Historically, prior to the partial recycling, all WW was jettisoned and polluted outer space [1–3,17,32].

State-of-the-art research has shown that for such deep-space scenarios, it is obligatory to apply an LSS based on biological principles rather than on the basis of physicochemical processes [4,6–8]. This is one of the options to achieve 98% recovery of all O₂, water, and nutrients, which is required for long-duration missions [48], and the desired >90% recovery rate of WW [5,47]. However, the BLSS should contain more than a microbial monoculture to provide for humans, which would be an unstable system [25]. Long-term regenerative systems of completely independent isolated environments need to be closed-loop and are ideally hybrid, combining biological components and physicochemical processes. The latter may be used as a failsafe or for redundancy, or may be an integral working system [1]. For example, part of the CO₂ would be recycled to O₂ by microalgae and part by the Sabatier reaction and electrolysis [18]. With a hybrid LSS, only an initial supply of resources and later a minimal amount of consumables would be needed [1,6,10,32].

According to Ma et al., a CELSS is a closed ecosystem with an integrated sum of interconnected unit processes [5]. In theory, the feasibility of constructing a closed BLSS that meets all the needs of the astronauts has been proven, and the current research aims to overcome the technical challenges of implementing it into practice [1,4,25]. Over multi-year timescales, the biological system would be more economically feasible [8], would necessitate lower operating temperatures and pressures, less hazardous chemicals, and produce less dangerous waste and byproducts, which is especially valid for WWT. According to Abdullah et al. [13], WW recycling is essential for celestial body bases, not merely a sustainable convenience, given the technical and financial challenges with water transportation. Therefore, the new generation WWT in extraterrestrial settings has to recycle the nutrients from the increasing streams of WW and utilize them in circular processing, for example, in an edible biomass. It has to reduce the operating chemicals, labor, and (most challenging) the energy consumption, as well as the waste output [48]. Bioregenerative systems make this possible [10,48].

For celestial body bases, it must be considered that not all components of an LSS are likely to be manufactured in situ, so systems that have to be flown in transit have to be scalable—able to scale down or de-compartmentalize for transport. They also have to be flexible to operate in reduced or full gravity and be transported in zero gravity [48].

A conclusion has been reached today that it is obligatory for a BLSS to involve photosynthesizing autotrophs [1]. Their CO₂ fixation ability is especially useful for the high CO₂ levels in confined space habitats [49]. Every gram of the O₂ that they recycle would replace the launch of 1 kg of bottled gas for a prolonged space mission [24]. Similar to their ecosystemic services on Earth, where photoautotrophs participate in the biogeochemical cycle of matter or substances, in smaller extraterrestrial ecosystems, they could enable not only the cycle of O₂ and CO₂ but also simultaneously recycle nutrients. To rephrase, their multifunctionality would allow coupling air revitalization with waste (solid, WWs, and

mixes) treatment and turning it into edible biomass, while only requiring light from outside of the system [1,50].

Microalgae are a critical and even irreplaceably beneficial photosynthetic component of a closed-loop LSS [13,14]. PBRs could provide a linkage and interface between the abiotic and bioregenerative LSSes [28]. In all types of closed ecological LSSes, they would be a basic element or a link, which would allow a complete or almost full regeneration of air and water [4,40].

5. Advantages of Microalgae in Comparison to Land Plants for LSS

Many credible scientific sources still consider cyanobacteria to be within the informal polyphyletic term microalgae [51–54], although there is no complete scientific consensus. In this review, for convenience, cyanobacteria will be considered microalgae, unless they are referred to separately.

An initial comparison between Embryophytes and microalgae reveals the advantages of the latter regarding surface-to-volume efficiency and less area needed. This is related to the described processes: O₂ production, CO₂ scrubbing, water usage efficiency, waste nutrient recycling, and high productivity of a fully edible biomass [1,8,17]. Mainly because all microalgal biomass is photosynthetically active, microalgae have a high solar (or suitable artificial light) energy conversion efficiency of 3–10% versus 1–3% in higher plants [46,55]. Microalgae and cyanobacteria, collectively referred to as phytoplankton, produce the majority of the atmospheric O₂ [56,57]. An experiment on the ISS even showed that microalgae converted three to five times more CO₂ into O₂ under microgravity than on Earth [58]. Cyanobacteria have especially high photosynthetic efficiency and O₂ production rates, outperforming eukaryotic microalgae [8].

However, in the BLSS context, the advantage of microalgae over land plants in O₂ production is valid only for engineered PBRs and not open ponds, where self-shading disturbs light distribution [1,17]. In fact, considering the algal productivity of a pond (10–30 g O₂ per m² per day) and human respiration (0.5–0.9 kg O₂ per day), the required area of a pond is estimated to be about 20 square meters per person [25]. This is close to the estimated minimum of 20–40 m² of ground area for land plants [7].

The fast growth and reproduction rates of microalgae [11], leading to a short harvest cycle [59], their adaptability [13], resilience to stress [40], ease of handling [11], cultivation length, as well as the water, illumination, and nutrient requirements [1,60] are other benefits. Moreover, in the unicellular microalgal population, every cell is a separate organism, and their vast number ensures survival of the population if part of the organisms die [40]. In addition, they have a short start-up phase of 3 to 5 days until reaching their full photosynthetic potential [46], high biomass yield with comparatively small volume [60], nutrient richness [59], and they can be continuously harvested [17]. Microalgae are fully ingestible with reduced waste production (no stems, straws, etc.) [17], and although the biomass of some species needs processing [10], it is less complex than that of, e.g., grain crops [17]. Especially important to the LSS context is their ability to flourish under extreme environments [59].

Therefore, microalgal PBRs would reduce the need for a large area for land plants and make an LSS more compact, faster, and more reliable. Microalgal PBRs are among the most advanced technological concepts for bioprocesses in space settings [29]. They have the advantages of automatic cultivation, harvesting, and control [46], and minimal maintenance for the crew, e.g., without daily watering [18]. The biomass increase and the level of the microalgal secondary metabolites can be controlled by modifying the light intensity, the culture media nutrients, and other bioprocess optimization factors, which can be mathematically modeled. This is especially valuable for use and production in space [28].

Regarding WWT, an advantage of microalgae is their ability to be cultivated in the (diluted) liquid waste stream as suspended cells in a compact aqueous culture. This means they do not need heavy soil (with a soil ecosystem that adapts for a long time) or artificial media like Arcillite for plants [18], which is critical for space missions requiring minimum launch mass.

In terms of WW tolerance, indeed, vascular plants, macro- and microalgae participate in the biogeochemical cycles of utilizing and recycling animal and human waste, and they can tolerate solid or liquid waste to a certain extent. Regarding human waste, the famous example of fertilizing rice in Japan during the Shogun times is not an exception, as, historically, human urine and/or feces have been used in other parts of Asia and in Europe. However, this waste was aged, composted, and soil-buffered, otherwise the plant root system would experience chemical burn injury [61–63].

Microalgae can function in and tolerate waste streams to a greater extent than vascular plants [1,11,16]. They also outperform macroalgae in this regard, with a few exceptions of waste-tolerant macroalgae [64]. Urea is hydrolyzed to ammonium (NH_4^+) by bacteria, thus NH_4^+ and urea are the main N-sources present in urine [35]. Both microalgae and land plants tolerate high concentrations of NO_3^- . It is the preferred N form for land plants, which often experience NH_4^+ toxicity at concentrations above 0.1–0.5 mM [65]. In contrast, microalgae, e.g., *Chlorella* sp. and *Arthrospira*, take both NO_3^- and NH_4^+ , and many microalgae tolerate ammonia species (i.e., $\text{NH}_3/\text{NH}_4^+$) at concentrations of 5–10 mM [66,67]. Microalgae and cyanobacteria can also utilize urea directly [35].

For these reasons, microalgae have been practically involved in terrestrial WW processing [15,67]. Accordingly, numerous researchers agree that microalgae, as an additional function besides air revitalization and food supplementation, can be used for a new, alternative, and sustainable approach to WWT in space [9,13,16,30,45,48,59,68–80].

6. Advantages of Plants in Comparison to Microalgae and LSS Artificial Ecosystem Biodiversity

The terrestrial closed ecosystem experiments showed that land plants are also valuable and irreplaceable for LSSes [4,10,30,32,33], even labelled as the most promising for LSSes, according to Duri [9]. The microalgal biomass produced from the WW of one crew member (approximately 5 L a day) is too little as an amount [14,18] and with a sub-optimal nutrient ratio for the dietary needs of one person [34]; therefore, microalgae can be a supplement, not the sole source of food. Both plants and microalgae are vital protein sources [10]; however, fresh plant produce is tastier and more complex than algal biomass and thus is an essential part of long-term nutrition. Land plants, i.e., the sight of foliage, are also important for the psychological support of the astronauts against isolation [6,9]. Because of the limited size requirements, they are usually harvested early in the form of microgreens with one set of true leaves, but the exact time of harvest is a subject of studies [49].

Besides air revitalization and nourishment, plants recycle and purify water through transpiration [8]. In addition, similar to microalgae, vascular plants are also studied for WWT [60]. They are considered to play a crucial role in these functions. For example, the smallest plant, duckweed, or more precisely the water plant *Wolffia globosa*, has a natural capacity of assimilating high quantities of NH_4^+ , and experiments suggest they are suitable for alternative WWT and further bioethanol production (as microalgae are). The authors suggest that it is possible that *Wolffia* can establish a symbiotic relationship with cyanobacteria and microalgae in the same way as it does with plant-promoting bacteria [50].

All this leads to the realization that the best approach for an LSS is the integration of different groups of organisms [8,81–83]. Fish in aquaculture, insects, and filamentous fungi have also been proposed as alternative biological sources of food and protein and

as waste recyclers [29,81]. All organisms could be divided into three groups: biological “producers” (e.g., plants and microalgae), “consumers” (e.g., humans, fish, insects), and waste “degraders and recyclers” (bacteria, microalgae, fungi, insects). Mirroring natural ecosystems, vascular plants in the form of microgreens and hydroponic culture, microalgae, cyanobacteria, as well as bacteria and fungi, would all have important roles in water management and decomposition of organic matter [8,82,83]. However, the miniature ecosystem of an LSS should not contain too much biodiversity, as this causes instability and makes modeling more difficult [84].

7. Microalgae for WWT on Earth

Using microorganisms to treat WW is, in fact, the global standard today, as most modern municipal plants employ aerobic biological treatment with bacterial activated sludge. The excess sludge is frequently stabilized later in anaerobic digestion reactors where biogas (mainly methane and CO₂) is produced [85].

Adding to or replacing the bacteria with photoautotrophic microalgae has given promising results in research [4,67]. This experimental pursuit was likely inspired by the microalgal blooms in streams and ponds contaminated by fertilizer, which contains NO₃⁻ and PO₄³⁻, and so do human and animal urine [86]. Microalgae, such as *Chlorella* and *Scenedesmus*, grow prolifically in WW oxidation ponds and sewage trickling filters, meaning that the byproducts of an activated sludge process stimulate their growth. The addition of urea and iron is even required if the microalgae are cultivated only in these byproducts [44]. WWT by microalgae has its limitations if the WW is not transparent enough and if the bacteria in the system produce too much turbidity, which reduces light utilization efficiency [44].

Nevertheless, the application of microalgal WWT in practice still remains an emerging field and a new niche industry. Microalgal–bacteria consortia are predominantly used not in closed PBRs but in large-scale outdoor open reactors, mainly in the form of high-rate algal pond systems. Globally, these systems are still under fifty. Pilot-scale (up to 0.4 m³) microalgal biomass production operations using different types of WWs, i.e., companies that apply this technology for commercial applications, are reported to be under twenty and are concentrated mainly in Western Europe and the USA [15].

The microalgae systems perform combined secondary and tertiary treatment because they (with, without, or after bacteria) may be applied at an early stage after the primary settling, but they are better at executing advanced purification of previously treated low chemical oxygen demand (COD) WW. That purification removes the remaining inorganic N and P (in the form of ammonium species, and/or NO₃⁻ and PO₄³⁻, respectively) [35,85,87]. Thus, microalgae are mostly used in xenic cultures in outdoor ponds in consortia with bacteria or after bacterial treatment [15]. The pilot-scale growth rates (about 0.54 per day) are half of those observed in laboratory research, indicating a scaling-up holdup, partly due to the need to maintain the cultures for long periods [15].

Moreover, apart from N and P absorption, microalgae are good at removing suspended solids and decreasing pathogens [35,85,87]. Both green microalgae [88] and cyanobacteria [71] are able to eliminate heavy metals and other micropollutants from WW. They, together with other microbes, are also capable of adsorbing methane, carbon monoxide, and other water-insoluble impurities on their surface [4].

On Earth, microalgae are used for direct WWT; however, they are most often in a consortium with bacteria [15]. In such liquid media, the micro hydrobionts exchange substances without direct contact [4]. The bacteria oxidize organic substances to CO₂, which the microalgae fix photosynthetically. In turn, the latter provides oxygenation valuable to bacterial respiration, (partially) replacing aeration. Needless to say, O₂ is distributed

throughout the WW very well, because the algae are dispersed throughout it [44]. Therefore, consortia usually perform better than microalgae or bacteria alone [15,89].

Notably, some microalgal species are capable of heterotrophy, i.e., they can utilize simple carbohydrates, acids, and amino acids in the dark; when light and organic C are present, some species may apply mixotrophy, which is common in WW. However, if bacteria are present, they may outcompete the algae in the oxidation of organic compounds [18,90].

Microalgae tolerate waste streams better than land plants, but axenic cultures still may not function optimally when put directly in raw WWs because they are inhibited by high concentrations of nutrients and high COD concentrations [3]. For example, the inhibition thresholds for *Chlorella vulgaris* have been found to be about 150–230 mg/L for NH_4^+ and 120 mg/L for P [18,91], translating to about 80 mg/L of COD [3].

8. Microalgae on Board Space Flights and Their Suitability for Space

From 1960 until today, microalgae have been sent to space flights mainly for studying pure biological effects, such as growth rate changes and photosynthesis efficiency in the two most unique conditions for space: microgravity and radiation. The review of Niederwieser et al. [19] lists over 50 algae experiments where simple culture formats such as agar plates, culture tubes, or flasks were used. Generally, no significant influence of microgravity or other flight conditions was found, but with a few exceptions [19]. Some tests show radiation resistance to an extent; e.g., *Limnospira indica* is relatively resistant to acute radiation exposure [92], but the effects of radiation have not yet been sufficiently studied [19]. Algae were also launched in spaceflight, contained in structures similar to aquariums in a system with aquatic animals [78,93–95].

In regard to PBRs, to the best of our knowledge, only two microalgal PBRs have been flown in space, both on the ISS (one of them was a system of four PBRs). They did not perform air revitalization, let alone WWT, as the early stages of bioprocess validation were examined.

Under the Micro Ecological Life Support System Alternative (MELiSSA) project of the European Space Agency, a system of four PBRs with an axenic culture of *L. indica* strain (synonym *Arthrospira platensis*, former *Spirulina platensis*) was run on the ISS in 2017, an operation named ARTEMIS-B flight, organized by the Belgian Nuclear Research Center (not to be confused with the Artemis Moon exploration program of NASA). The PBRs were only 50 mL each, and operated in a batch regime for one month. They were sealed from the outside atmosphere and not used to support the astronauts' breathing, but still, the O_2 productivity was monitored online. The goals were to study not only the biological effects on the microbial physiology but also how the space factors influence the bioprocesses in the PBR. The test was performed in parallel on the ground. There were technical issues with the start-up, the stirrer, and the gas pressure measurements for part of the PBRs, which reduced the number of batches. The results showed that, except for PBRs with stirring problems, O_2 production and therefore biomass growth were comparable both in the mechanistic model on the ground, and on board ISS. Due to the reduced batches, a definitive conclusion on the influence of microgravity on that particular strain remains difficult to assess [96]. Nevertheless, ARTEMIS-B is the first successful dynamic culture experiment in space. It proved the viability not only of building and operating a PBR in space with the same O_2 release as the ground control but also of applying ground commands to adapt PBR conditions and allowing several crew interventions [2].

The other PBR, loaded with *C. vulgaris*, was launched in 2019 by the German Aerospace Center (DLR). The purpose was to prove the feasibility of its non-axenic long-term (for 180 days) cultivation in space and to demonstrate for the first time the technology and performance of a hybrid LSS. The PBR was kept in the Life Support Rack (LSR), and the

plan was to integrate it within its operations. However, the connection was not performed because the LSR was not fully operational but still in the commissioning phase. The PBR used a backup bottle with CO₂. In addition, O₂ production and CO₂ consumption could not be evaluated, since both were being absorbed due to a leakage in the O₂ absorber. Moreover, an unexpected and not yet fully understood failure with the power supply ended the experiment after two weeks of running—earlier than planned [20].

These stand-alone PBRs, not connected to the LSS [18], only show how the very extreme harsh environments are still a challenge to our technologies.

As discussed, many researchers undoubtedly state that microalgae, besides O₂ and food provision, could perform WWT in space [9,13,16,30,45,48,59,68–80]. The research of some of them is not related to WWT per se, but to the suitability of the algae for space conditions.

It is well known that microalgae can grow and function well in microgravity [3,14], but research on different strains is still conducted. For example, a strain from the *C. vulgaris* species demonstrated growth and CO₂ consumption rates not significantly different under microgravity and under Earth gravity (1 g) [56].

L. indica is found to tolerate irradiation of 660 Gy within three hours, and repeated cycles of irradiation and subsequent recovery of the surviving fraction is proposed, a.k.a. “training-effect” [97]. An effective cryopreservation method for this cyanobacterium that is adapted to spaceflight experiments has been developed [74]. Simulation chambers are proposed [73], as is a bioreactor design for testing cultures in the stratosphere [98]. A monitoring system for *C. vulgaris*, using an image capture device at microgravity for the satellites CubeSats, is recommended [72]. The ISS orbits Earth roughly every 90 min, and 45′:45′ light–dark cycles did not affect the growth of green algae strains and even improved the growth of strains of cyanobacteria [76].

9. Microalgal Potential for WWT in Space

Water is the heaviest necessity in astronauts’ daily life, with a requirement of 2250 kg vs. 1359 kg of food per crew member for a 30-month mission [48]. The WW that would be generated in a crewed space vehicle or a planet colony includes sewage from the toilets, which could be separated into urine and solid waste or could be mixed; hygiene WW, a.k.a. grey water (streams from showers, laundry, and sinks, e.g., dishwater); and condensate (including exhaled breath, perspiration, and transpiration water from plants) [3,14,69].

Urine is the main source of N in space WWs, mainly as urea (85% of the total potentially recoverable N) [10]. Because of the bone demineralization, including high calcium (Ca²⁺) loss in microgravity, crew urine has a high Ca²⁺ concentration (220–230 mg/L), which causes high scaling in the ISS processing systems and a lower water recovery rate (85%) than the desired (>90%) [5,47].

The reports, calculations, and estimates are presented differently—in kg or in L. For example, the urine is reported to be 1.7 L/person/day [5,48], or 1.39–2.11 kg [14]. Notably, the condensate from humans is more—2.9 L/person/day, and it also contains organic substances, ammonia, and minerals [5,48]. The urine and its flush water of 0.30 kg/person/day [14] on the ISS are estimated to contain 5.98 g carbon (C) per L (from carbonate, urea, and other organics), 0.05 g phosphorus (P) per L and 8.04 g N/L (mainly from urea) [99], making the average daily excretion of N 7–16 g per crew member [10,100].

The calculations for hygiene water vary from 2.7 L/person/day [5,48] to 13.5 L/person/day [44]. The grey water in space contains higher detergent concentrations than those on Earth, due to the reduced water consumption. It is expected to increase significantly in future extended missions or celestial body colonization to 25 L/person/day, and even more

for a mature planetary base because of the tendency to increase the astronauts' comfortable daily life [3,5].

In addition, the WW composition is expected to become more complex because of the hypothetical plant cultivation, animal breeding, and even cell-free starch synthesis from CO₂ exhaled by crew members, which would produce more nutrients in the WW and specifically more surfactants in the grey water [5]. Therefore, intensive extraterrestrial missions and colonies would require a higher degree of closure of the water cycle.

Undoubtedly, the prototypes for space microalgal WWT have taken inspiration from terrestrial research or applications and can be used in parallel both in space and on Earth, e.g., in a circular bioeconomy [13].

In space settings, if microalgae are to be applied to perform WWT, the need to maintain algal cultures for long periods also exists, as on Earth, but size is critically limited, so the scaling-up size problem does not exist. In addition, the lower volume of the effluent in a spacecraft—low loading conditions—facilitates its easier, precise prediction for modeling and simulations [4]. However, the closed small system within a vehicle or an extraplanetary colony has to be designed to handle emergency shock loadings [48].

The ability of microalgae and cyanobacteria to remove or decrease heavy metals, other micropollutants, suspended solids, and pathogens, as well as to adsorb methane, carbon monoxide, etc., are also relevant to spacecraft and a closed LSS context [4,35,71,85,87,88].

Microalgal mixotrophy may be relevant in space mostly for axenic cultures and dark periods—e.g., alternating with autotrophy during light periods [14,18,35].

The water in a PBR could be a breeding ground for microbes, as it contains poly- and monosaccharides, cell fragments, and their degradation products. Consequently, if the human-derived WW after microalgal WWT is to be recycled as potable water, and especially for drinking, disinfection is necessary, as microalgae do not guarantee a complete eradication of the pathogenic bacteria [86]. Abiotic technologies such as multifiltration, ion exchangers, reverse osmosis, and distillation have to be used to remove the listed components [101]. Disinfection of the edible algal biomass is also needed [99].

In space contexts, the problem of inhibited microalgal cultures after direct contact with raw WWs, because of the high COD and nutrient concentrations, also exists. The first possible solution is dilution, which, as Afolabi explains, requires more potable water and limits overall efficiency [3]. The second solution to reduce the nutrient concentration to an acceptable level is pretreatment of the urine and other waste streams in bacterial bioreactors, which is more viable and would minimize energy expenditure [3]. Although there are many projects of space agencies that plan for dilution and direct WW–algae contact, the most primary and recent ones tend to opt for bacterial biotreatment to mineralize the nutrient load of the WW before reaching the algal PBR. Often, the PBR is the final step of WW utilization, where the algae receive effluents that are purified to a great extent [3,11,12,102,103]. Although separate, the reactors are connected in a closed gas loop, so that the microalgae would still provide O₂ for the bacterial oxidation of waste nutrients [3].

Often, the first pretreatment bioreactor, if more than one, contains anaerobic bacteria. Their benefit is better processing of concentrated WW, and no requirements for aeration, which is valuable for a station or vehicle in a vacuum medium [3,5,11,103].

In addition, an innovative approach worthy of investigation would be the inclusion of microaerophilic bacteria in the pretreatment step. It could prevent bacterial decay and predation, which are usually increased under aerobic conditions. Research shows that the microaerophilic enrichment has the potential to intensify bacterial metabolism and reduce aeration energy demand. Notably, this technique may rely only on control of oxygen levels, as a key design variable, and would not require any hardware modifications, thus

further reducing operational costs [104]. The most suitable placement of a microaerophilic reactor should be after an aerobic one, so that the former could utilize the O₂-depleted gas effluent.

While both bacteria and microalgae could do WWT on their own, e.g., bacterial NO₃⁻ production from urea has been demonstrated under simulated space gravity conditions [105], other authors and projects prefer algal–bacterial consortia to bacterial pretreatment. They find the most common strategy on Earth, the combined action of a consortium, to be more beneficial [8,82,83]. Archaea and fungi are also suggested to contribute to waste and matter recycling, as they do on Earth [82,83].

Microalgae also require the elements S, K, Mg, Ca, Na, Cl, Fe, Zn, Cu, Mo, Mn, B, and Co, which are estimated to be present in WW streams in space, e.g., on board the ISS, except Co, which would need to be supplemented [14]. A bacterial pretreatment often diminishes these elements, so additional supplementation is likely necessary.

Although microalgae are capable of utilizing both NH₄⁺ and urea, often the process estimates show benefit in the initial bacterial nitrification of these compounds in human waste. It is preferable that only a nitrate-rich solution reaches the PBR [2,35,103], although this subject remains to be fully elucidated [35].

Similar to the biomass growth and metabolite content, the efficiency of microalgae for WWT is also heavily influenced by the environmental conditions, with the additional factors of microgravity and radiation in space. Therefore, an optimized PBR hardware design is a prerequisite for LSS. The engineering efforts should consider gas and fluid exchange and mixing in microgravity, temperature, biomass harvesting, radiation shielding, etc. [106]. When sunlight is not available in the spaceship, the ambient light is not sufficient [28], and a light source and its intensity and regime should be considered.

Because of the important tasks planned for unicellular organisms (both eukaryotic and prokaryotic), despite their relative resistance to cosmic ionizing radiation [19], the hazard of long-term exposure to and insufficient shielding should be prevented [97].

Macroalgae also have the potential to perform WWT on Earth, but in space they would not be viable because they grow more slowly, require attachment surfaces and complex flow geometry, self-shade easily, etc. [107]. Therefore, the controllability and predictability of microalgae in a PBR make them a preferred choice in space research.

While fungi are capable of outperforming bacteria in WWT [108], their potential in space is better directed at the production of substances, such as enzymes and antibiotics [81]. Fungi consume O₂, and those that could perform WWT are less suitable for food generation, while being more prone to cause biological contamination [109] and biofouling [110] in an environment with fluidics already complicated by microgravity. The additional functions of microalgae for air revitalization make them better candidates for WWT.

10. Fluid and Gas Exchange Characteristics and Problems under Microgravity and Optimal Solutions

In weightlessness or microgravity, there are no convection forces, so fluid flow dynamics and gas mass transfer are restricted to diffusion [18,32]. This causes limited O₂ distribution in a PBR [32,111]. Also, there is no cell settling and no separation of the gas and liquid phases in a PBR [18]. Buoyancy is absent in no gravity [112] and reduced in microgravity [106]; i.e., warm fluid or gas does not rise, and cold does not sink. Surface tension is maximized, thus surface area is minimized, and diffusion produces larger gas bubbles. Capillary forces become enhanced, and fluid flow must be forced through pumping [106]. There is also no gravity-driven shear, meaning that in microgravity, adjacent layers of fluid move relative to each other [113].

An airlift PBR cannot be used because it depends on gravity—sparging air from below, which lifts through the heavier liquid, thus dissolving CO₂ into it and mixing the

culture [112]. Therefore, since gas flow cannot be used for mixing, Belz et al. [114] and Pickett et al. [106] list some alternative physical mixing methods—hydrodynamic jet nozzle, moving fixtures for stirring, low shear pneumatic flow, etc.

Membrane bioreactors on Earth reduce the volume and footprint of a biological WWT plant [5]. Notably, they are also a solution to the absence of free convection forces and the limitation in available O_2 in space because the membrane is capable of gas exchange without exploiting convection [112]. A membrane PBR can technically have any geometry [112]. A more detailed design, given by Verbeelen et al. [32], is a membrane-aerated biofilm reactor (MABR) where gas-permeable membrane tubes ensure the aeration. Specifically, it was designed for a biofilm of nitrifying bacteria on the outer surface of the membranes, but it could be optimized for microalgae.

The magnetic forces, e.g., magnetic stirring, are also a promising solution to the challenges of gas–liquid transfer phenomena and the resulting mass transfer effects, such as the lack of or reduced buoyancy [112].

For example, the requirement to use membranes for phase separation also has disadvantages, such as low specific gas diffusion rate, biofilm buildup, and the need for mechanical pumping to drive a membrane phase separation system. This pumping not only inflicts stress on the algal culture but is also prone to deterioration, thus requiring spare parts.

Therefore, Kura [60] proposes two magnetic phase separation mechanisms to replace membranes and pumps—the Lorentz force and the Kelvin force. Through the Lorentz force, magnetic fields acting on the microalgae medium are able to create a vortex in a circular reactor, creating a centrifugal force which drives liquid to the outside and bubbles to the inside of the reactor, where a liquid–gas boundary is formed. Ground tests of a small-scale magnetohydrodynamic PBR indicate the feasibility of the system. The Kelvin force arises when applying a magnetic field to liquids and gases with different magnetic properties, and is capable of attracting gas bubbles, leading to a passive phase separator. The author introduced concepts for a diamagnetic separator device, a bubble reactor, and a raceway reactor using this mechanism. The results were also promising [60].

Space aquatic chemistry focusing on water characteristics is an emerging field [5], and while the physical phenomena of fluids in microgravity are known to an extent, their influence on gas, water, and solute transport in live organisms has not been completely investigated [18]. One of the limited number of studies involved *L. indica* in culture bag membranes, used routinely for space cell culture experiments. It utilized a random positioning machine (RPM), a.k.a. a clinostat, set up for a simulation of microgravity in a ground analogue. The research showed that there was a thicker stagnant liquid boundary layer over the cell membrane and the culture bag membrane. Also, paradoxically, a less efficient diffusive O_2 gas release from the liquid leads to high O_2 partial pressure, i.e., high dissolved O_2 concentrations. This caused C limitation, which inhibited *L. indica*, slowed its growth, and changed its protein expression [113].

11. Microalgae Treating the Waste of Aquatic Organisms in Space Contexts—A Prototype of WWT

Closed aquatic systems are important for ecosystem experiments. In space settings, if microalgae are in the same compartment as other aquatic organisms, e.g., fish, or are separated by a permeable membrane, they would perform WWT by recycling the waste in the water, not only produced by the cabin crew but also by aquatic animals.

As early as the Cosmos missions were carried out, before 1990, *C. vulgaris* was grown in a three-component aquatic system, including fish and bacteria, during the space flight for 13 days, thus it participated in fish waste nutrient recycling [94].

On board the Russian FOTON-M3 satellite, an aquarium chamber with 15 snails (*Bi-omphalaria glabrata*) and four fish (*Xiphophorus helleri*) that were fed automatically was separated from a chamber with illuminated *Euglena gracilis* via a membrane exchanger, which allowed transporting the O₂ to the algae and CO₂ and waste products in the opposite direction. Notably, the O₂ produced was enough for the animals throughout the experiment. Cell density of *Euglena* increased over the first 150 days but then declined fast and sharply, while the cells retained their motility and velocity [78,95].

In Germany, in 1987, a complex approach of a microalgal bioreactor with the green microalga *Chlamydomonas reinhardtii* was proposed. This idea led to a truly innovative concept, the AQUARACK animal maintaining system. A zoological component or compartment with the fish *Xiphophorus hellerii* would have been combined with a PBR, with a botanical component, and with a filter system containing nitrifying bacteria (a microbial component), in a stable, long-term biological equilibrium of the closed system. In order to close the food loop, i.e., for feeding the fish, a co-culture of microalgae and suitable crustaceans (e.g., *Daphnia* or *Cyclops* sp.) was proposed for the adults, and a microalgal-rotifer system was proposed for the juvenile fish. However, the buoyant water higher plant *Ceratophyllum demersum* replaced the PBR in the concept, likely due to its high capacity for NO₃⁻ and PO₄³⁻ uptake. In the 1990s and the 2000s, the ideas were implemented in the experiments of the Closed Equilibrated Biological Aquatic System (CEBAS) by the DLR. Remarkably, in one of the CEBAS ground experiments, *Microcystis* cyanobacteria replaced *C. demersum* naturally and took over the producer role without any external influence. This happened in a mini-module and monolithic setting where all subcomponents were united in one tank system separated by transparent and non-transparent walls with a special arrangement of small holes for controlled water flow. The whole system displayed fluctuations, possibly compensating reactions to natural disturbances that arise in ecosystems [115]. The CEBAS mini-module, which was later flown in space, contained *C. demersum* and was not inoculated with microalgae [116].

Near the year 2010, China launched a system named Closed Aquatic Ecosystem aboard the spacecraft Shenzhou 2. It included the green microalgae *Chlorella pyrenoidosa* and freshwater snails (*Bulinus australianus*) in a sealed aquatic unit. After the flight, the algae survived, but this time the snails had died, a proof of challenges in preserving multi-trophic systems in space [93].

The microalgae *Tetraselmis chui* was used to purify WW from zooplankton as well as its feed in a ground experiment for intensive production of the harpacticoid copepod zooplankton *Tigriopus californicus*. An invertebrate aquaculture is a promising source of food in deep-space travel. *Tetraselmis* and other resident microorganisms maintained water quality in the “green water” closed-loop bioreactor, achieving zero effluent discharges. As the authors suggest, such a system should be a closed circuit, produce food for the invertebrates on-site, and facilitate the maintenance of water quality [117].

PBRs are also suggested to be combined with aquatic systems, hydroponics, and aquaponics to improve their water purification [8].

12. Indirect WWT Including Microalgae in Programs and Projects of Space Agencies and Academia

12.1. AES of NASA

The AES program of NASA aims to develop extended missions with a duration of several months to several years. Under this program, a study that focuses on the integration of subsystems established a ground prototype system for a crew of four. It included three bioreactors: an Organic Processor Assembly (OPA) with anaerobic bacteria, the first-line treatment of simulated solid waste, and its flush water. Its effluent would go to a

Reservoir Tank that would act as a failsafe and then to a PhotoMembrane BioReactor (PMBR) with *Chlorella sorokiniana*. A Suspended Aerobic Membrane BioReactor (SAMBR) would process synthetic urine. PMBR and SAMBR formed the Nutrient Processor Assembly. *C. sorokiniana* was chosen to be in the PMBR in this ground demonstration because it has a high nutrient threshold (for example, it can grow in urine, although its two-fold dilution gives better results) and potential for NH_4^+ removal from urine-derived WW [118]. The PMBR was able to process 2.5 L/d of effluent from the OPA, and the PMBR's effluent could be used after further treatment as potable water or as a fertilizer for downstream plant growth. The researchers redesigned the cylindrical PMBR into a flat-panel configuration due to self-shading and other concerns.

However, when the OPA and the PMBR were conjoined and integrated, unforeseen issues with the system power consumption arose, and due to that, upstream instability and multiple interruptions happened. One of the main lessons learned from this early-stage development and early integration validation was the need for software capable of system-to-system communication. Discussions are ongoing to develop a “master-style” overview software that can remotely operate and monitor these complex systems [11].

12.2. NASA and the University of South Florida

A microalgal system is also the final stage of WW utilization and WWT in an Earth-based project of NASA and the University of South Florida. The system, together with a bok choy cultivation system, would use a nutrient-rich permeate as a medium from a novel device named Anaerobic Membrane Bioreactor (AnMBR). It is described as a bioregenerative fecal WW management hybrid system for early planetary habitats. It deploys an anaerobic microbial consortium coupled with an ultrafiltration membrane module for retention of solids and cells. AnMBR has a productivity of 2.5 L/day of nutrient-rich permeate. The device has been operating since 2021 with canine fecal waste as a surrogate for human waste [12].

12.3. BIOSYS of the University of Louisiana

The BIOSYS project or system is under development by the Energy Institute at the University of Louisiana, Lafayette. It is a laboratory-scale ground prototype of a waste management system with a low energy expenditure, envisioned for lunar and Martian habitats [3]. The effluents of the sewage, food waste, and grey water would firstly pass through an anaerobic bioreactor, then through an aerobic one with activated sludge, and then, possibly after ultraviolet and/or ultrasound treatment, through an algal PBR with *C. vulgaris*. This species was of particular interest to the researchers due to its high photosynthetic efficiency, lipid and carbohydrate content, and ability to utilize waste nutrients for growth [119]. The algal biomass, along with the bacterial one, would participate in the final production of lipids for food, fuels, and lubricating oils, proteins, and microbial cakes [3].

A conceptual research originating from the same University of Louisiana, examined both scenarios of WWT by microalgae: after the two BIOSYS bioreactors, or a direct microalgal WWT. The authors used stoichiometric mass-balance feasibility calculations and a process estimate based on, e.g., urine composition, microalgal optimal C:N:P ratio, and other growth parameters and requirements.

First, the authors summarized previous findings that in saltwater, a C:N:P mass ratio of 41:7:1 (if NH_4^+ is the N source) and 48:7:1 (if NO_3^- is the N source) is the ideal requirement for growth of microalgal species.

To determine the threshold level of C, *C. vulgaris* was cultivated at fixed N and P concentrations (both low levels) with increasing C concentrations. The content of the f/2 maintenance medium (1 mg C/L, 64 mg N/L, and 13 mg P/L) was used as the low levels

of the macronutrients. The C level, which resulted in the highest biomass concentration, was named High C. The high level of C and the low level of P were then fixed, and N was increased until the level above which the biomass concentration started to decline (High N). Lastly, the high level of P (High P) was determined by increasing P concentrations at fixed High C and High N levels.

The researchers experimented with synthetic domestic WW, a simulation of the effluent from the two bioreactors in BIOSYS, thus containing macronutrients mainly in the forms of carbonate, NH_4^+ , and PO_4^{3-} , and without organic C (even with inefficient treatment in the two bioreactors). The ranges until microalgal growth inhibition is reached were 0.1–71 mg C/L, 64–127 mg N/L, and 13–34 mg P/L. Within them, 71 mg C/L, 64 mg N/L, and 13 mg P/L resulted in the highest biomass concentration (approximately 1.4 g/L), i.e., at high C, low N, and low P (named treatment HLL), which corresponds to a C:N:P mass ratio of 5.5:5:1. Although closest to the theoretical among the tested C:N:P ratios, it was still C-limited by at least 7-fold. This was also supported by 99.9% C consumption, but only 56% and 72% of N and P consumption, respectively. Therefore, an incremental supplement of C must be added for optimal growth and to enhance N and P consumption.

In the ideal case, the influent to the PBR will contain macronutrients below the maintenance low levels. The PBR would be the final WWT system. If revitalizing the air is the goal, based on treatment HLL, the PBR will require daily addition of ~256 g N and 52 g P. In the case of the first two bioreactors failing or being inefficient, dilution of the PBR influent is obligatory.

The scenario of direct use of actual liquid wastes for the PBR will be discussed in the corresponding section (13.2.3.) [99].

12.4. MELiSSA of European Space Agency (ESA)

The MELiSSA program of the ESA is one of the largest LSS studies. It was launched in the late 1980s, making it the longest-running BLSS research project [10], and is based on the principle of an “aquatic” lake ecosystem that aims to be a closed-loop LSS [23]. This is a different approach from the large analogue human test facilities. The solid waste is planned to pass through three bacterial bioreactors (compartments), while the urine only through the last of them, before the resulting solution, rich in NO_3^- , would reach a compartment with vascular plants and *L. indica* [103]. The first compartment would be with thermophilic anaerobic bacteria, the second with the purple bacteria *Rhodospirillum rubrum* in a photoheterotrophic mode of its metabolism, and the third with nitrifying bacteria [10]. Previous versions included mixing of solid waste and urine together before passing through all the bioreactors [35,83,120]. As expected, the CO_2 produced from the crew and the other bioreactors would also enter the photosynthesis compartment, which would generate O_2 , food, and water for the astronauts. ESA introduced and prefers spirulina in LSS studies for several reasons: light conversion efficiency, alkaline tolerance, high nutritional value, adaptability to different conditions, including radiation [28], and better digestibility than *Chlorella* [23].

According to some researchers, MELiSSA is similar to BIOSYS, except that the process steps are differently oriented, and BIOSYS is engineered to manufacture many more products [3]. MELiSSA’s pilot plant and compartments are still testing closure and reliability in modules on Earth and gradually in spaceflight experiments, but the system is not yet fully operational as a complete system on a crewed mission [32,121]. For example, a PBR with *L. indica* was flown on the ISS as a preliminary bioprocess validation, which did not utilize waste or create O_2 or food for the astronauts [96]. In addition, only the nitrifying microbial consortium has been launched in space [122].

12.5. MELiSSA Pilot Plants, Ground Demonstrations, and Associated Academic Work

12.5.1. MELiSSA Pilot Plant in Barcelona

The MELiSSA Pilot Plant in Barcelona included a test mock crew of three rats, whose waste would first pass through a nitrifying bioreactor and then to an air-lift PBR with a capacity of 83 L and loaded with spirulina [123]. The gas phases of the three compartments were connected in a closed gas loop; thus, the PBR simultaneously provided the O₂ for nitrification and animal respiration in the rats' isolator [2].

12.5.2. Ground Work with Different N-Sources

Although the multiple reactors and microbial processes increase the complexity of the LSS, the direct connection of non-nitrified urine to the PBR in the photosynthesis compartment seems not to be feasible if the urea is left to be hydrolyzed to NH₄⁺ by bacteria during storage time. An experiment with a simplified version of MELiSSA using a mouse as a consumer and a test mock crew showed that the system could meet the desired O₂ levels of 20.3% under NO₃⁻ and under urea regimes, but it could only reach a maximum O₂ level of 19.5% under an NH₄⁺ regime. This 35-day-long ground demonstration study was performed on a simplified version of MELiSSA [35].

The authors find it important to also investigate the cumulative effect of different N sources and high urine salinity in the fluctuating streams of human urine on cyanobacterial cultivation and air revitalization. Different organic compounds (metabolites, hormones, etc.) and personal hygiene products present in urine are an influx of external organic C sources and will change the cultivation mode from photoautotrophic to mixotrophic, so a study on their influence is even more important [35].

12.5.3. The University of Ghent

The researchers made a ground demonstration, which included a physicochemical treatment and a microbial electrolysis cell (MEC), which turns urine into a stable nitrate-rich solution for a PBR [2]. With an emphasis on urine nitrification, and in collaboration with other Belgian and Spanish colleagues, they developed in detail a combination of physicochemical treatment, MEC populated by a ureolytic heterotroph, and MABR nitrifiers. Ultimately, a low COD, nitrate-rich effluent suitable for cyanobacteria and plant growth would be obtained in a terrestrial experiment. The spirulina biomass could be used as a source of microbial protein [32,124].

12.5.4. Beijing University of Aeronautics and Astronautics

Inspired by MELiSSA, a Chinese study examined a continuous cultivation of *L. indica* with 12.5 mL synthetic human urine per liter in a PBR. Under these conditions, 1.05 g biomass was obtained, the consumption of N reached 99%, and the consumption of P even more, which is very important to increase the degree of closure of WW recycling. The consumption of Cl, K, and S was 75%, 84%, and 96%, respectively. There was a large quantity of Cl⁻ and NaHCO₃ that remained in the medium, and the author commented that further studies should find ways for the culture to consume them [125].

This research contrasts with the BIOSYS experiments, where 99.9% C consumption, but only 56% and 72% of N and P consumption, were observed. However, the authors had used a specific C:N:P mass ratio of 5.5:5:1 [99].

12.5.5. The Stephan Angeloff Institute of Microbiology

Although Bulgaria is not a member of ESA, the Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences hosts research on microalgae [126] and cellulose-degrading bacteria [127]. The institute has the potential and capacity to test bacterial and microalgal strains in microgravity conditions with a random positioning machine

(clinostat), as well as to apply them in real space conditions. Degradation of flush water containing cellulose (hygiene paper, clothes, etc.) by bacterial–algal consortia in separate bacterial bioreactors and PBR with subsequent accumulation of microalgal biomass is a promising research direction that the institute aims to follow [2].

12.6. China Astronaut Research and Training Center and China Manned Space Program Agency

Researchers from Chinese space facilities also envisioned the PBR in an LSS to be one of the end stages of WW utilization in a process estimate of an installation. The PBR would be used in space stations and extraterrestrial missions with a duration of 2 to 5 years, as well as in a future Lunar base, but only in the preliminary stage, because in the next stages, higher plants would provide O₂. A bioreactor for pretreatment of urine WW (urine and its flush water) and a brine/feces processing unit would send their effluents to a second bioreactor, which in turn would feed a PBR and/or fertilize higher plants with a solution with stabilized N and provide water to the crew, after purification by a water recovery subsystem. Material flow balance calculations showed that the PBR would need supplemental water, and biomass from it would proceed to a dewatering unit, whose effluent would feed the second bioreactor. The authors concluded that, in theory, a circular ecosystem consisting of multiple species within a “human–plant–animal–microbe” concept with a total material closure degree of 98% could be built [102].

13. Direct WWT Including Microalgae and Their Bacterial Consortia in Programs and Projects of Space Agencies and Academia

13.1. Direct WWT Using Microalgal–Bacterial Consortia or Polyalgal Microalgal Cultures

13.1.1. Biosphere 2 of NASA and the Technical University of Munich—a Terrestrial Closed Ecosystem Environment Experiment

Human urine was deposited in the 1990s in the marshland biotope of this project, already described in Section 3, where the microbial community digested it, and the bioavailable N could be redirected towards the remaining biomes. Although not in an engineered PBR, microalgae almost inevitably appeared in this marshland biotope and were almost certainly a part of the communities of the other biomes, too, even if scientists did not intentionally add them [32,33].

In the Biosphere 2 system, the University of Arizona and the Arizona State University are currently setting up a facility named Space Analog for Moon and Mars (SAM). The Technical University of Munich has designed a PBR to be integrated into it. Its main intended application was air revitalization, but it had to maintain capabilities for secondary applications in WWT and biomass production. The design included an interface compatible with a ball valve, so that WW influent could enter the PBR [112]. The chosen configuration, which complied mainly with the air revitalization goal, was a Flat Plate Airlift (among the most frequently used types of PBR), a modular PBR with a volume of 300 L. As mentioned, an airlift mode depends on gravity and convection, thus a mechanism to ensure mixing in their absence would be needed. Whether batch or continuous operation is more appropriate was left for future investigation.

The authors commented that axenic cultivation, in the case of *C. vulgaris*, while keeping the chamber entirely free of other organisms, is feasible but challenging and not proven for long-term stability. In contrast, non-axenic cultivation of *C. vulgaris* is possible, long-term stable, and more feasible in terms of designing and controlling the cultivation process [112,128].

13.1.2. AlgaTron of the University of California, Berkeley, in the 1960s

As early as the 1960s, ground experiments proposed a bacterial–algal consortium with predominating microalgae, grown as a film on the wall of a transparent rotating drum, named Alгатron. This allowed shallow 3.5 mm deep cultures to ensure efficient illumination with a minimum of equipment. The rotating suspension is a solution to the problem with the gas exchange arising because of the shallow culture—there was fast and continuous renewal of the culture surface and high gas exchange between the culture and the ambient air. This provided additional aeration of the activated sludge besides the photosynthetic aeration. The instrument also allowed a high concentration of culture biomass and high contact between wastes and the culture.

The algae in the consortium were mainly from the *Chlorella* group, but when the retention time of the biomass was prolonged, the cyanobacteria *Oscillatoria* predominated. Domestic primary WW, even enriched with fresh feces and urine in some cases, was used as the test medium.

The results were encouraging and showed that the wastes of a crew member could be adequately treated with the use of 3 or 4 m² of Alгатron surface area. Optimizations, i.e., culture concentration, could ensure even smaller areas and a minimum volume and weight of liquid in the Alгатron. The system was proposed to be especially useful for degrading complex polysaccharides and other compounds that are challenging to biological WWT in closed systems [44].

13.1.3. The Kennedy Space Center of NASA

A ground prototype of a PBR with a microalgae–bacteria consortium with *Chlorella* sp., which was hypothesized to process raw, undiluted crew WW, was developed by Pickett et al. [106] at the Kennedy Space Center, Florida. Rather than relying on gravity-dependent phenomena like buoyant gas lift for mixing, this work used strategies to tackle a reduced gravity environment—pump-induced recirculation for internal mixing and tubular membrane-based gas exchange—to maintain critical processes (mixing, gas transfer, biomass, and cell suspension). Simulated cabin air was pumped in for CO₂ delivery. Downstream, it would use an ultrafiltration membrane for solid–liquid separation of the algal biomass and the liquid permeate. Backwash for minimizing membrane fouling, harvesting at high densities to minimize downstream dewatering, and a high level of illumination because of the high biomass densities were also employed. The PBR was Technology Readiness Level 3, meaning lab-scale early experimental validation. The research focus was gravity-independent gas exchange mechanisms, and subsequent testing is needed in order to investigate the effects on WWT [106].

13.1.4. Eu:CROPIS

The Eu:CROPIS mission of the German Aerospace Center involved a microalgal–bacterial consortium in direct contact with synthetic urine used as fertilizer. A symbiotic community of *E. gracilis* and nitrifying bacteria, using synthetic urine as a fertilizer, was intended to grow tomatoes under the gravitational conditions found on the Moon and Mars in a closed loop. The community was planned to populate a trickle filter where the *Euglena* was to provide O₂ for the nitrification activity. Tomato plants were to be cultivated with the help of the nutrient stream from the trickle filter. The system flew in space as a small satellite mission. Unfortunately, due to a computer failure, irrigation could not be initiated, and the dormant *Euglena* and tomato seeds could not be activated [129]. Yet, the ground demonstration was functional and has been used as an analogue for long-term extraterrestrial biological research. It has been established that, depending on the dilution of the synthetic urine, a 66–87% N recovery efficiency was realized. As expected, an undiluted urine negatively impacted the NO₃[−] production rates, while 60 and 80% diluted urine for feed was within the optimal range for N recovery [21,22].

13.2. Direct WWT Using Axenic Microalgal Cultures

13.2.1. BIOS-3 of the Soviet Union in the 1970s—a Terrestrial Closed Ecosystem Environment Experiment

In the BIOS-3 system, already described in Section 3, due to its high salt and urea content, urine was treated separately from the other waste streams. It was, directly or after pretreatment, directed to the PBR loaded with *C. vulgaris*, utilized for higher plants, or disposed of. BIOS-3 was a milestone for LSS because, for the first time, a system recycled air and water and accommodated three persons for four to six months [25].

13.2.2. State Science Center of the Russian Federation—Institute of Biomedical Problems of the Russian Academy of Science

Ground-based experiments that included a system of 15 m³ in volume containing a PBR with 45 L algal suspension (most likely of *C. vulgaris*, because of the Russian tradition) showed that the PBR was able to fully regenerate the air and water for one person and ensured a partial N cycle due to complete consumption of the urine N. Assuming that the urine processing in the PBR would provide 30% of the O₂ demand and consume 30% of the CO₂, the authors proposed a dual LSS with an abiotic and biological part, each one performing a specified portion of the regenerative functions. In the event of failure of one system, the other one will take charge; e.g., if the abiotic component does not generate enough O₂, its production by the microalgae will be raised up to 100% within 24 h [40].

13.2.3. NASA's EPSCoR Supported Research Developed at the University of Louisiana

Aiming for direct treatment of urine and flush water, the University of Louisiana team proposed a round-robin PBR system after research supported by the NASA Established Program to Stimulate Competitive Research. The modular nature of the PBR allowed the removal of a single problematic PBR unit in the middle of the cascade without total system shutdown [99]. This modularity of the system is highly recommended in extraterrestrial missions [48].

The round-robin PBR will require dilution of liquid waste to reduce the level of macronutrients within the tolerance threshold of *C. vulgaris*.

The authors worked with the assumption that on the ISS, 2.05 L/day average daily liquid waste (urine and flush water) per crew member is produced, with average daily concentrations of 5.98 g C/L, 8.04 g N/L (mainly from urea), and 0.05 g P/L, forming a C:N:P mass ratio of about 128:173:1. On that base, the required dilution of the liquid wastes as well as the different required bioreactor (wet) volumes that serve different macronutrient goals could be estimated. For instance, to ensure C provision in the high range, a volume of 173 L is required, but it would result in N and P concentrations of 95.5 and 0.6 mg/L, respectively; therefore, P amendment would be needed. Interestingly, with the sequential depletion of C in the media during cultivation in the system of units, the authors propose that the respiration-derived CO₂ should be used to increase the input of C. If the aim is a complete PBR absorption of the CO₂ generated per crew member (1040 g/day), a daily supplement of ~250 g N and ~54 g P will be needed, based on treatment HLL (see Section 12.3).

The actual PBR design would depend on the specific microgravity scenario (e.g., orbiting space stations, deep-space travel, or partial gravity on lunar/planetary surfaces). Finally, as planned in other projects, a further purification of the PBR effluent before it is turned into potable water is needed [99].

The authors also modeled a treatment of both the urine and exhaled CO₂ within the context of integrating a closed-loop microalgal system into the existing LSS within the ISS.

Because these species are extensively studied, *C. vulgaris* and *L. indica* were selected for use.

Depending on the proportion of N source (NH_4^+ or NO_3^-), a C:N:P mole ratio between 106:16:1 and 124:16:1 is recommended for their sustainable growth [14]. The first ratio turned out to be the Redfield C:N:P ratio, which states that marine phytoplankton and deep-ocean organic matter contain a relatively constant mole ratio of C:N:P at 106:16:1. Discovered by Alfred Redfield in 1934, this ratio acts as a point of reference for nutrient availability and biological processes in aquatic ecosystems [130]. Even with a perfect Redfield C:N:P ratio, the too high absolute concentrations in raw urine would be stressful for algae, thus appropriate dilution is assumed.

It was estimated that the exhaled CO_2 (1.04 kg/person/day) and the urine could provide a C:N:P mole ratio of about 7836:324:1 and 8014:382:1 for inorganics plus urea and overall resources, respectively. This indicated that P was the limiting nutrient and would limit microalgal biomass production to approximately 11 g/person/day and O_2 generation to approximately 10.4 g/person/day, according to the calculations. These yields were significantly lower than the estimated food and O_2 requirements of 1510 and 816 g/day/crew member, respectively. The authors predicted mixotrophic cultivation because of the organic C in the urine.

Cultivation mode affects reactor size, and reactor space requirement is critical for extraterrestrial flight applications. Both species were considered for both suspended and attached-growth cultivation. Although attached growth systems have higher installation costs than suspended growth systems [18], the biomass productivity of attached-growth is 1.6–5.5 times higher than suspended-growth [131].

The assessment suggested that both species should be cultivated in attached-growth cultivation. They would either be operated under limited N/P conditions and produce low amounts of biomass and O_2 , or require continuous supplementation of N and P from external sources or from other waste streams, e.g., feces, food waste, etc., so that both could provide significant and sufficient amounts of reclaimed O_2 and biomass. However, only *L. indica* turned out to be able to function in the wet volume of the smallest estimated reactor. The authors admitted that this estimation was rather simplistic compared to the complex relationship among the cultivation parameters [14].

In another ground experiment, *C. vulgaris* was grown in a series of PBRs in a round-robin scheme. Samples were collected, and N and P nutrient removal was calculated based on measurements and used as performance metrics for WWT.

An initial biomass concentration of 0.5 g/L, with an average biomass productivity of 74 mg of biomass/L-day, resulted in 89% N removal and 76% P removal, and an average of 12% CO_2 removal per PBR.

Notably, the initial biomass concentration from 0.5 g/L to 1.8 g/L did not increase nutrient removal and CO_2 uptake. Seed culture from a previous PBR even decreased it, while seed culture from maintenance media decreased only the N removal. These differences were due to mass transfer limitation for mineral uptake and exchange of gases when the microalgal cell culture was denser. Increasing illumination up to the sunlight's intensity for six days did not increase biomass concentration.

The reduction of at least 87% of N and 68% of P is also expected over six days under continuous illumination. Any residual N and P in a PBR unit can be fed to the next PBR unit in the cascade for further reduction. To achieve the target biomass productivity of 74 mg of biomass/L/day, the illumination can be increased to the optimum for this *C. vulgaris* strain, and agitation can be increased by adding baffles where the bubbles are introduced. This would also increase the mass transfer rate of the nutrients and CO_2 to the microalgae cells.

At least ten modular units are necessary because of the CO₂ scrubbing requirements, but the number and volume of PBR units depend on the priority needs for life support.

The absence of cell settling and the absence of separation of the gas and liquid phases in a PBR under microgravity, i.e., the gas mass transfer being limited to diffusion, may cause problems with the automated pumping of the culture, as well as with the nutrient uptake in the round-robin scheme, which needs to be validated during space explorations.

The modular design and operation of the PBR units in series is especially important for deep space or colonies on the Moon or Mars because, as discussed, it would allow problematic units to be uncoupled from each other for safety and reliability. Changing the feeding rates of the macronutrients and CO₂ is an example of future optimization of the system.

The authors discuss that the Define, Measure, Analyze, Improve, and Control (DMAIC) approach can be applied in the commercialization of microalgae cultivation and processing systems, while the Define, Measure, Analyze, Design, and Verify (DMADV) approach can be used for integration of microalgae in the space LSS [18].

13.2.4. Virtual Habitat (V-HAB)

A joint collaboration of the Technical University Munich and the University of Colorado made a simulated model of replacing components of the life support rack of the ECLSS on the ISS with a flat-panel PBR with membrane air exchange and *C. vulgaris*. They used the dynamic life support simulation tool V-HAB, which has been under development at the Technical University of Munich since 2006. The model planned for adding a buffer in the urine before entry in the PBR, and that NO₃⁻ salt would be provided to the algae when urine was unavailable. The analysis showed that, for a crew of five astronauts on the ISS and in other space contexts, the PBR, together with a CO₂ buffer system, was capable of replacing the Urine Processing Assembly (UPA) and the Oxygen Generation Assembly (OGA) and reached 100% urine processing capability. UPA is a part of the Water Processing System (WPS), and OGA consists of the Sabatier Assembly and the Electrolysis Cell Stack. The other parts of the WPS, however, are still required for the treatment of the effluent from the PBR back to potable water. This *in silico* model led to increased recovery rates of the water from urine, thus reducing resupply, and the model was beneficial in terms of volume. However, it had a few disadvantages in mass and major drawbacks in power consumption. Whether the trade-off is worth the cost would depend on the mission duration and destination. This conceptual integration is not tested in a physical ground experiment yet [46].

13.2.5. The University of Colorado

Matula and Nabity [86], based at the University of Colorado, made a high-level simulation including microalgal PBRs to perform all the functions of air revitalization, WW management, food production, radiation shielding, and thermal control on the ISS for a crew of six. The algal PBR volume, mass, power, and consumables necessary to meet relevant ISS environmental requirements were calculated.

For all functions, the bioregenerative systems proved their capability of supporting human spaceflight again, and that algal PBRs must be considered for ECLSS purposes in future missions. However, a PBR sized to fulfill all ECLSS functions for six crew members would require from 10 to even 100% more mass, power, and volume than the current technologies. The illumination systems contributed to a great extent to the excessive power requirements, and this was also valid for WWT, where the power consumed was much higher (about 280 times), compared to that of the current WWT technology on the ISS. It could be reduced with the replacement of the fluorescent lights with LEDs tuned to algal growth.

The authors found that the amount of biomass and algae medium for the suitable species *C. sorokiniana*, which is required to process the urine and grey water of six crew members, may be excessive. The volume of the PBR would be 21 m³ or about seven times more than the WWT system existing on the ISS, while the mass would be about 34 times greater than the existing system. This higher volume was required because of the rate-limiting step of the reduction of N- and P-based compounds in human urine. The slow turnover and large volume may be resolved through increasing the biomass density; thus, the authors propose increasing the lighting and/or magnesium supplementation, which has shown promising results.

Disinfection of the PBR effluent before being used as potable water and also pasteurization of the urine stream before feeding it to the PBR is also strongly recommended, an enhancement replacing the harsh chemicals used now for the urine brine on the ISS. To justify the feasibility of using a benchtop PBR in a space vehicle cabin, the authors envision a real microgravity test flight [86].

13.2.6. S.A.B.E.R. Developed in BASIS Peoria

A work, originating from the BASIS Peoria School in Arizona, describes a ground demonstration experiment, named Sustainable AI-automated Bioreactor for Environment Remediation (S.A.B.E.R) with a Random Forest-based artificial intelligence (AI) control system, testing *C. vulgaris* in nutrient media containing urea and coffee ground waste. The algae were loaded in a three-liter container in a weatherproof tote of approximately 70 L. The magnetic stirrer at a low setting speed had an additional function of simulating microgravity, although imperfectly. The high N content of urea, when combined with the P and micronutrients of coffee waste, produces a balanced medium that enhances algal growth and O₂ production. The concomitant rise in pH is counter-balanced by the buffering capacity of the coffee's organic acids. The results showed that waste-derived media performed comparably to standard nutrient media. The AI-driven approach exhibited significantly higher O₂ production, more stable and reliable biomass growth, and more efficient CO₂ reduction than the manual control and the threshold-based method, but the energy consumption of S.A.B.E.R.+AI mode was slightly higher. This trade-off was found to be largely beneficial, especially when considering that the efficiency of a system is not measured solely in cost in cases where an LSS cannot fail, such as space missions, lunar outposts, bunkers, or remote research facilities (S.A.B.E.R is also suitable for ground facilities). In such scenarios, efficiency is also measured in reliability, adaptability, and resource independence.

The authors also did closed-loop simulations with WW recycling, coupling S.A.B.E.R.+AI with metabolic cascade membrane bioreactor units, where NH₄⁺ from grey water nourishes the algae, whose O₂ is used by nitrifying bacteria downstream. The results of cascading three such units showed net O₂ autonomy exceeding 85%, water reuse of 60%, and N recovery over 90%, which significantly exceed current ISS figures and align with MELiSSA targets.

The studies showed that integrating a completely automated adaptive control system and waste-derived media noticeably increases the biological and energetic performance of a PBR [24].

13.2.7. The University of Stuttgart

A concept of noninvasive process control of a 100 L PBR functioning for WWT (and most probably air revitalization) in space was proposed, which consists of a sunlight collection unit that includes mobile mirrors around a tubular PBR (among the most frequently used types of PBR) with *C. vulgaris*. By changing the closure or openness of the mirror unit and the intensity of the artificial light, and by adapting the degree of focus,

constant levels of illumination and temperatures can be achieved. To elaborate, this unit would collect sunlight at times of low intensity and partially obscure the PBR at times of high intensity. It would also thermally insulate when the temperatures are low and homogenize the intensity distribution on the PBR surface, thus assisting the illumination. The authors hypothesize that if closed, this mirror collector system in an LSS, when heated by LEDs, could secure temperature levels for the PBR even outside of the habitat on the celestial bodies, and even during the nights. However, the system has to be optimized to withstand low outside pressure, space radiation, and sandstorms [112,132].

14. Summary of the Projects

General depictions of direct and indirect WWT with the participation of PBRs are presented in Figure 1.

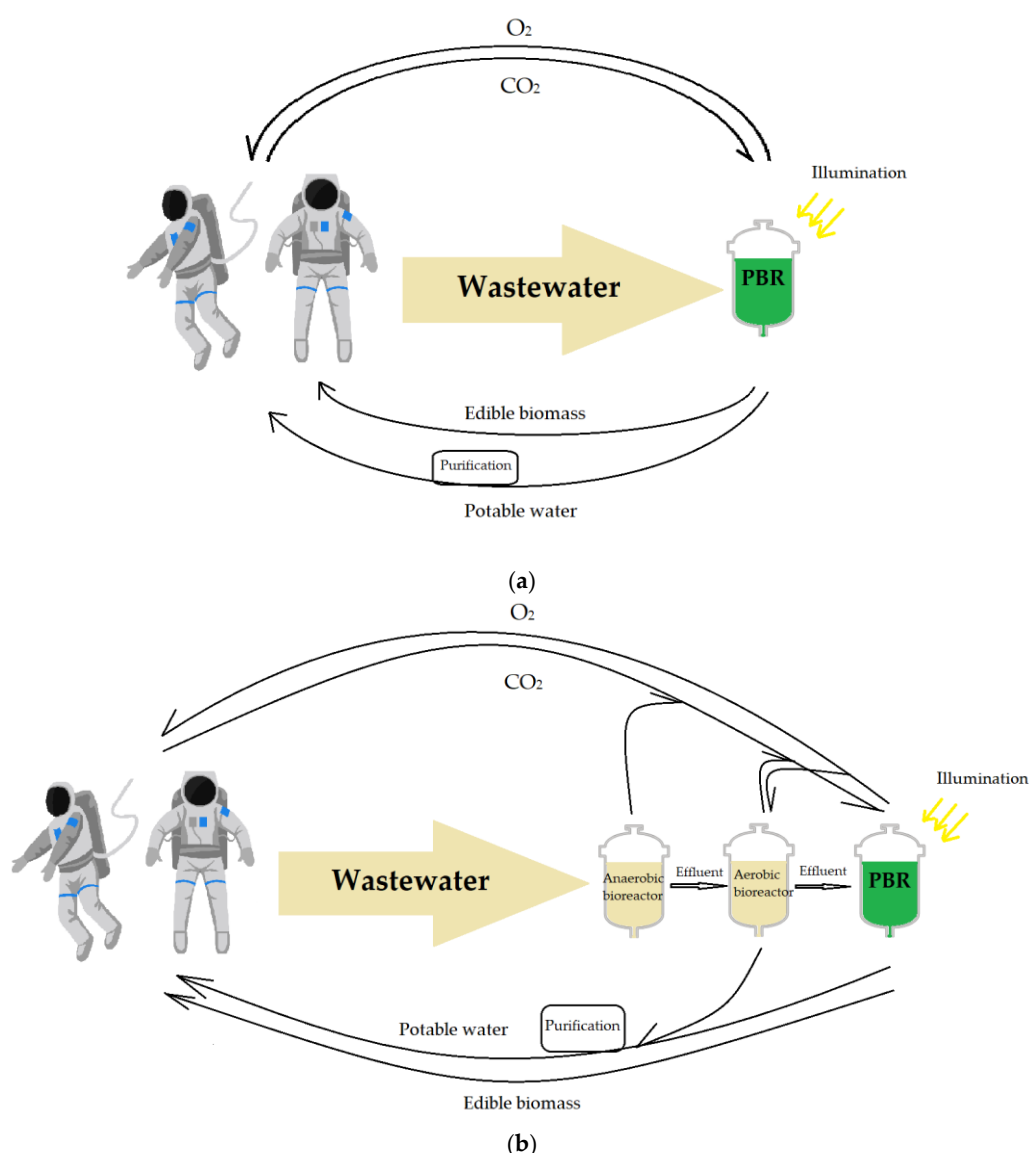


Figure 1. General schemes of (a) direct wastewater treatment (WWT) with the help of microalgal photobioreactor (PBR) in a space station or space mission; (b) indirect WWT with the help of microalgal PBR after bacterial treatment in a space mission, adapted from [3,11,12,102,103].

Table 1 summarizes the projects and programs outlined in this work. As can be seen from the Table, parameters such as mass, power, volume, crew time, robustness, etc., are

interrelated, and each of them has limits of applicability. Therefore, the optimization procedure, or the decision criterion, is actually the objective function. Depending on the formulation of the objective function, the researcher can search for a minimum or maximum of a function of many variables. Therefore, the decision-making procedure is actually a conditional optimization procedure.

Table 1. Summary of each reviewed project or program (system). The projects are arranged by type of gravity condition.

Reviewed Project	Organism	Wastewater (WW) Type	Pre-treatment	PBR Design	Scale	Mission Relevance	Main Limitation
Analogue Ground Human Test Facilities Attempting to Achieve a Closed Mini-Ecosystem							
Biosphere 2	Microalgal consortia	Human urine	No	Not a PBR but microalgae in a marshland	1.3 ha analogue human test facility	-	Not discussed
BIOS-3	<i>Chlorella vulgaris</i>	Urine	No	Not discussed	For three persons	Four to six months	Not discussed
Process estimates, conceptual designs, simulation models							
China Astronaut Research and Training Center	Not disclosed	Urine, flush water, brine, and feces	Yes	Not discussed	Material flow calculated for one person	Missions of 2–5 years, and a preliminary planetary base	Need supplemental water
Space Analog for Moon and Mars in Biosphere 2	<i>C. vulgaris</i>	General WW	No	Modular flat plate airlift	300 L volume	Planetary habitats	Unclear whether batch or continuous operation is more suitable
Virtual Habitat (V-HAB)	<i>C. vulgaris</i>	Urine	No	Flat panel PBR with membrane air exchange and	For a crew of five	Space stations and other space contexts	A few disadvantages in mass and major drawbacks in power consumption
The University of Colorado	<i>Chlorella sorokiniana</i>	Urine and grey water	No	Not discussed	PBR with 21 m ³ , for a crew of six	Planned for the ISS but likely suitable for other space contexts	Requires from 10 to even 100% more power (because of illumination), mass, and volume than the current technologies
The University of Stuttgart	<i>C. vulgaris</i>	General WW	No	Sunlight collection unit of mobile mirrors around a tubular PBR	100 L PBR	Planetary bases	Not discussed
Ground prototypes or experiments							
AES of NASA	<i>C. sorokiniana</i>	Simulated solid waste and its flush water	Yes	Flat-panel	Can process 2.5 L/d of effluent for a crew of four	Several months to several years	Power consumption failure, absence of software able to perform system-to-system communication
NASA and the University of South Florida	Not disclosed	Canine fecal waste	Yes	Not discussed	Produces 2.5 L/day of permeate	Early planetary habitats	Not discussed

MELiSSA Pilot Plant in Barcelona	<i>Limnospira indica</i>	Solid waste and urine of rats	Yes	Air-lift PBR of 80 L	For three rodents	-	Not discussed
AlgaTron	<i>Chlorella</i> or <i>Oscillatoria</i>	Domestic WW, in cases enriched with feces and urine	No	Transparent rotating drum with culture in the walls	Three or four m ² of AlgaTron surface area for one person	Not discussed, most likely for years	Not discussed
The Kennedy Space Center of NASA ¹	<i>Chlorella</i> sp.	Planned for raw undiluted crew WW	No	Pump-induced recirculation and tubular membrane-based gas exchange	Not disclosed	Not discussed, most likely for years	Not discussed
State Science Center of the Russian Federation	<i>C. vulgaris</i> most likely	Urine	No	Not discussed	PBR with 45 L of algal suspension for one person	Not discussed, most likely for years	Not discussed
Process estimates, simulations, or conceptual research, followed by ground experiments							
BIOSYS	<i>C. vulgaris</i>	Synthetic domestic WW, planned for the effluents of sewage, food waste, and grey water	Yes	Not discussed	Laboratory-scale	Lunar and Martian habitats	Addition of carbon, or nitrogen (N), and phosphorus (P) required
NASA's EPSCoR Supported Research	<i>C. vulgaris</i> and <i>L. indica</i>	Urine and flush water	No	Modular round-robin PBR with at least 10 units or attached-growth cultivation	Different PBR volumes according to the goal, e.g., for high C provision a volume of 173 L	For orbiting space stations, deep-space travel, or planetary bases	The estimate is simplistic and may not be equivalent to the real conditions; large volumes of PBR may be needed; N and P from external sources or from other waste streams needed; microgravity may cause problems with the automated pumping and with the nutrient uptake
S.A.B.E.R.	<i>C. vulgaris</i>	Urea for the ground test, grey water for the simulation	No	A simple container for the ground test, cascade membrane reactor units for the simulation	A three-liter container for the ground test	Space missions and planetary bases	The energy consumption of S.A.B.E.R.+AI mode was slightly higher
Spaceflight or spaceflight launch of parts or modules of the system							
Eu:CROPIS	<i>Euglena gracilis</i>	Synthetic urine	No	Trickle filter	Small-scale (vegetable garden); parallel	Lunar and Martian habitats	Due to a computer failure the irrigation could not be initiated

				ground proto- type			
MELiSSA ²	<i>L. indica</i>	Planned for solid waste and urine	Yes	Not determined yet	Most likely variable	For years	Not discussed

¹Laboratory-scale early experiment. ² Mostly ground experiment, a very limited number of modules in spaceflight.

As can be comprehended from the Table, the projects with the configuration of direct WWT using microalgal–bacterial consortia or polyalgal microalgae cultures are the fewest. Likely for that reason, they have been largely developed and planned for long-term missions or planetary bases. The other two modes—bacterial pretreatment followed by PBR and direct axenic microalgal WWT—can be attributed to both short-term and long-term missions, as well as to planetary bases.

15. Other Research Related to Microalgal WWT in Space

Demey and Vandermies [133] from the Redwire Space Europe facility in Belgium propose the urine from the cabin crew to be partially treated abiotically and purified from salts and some organic matter into a urea-rich solution and to be used as a nutrient for *L. indica* [79].

Verbeelen et al. [32] summarize that different strategies in research are applied to utilize urine so that plants and microalgae have a suitable form of N to assimilate, either by directly combining urine with a nutrient medium or indirectly through the production of an appropriate fertilizer.

Nostoc entophytum is a promising candidate comparable with other biosorbents for the removal of cadmium from polluted WWs. Under microgravity conditions, it was capable of absorbing cadmium at amounts up to 29% higher than the amounts taken up by the controls under Earth gravity [71].

The presence of hydrazine, a highly toxic and reactive rocket fuel, in closed-loop systems can endanger biological LSSes. *C. vulgaris* alone reduced hydrazine levels by 81% within 24 h and was hypothesized to act synergistically with extremophilic *Bacillus* spp. The results, in addition, support the feasibility of converting spacecraft fuel tanks into PBRs. Titanium, a biocompatible material common in aerospace hardware, did not inhibit *C. vulgaris* or hydrazine detoxification. Hydrazine poses a substantial environmental risk on Earth, an example of harmful space industry footprints on the planet. Therefore, industrial WWT would also benefit from the microalgal degradation of the compound [134].

An innovative approach and another possible extraterrestrial function of microalgae is electricity production through the algal fuel cell (AFC). It converts light energy into electrical energy. At an experimental scale, the AFC could utilize WW and transform it to electricity, so it is hypothesized to be operational in a similar manner in space, and in addition, release O₂ to the astronauts. The current low efficiency of an AFC can be overcome through optimizations, e.g., refining the electrodes [135,136].

The ability of cyanobacteria such as *L. indica* [49] and green microalgae, such as *Chlorella* and *Dunaliella*, to remove heavy metals and some organic compounds in WW is especially useful for the hypothetical extraplanetary colonies [69]. The heavy-metal removal would also be useful for plant cultivation on regolith, the outer surface rock and dust layer of a planet [49]. Needless to say, during such habitation, an LSS that can utilize local resources (e.g., CO₂ on Mars and water, if found), a.k.a. in Situ Resource Utilization (ISRU), would demonstrate a dramatic improvement [14].

Regolith simulant is a material developed to mimic the structural and chemical behavior of the original material. It is a commercially offered mineral mix consisting mainly of silicon dioxide (SiO_2) and aluminum oxide (Al_2O_3) with a grain size <1 mm [137,138].

Different microalgal species have been reported to be able to utilize Martian regolith simulant [1]. The addition of leachate of Martian regolith simulant and diluted synthetic human urine to the culture medium of *C. vulgaris* did not significantly affect final biomass concentrations. Carbohydrates, proteins, and carotenoids increased at the expense of lipids, thus suggesting that Martian regolith and urine can be transformed into edible biomass [137].

A very innovative concept of “3D green bioprinting” or incorporating and embedding live microalgae cells into 3D printed hydrogel and using it to treat WW in space is introduced by Windisch et al. [138]. This would be one of the long-term goals after O_2 production. They propose adding Lunar or Martian regolith to the alginate and methylcellulose hydrogel as a nutrition source for the algae for ISRU. *C. vulgaris* was 3D printed in such a “bioink” hydrogel, together with regolith simulant. The algae displayed high viability and growth, and kept their photosynthetic efficiency [138].

Lemos finds marine microalgae especially suitable for WWT in space because of their varied metabolic characteristics, and since *C. vulgaris* and *Tetradismus obliquus* are efficient in WWT, their marine strains or strains adapted to saline medium, respectively, hold promise in LSS participation. The author speculates that their multifunctionality would be implemented in planet or satellite colonization, where their WWT would be interconnected with regolith to soil conversion, which, together with atmosphere formation, would make the base of terraforming the planet [82] into a life-sustaining ecosystem [13].

16. Other Functions of Microalgae in Space Settings

As already discussed, microalgae could play a multifunctional role in an LSS—apart from treating gas and liquid waste streams and producing food—in an intricate integral biorefinery concept.

Although they are unable to completely disinfect the water, they could decrease the pathogenic bacterial load in it before pasteurization and/or other steps of turning it into potable water [86]. This is through their antimicrobial metabolites [139] and the rise in pH, associated with the photosynthetic process [28,140]. The microalgae themselves, or the PBR effluent stream, may be suitable fertilizer or compost for microgreen higher plants in space [79,82,106]. For instance, *L. indica* has had good results with Daikon radish microgreens using simulated Lunar and Martian regolith [49]. The microalgal biomass could also be used for rearing the alternative protein sources—fish [82] and zooplankton [117] in aquaculture, and even insects, emerging as high-value space-grown harvest [68].

The biomass could also be utilized for biodiesel and other biofuels, biogas [24,82], bioethanol [50], or other biomaterials [9], provided the respective technology could be applied in extraterrestrial missions. Microalgae were found to optimize the aerosol balance in terms of negatively charged ions in the cabin air in a ground experiment [40]. More unique proposals include thermal control (a large over 300 L PBR to be used as a thermal sink) and radiation shielding by PBRs [86].

Many projects plan the production of biofuel from microalgal compounds released through pretreatment, and the BIOSYS project envisions the extraction of the lipids into nutraceuticals and lubricating oils [3]. However, more sophisticated processing of the biomass and extraction of high-value compounds are more relevant in the hypothetical Lunar or Martian colonies with industrial technologies, in contrast to the technological constraints on a spacecraft [69]. On these habitations, microalgae and especially *L. indica* could also be a fertilizer for plant cultivation on the nutrient-poor regolith, neutralize its perchlorates, and mitigate its alkalinity and poor water-carrying capacity [49].

Microalgae could even be used as feed for bioreactor bacteria, as Verseux et al. [141] proposed. They managed to grow the cyanobacteria *Anabaena* sp. in chambers with low-pressure and high CO₂ (4%) and high N (96%) content. The biomass was successfully utilized as a nutrient source by *Escherichia coli*. In a colony on Mars, engineered *E. coli* would, as on Earth, be used to produce recombinant proteins (e.g., insulin) and chemical compounds.

17. Most Suitable Microalgal Species

Elite algae strains need to be selected and optimized [13] in order to tolerate waste streams, albeit diluted, and to endure harsh and extreme extraterrestrial conditions, e.g., radiation [79]. Abdullah et al. find the microalgal species that are most studied and successful in terrestrial WWT to be also suitable choices for interstellar applications, i.e., Chlorellaceae, Scenedesmaceae, and *Arthrospira* species [13].

Likely because of their cultivation flexibility and resilience to space conditions [72], the genus *Chlorella* is the dominant choice for model organisms of the space programs and agencies. Unspecified *Chlorella* sp. were chosen by NASA's Kennedy Space Center [106] and the Alcatraz of the University of California (although the cyanobacteria *Oscillatoria* naturally predominated under certain conditions in the latter) [44]. *C. vulgaris* takes the first place in the number of space research projects: BIOS-3 [25], BIOSYS [3], V-HAB [46], S.A.B.E.R. [24], the project of the University of Stuttgart [112,132], and NASA's EPSCoR-supported research in the University of Louisiana [18] (which also made in silico models with *L. indica* [14]). *C. sorokiniana* is the subject of the projects of NASA's AES [11] and the University of Colorado [86]. *E. gracilis* only participated in the Eu:CROPIS mission [21,22].

Cyanobacteria are hypothesized to have evolved during a period on Earth with very harsh conditions; thus, their vigorous biochemical processes enhance survival, they can grow in light-limited environments, and they can fix N [142]. Cyanobacteria also have higher photosynthesis efficiency and higher O₂ production rates than eukaryotic microalgae [92]. Apart from *Oscillatoria* in the Alcatraz [44] and the EPSCoR simulations [14], ESA's MELISSA has firmly stood with the cyanobacteria *L. indica*. ESA introduced and has preferred spirulina in LSS studies for several reasons: light conversion, alkaline tolerance, high nutritional value, adaptability to different conditions, including radiation [8,28], and better digestibility than *Chlorella* [23]. Its robustness, safety, and suitability for other mentioned functions, such as fertilizing, have given *L. indica* the reputation of an exceptional candidate for space [49,75]. Importantly, there are pre-clinical results showing radioprotective effect of its biomass [143] likely due to its effective DNA repair mechanisms and high antioxidant effect [97]. Although NASA does not actively research PBRs with *L. indica* for WWT or even for O₂ production in space, the agency provides spirulina to space travel crews as a nutrient supplement [133].

18. System Automation and Control

In space conditions, online monitoring of any test BLSS subsystems, albeit important, has been either too expensive or often incompatible with the space vehicle hardware so far [29]. Therefore, it needs special attention in future development.

Remote sensing techniques [73] and automated control systems [4] are also important and critical for long-duration missions [112] because minimizing crew intervention would make the BLSS autonomous [18]. In addition, control software capable of system-to-system communication would prevent failure of the whole system if issues with one component or its communication with the others arise [11]. A deterministic control system is obligatory in order to allow predictability and precise, consistent, and timely automation

[112]. The cultures in the PBR are recommended to react dynamically to changing environmental conditions [46], thus adaptive control strategies and machine-learning control, characterized by self-learning, would contribute to the robustness of the system [8,112].

19. Limitations

The extreme environment of outer space provides limitations for all biological and technological systems, and most of them have already been discussed.

High energy and power consumption are the main limitations for a successful BLSS and microalgal WWT within [4,6]. WWT facilities on Earth already have high power requirements and are also expensive, occupying large territories, and on occasion unreliable [10]. The smaller volumes of sewage in space would reduce these power requirements [39], but illumination and other microalgal necessities still require a lot of energy.

Other resource extraterrestrial constraints include launch mass, areas, and spacecraft cabin volume [6]. The interconnection of a PBR that can perform WWT with the different subsystems of an LSS in a cabin is explored in detail only in two studies [14,46]. More research into a unit- and system-level understanding [112] is needed to achieve integration of the PBR [17].

The effect of microgravity and radiation on PBRs and the effect of microgravity on simple microalgal cultures and PBRs are not sufficiently studied [6,29]. This is also valid for their combinatorial effect on cultures on agar or in flasks [28]. Therefore, gene mutation in the cells is possible, and its influence on the culture bioprocesses needs more studies [5]. More microgravity simulation experiments are necessary, as they are very limited at the present moment. They could be in clinostats, rotating wall vessels, completely novel types of simulations, or on parabolic flights—where the aircraft flies in a steep U-shaped, or parabolic, path, and each free-fall maneuver provides approximately 25 s of weightlessness [78].

The mentioned limitations in fluid exchange under microgravity cause challenges because their effect on the fluid and metabolite transport in living cells is not fully studied [18]. Although countermeasures to reduce the stagnant boundary fluid layer around cells that is caused by microgravity, to avoid overly long cultivation, are needed [113], the technology developed for that purpose or for general work with live cells or organisms in microgravity is limited [6].

As discussed in Section 7, the algal growth inhibition in raw WW due to nutrient overload requires dilution or bacterial pretreatment [3]. The algal biomass produced from the waste per crew member is not enough for one person, and neither is its nutrient content optimal, making land plants needed as sources of food [18].

Other specific limitations not mentioned so far include a higher CO₂ content than on Earth in the limited closed volume of the cabin air. The maximum acceptable level on the ISS is 2% CO₂, presenting challenges to increase the algal CO₂ scrubbing capacity by overcoming mass transfer limitations [18]. There is an insufficiently explored possibility of a phenotypic drift of a microalgal species or community over multiple generations in space, and thus a drift in process efficiency or end products [6,29]. In hybrid systems, biological processes and organisms may be sensitive to abiotic subsystems and their chemicals, and vice versa, biological products might foul or degrade the abiotic subsystems. The countermeasures are intermediate processing steps, buffer systems, and careful material selection, etc. [8].

No commentary was observed in the algal WWT sources about the high Ca²⁺ content of urine in space. Ca²⁺ concentrations above approximately 100–200 mg/L lead to precipitation of PO₄³⁻ and carbonate in a liquid [144]. Therefore, it can be speculated that, with an average content of about 225 mg/L in urine in space, Ca²⁺ would be another inhibitory nutrient, thus requiring dilution.

The cabin and the whole environment of a space station are small, closed, and multi-purpose. In addition, the microgravity does not allow settling of particles, droplets, etc., but they remain airborne and spread everywhere, thus enabling the mutual contact of the microbiotas of the human crew, the plants, insects, and other hypothetical members of the LSS [19].

For these reasons, and considering the different microorganisms in the different bioreactors, there is the hazard of unwanted cross-contamination from biological and chemical sources and of unwanted communication by quorum sensing between the unicellular organisms [17,19,28]. Axenic microalgal cultures might also exhibit insufficiently studied cell–cell communication and biofilm formation. All this could disrupt the operation of the LSS and alter its operation [17,145]. On the other hand, the highly controlled environment of a space station makes sterilizing procedures easier [19]. Sterility or axenicity of the LSS compartments is planned to be ensured with microfiltration membranes [133].

Last but not least, to the best of our knowledge, only one source, authored by Aguda et al. [99], addresses the safety concerns of consuming microalgae cultivated on human-derived WWs. For the terrestrial context, there are scientific opinions supporting either of the two concepts: that the microalgal biomass or its products cannot be in the human food chain, or that they could, given they meet the safety regulations [146]. While the paper states that even the astronauts' exhaled CO₂ could be chemically or biologically contaminated, other researchers do not address that topic, probably because of the fact that even small, closed, and within an extreme outer environment, the inner environment of a space station is, as mentioned, highly controlled, with controlled import and supply of substances, including possible toxic ones and pathogens.

20. Future Outlook

The important features of an LSS are robustness and resilience to sustain long-duration missions, as well as reliability, performance, and sustainability to address all unidentified possible problems [27]. Bioregenerative LSS is still not yet achieved despite the decades of ground tests utilizing state-of-the art technology, not because it is unfeasible, but because the task is too complex, and experiments in space flight conditions are very challenging [1,13]. Long-term extraterrestrial data related to Bio-LSS and bioprocesses and their control are still limited [1,29]. Even with unmanned missions, online monitoring and data transfer have had challenges, [29]. Tests with bioreactors connected to life support compartments are still rare, and only on the ground [17].

Microalgae have been examined from the beginning of BLSS development, unlike other bioprocesses related to space, e.g., bacterial nitrification for urine treatment or regolith weathering for metal mining [29,32]. However, in a similar manner, due to the engineering constraints, only two microalgal PBRs have been sent to space for early process validation, which did not even perform air revitalization [20,96].

Even with those early-phase research and tasks, technical issues still arose, pointing to the challenges in such remote and extreme environments [20,96].

Nevertheless, space agencies from various countries are supporting theoretical work and targeted experimental research. The results obtained, regardless of the scale, are promising, motivate further research, and show that BLSSes are important and feasible despite the emerging challenges [1,7].

Space agencies and academia have been searching for configurations or scenarios for microalgal inclusion in space WWT and have developed three main configurations since the beginning of the new millennium. Axenic microalgal cultures have been developed from approximately 2016 to 2025, and these projects include NASA among their most influential creators [14,18,24,40,46,48,86,99,112,131,132]. Direct WWT using algal–bacterial consortia is developed from approximately 2018 to 2025 [21,22,106,112,128]. Consortia

may be the most used and effective configuration on Earth [15], but some of the major space agencies, such as NASA and ESA, are mainly focused on the method of bacterial pretreatment, developed approximately from the years 2022 to 2026 [3,11,12,102,103].

Considering these facts, the current data do not allow a determination of the most effective method and plausible scenario to be applied in space exploration, and this question is warranted to be elucidated in real space conditions. Each space parameter changes within a certain range, which means that finding optimal solutions is associated with the use of regression and statistical analysis, instead of a trial-and-error procedure. Possible scenarios include the participation of various factors, processes, interaction conditions, etc. These processes can also be influenced by unpredictable circumstances and random events in space, such as solar flares, radiation flows, etc.

In regard to the challenges in such complex research, they are related both to the metabolic processes of the used consortia of bacteria and microalgae, and in particular to the unexplored pure strains affected by space conditions. Due to the fact that space research is constantly changing in its scale, crew, etc., the challenges in experimental studies may continue to arise in the future.

Depending on the positive results of the research and the potential of the biological systems used, the perspectives are expanding and may, depending on the scale, include characterized and safe pure cultures of natural origin or genetically modified microalgae. A possible perspective is the integration of different groups of microorganisms, including plants.

Optimized microalgal PBRs have immense potential in LSS integration [13]. However, in regard to microalgal WWT, many challenges remain. The combination of gravity and radiation effects on microalgal cultures is not yet fully elucidated [28], while for bioreactors and PBRs, there are limited studies even for the separate factors [6,29]. The integration of a PBR in a vehicle cabin needs more studies of its interconnection with the different systems [17]. Other particular possible problems to be considered include, as mentioned, phenotypic drift [6,29] and unwanted bacterial contamination [17].

The creation and successful novel implementation of microalgal WWT within an extraterrestrial LSS would require interdisciplinary research—algal biology, algal biotechnology, bio and space systems engineering, computing, and environmental science [8,24].

The theoretical extrapolations for the development of BLSS are very difficult, thus more ground experiments are warranted [7]. Since the microgravity, radiation, and other specific space factor combinations cannot be fully and simultaneously replicated in simulations on Earth [7,19], long-term research for microalgal WWT in real space is even more crucial [1,28].

Figure 2 presents the processes of obtaining data and evidence under the influence of limiting factors to determine the optimal research priorities for the microalgal role in space WWT.

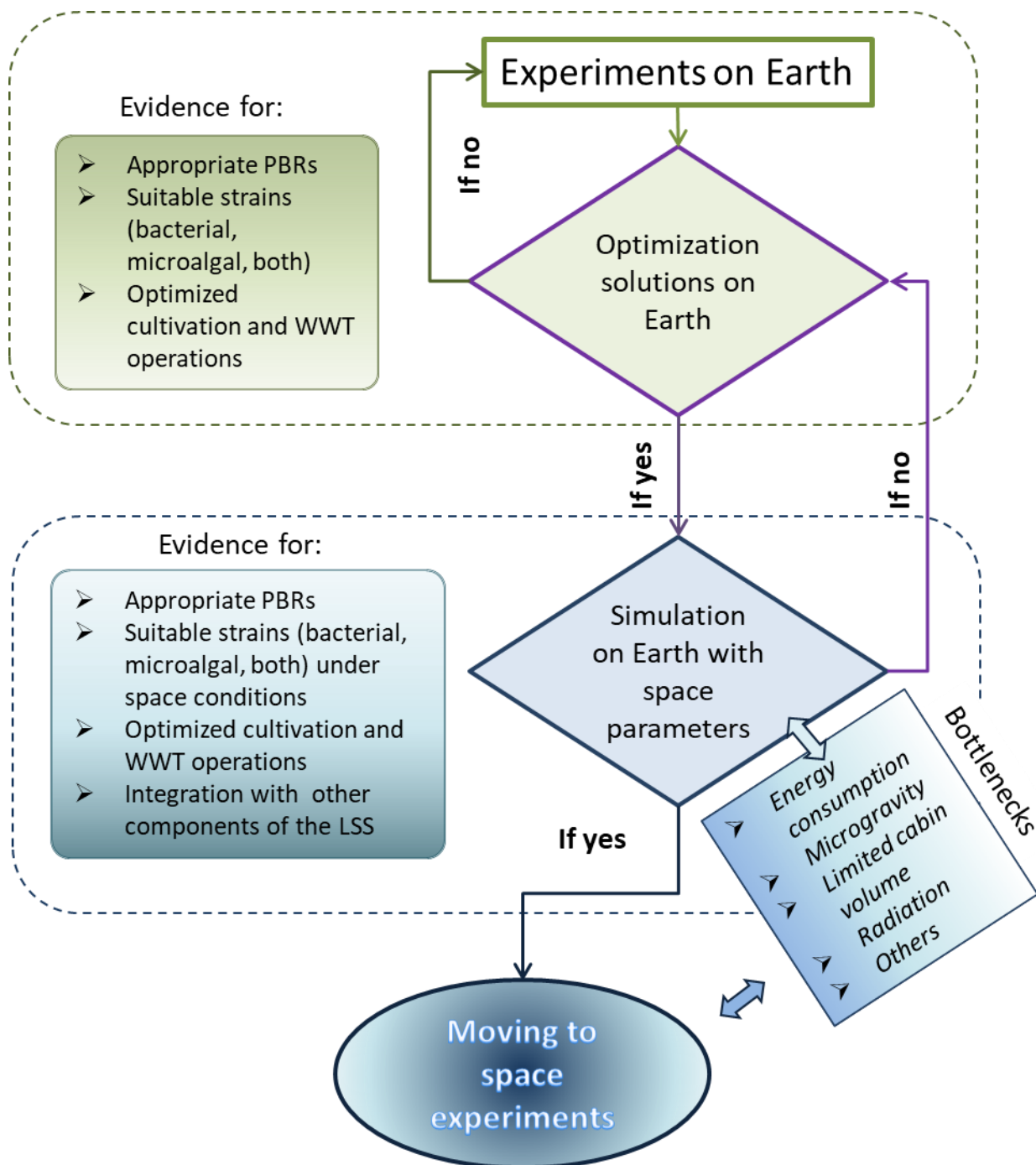


Figure 2. A roadmap figure connecting the processes of research into microalgal system involvement in space WWT.

21. Conclusions

Although a literature search and comparison show that a more realistic short-term role of microalgae in space is the provision of O₂ and food biomass, in the long-term, the coupling of these processes to WWT in an integral LSS would add immense benefit to the system and the degree of its closure.

When resupply from Earth would not be feasible, innovative and advanced technologies are required for an LSS that can perform WWT in deep-space missions. Microalgae and other photosynthetic organisms are the base of future next-generation hybrid LSSes, which would ideally include abiotic components, as well as bacterial reactors.

The technical challenges in space have not allowed realizing extraterrestrial involvement of microalgae in WWT despite decades of terrestrial research. Nevertheless, the ongoing research of NASA, ESA, other space agencies, and academia shows that innovative PBRs have great potential for WW utilization.

We have to continue on a difficult, challenging, and long path of engineering optimization, Earth-based simulations, and actual space experiments in order to succeed in building a closed-loop LSS with a bioregenerative architecture and WW recycling with microalgal system involvement.

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Abbreviations

The following abbreviations are used in this manuscript:

BLSS	Bioregenerative life support system
PBR	Photobioreactor
WWT	Wastewater treatment
N	Nitrogen
P	Phosphorus
CO ₂	Carbon dioxide
O ₂	Oxygen
WW	Wastewater
LSS	Life support system
Eu:CROPIS	Euglena and Combined Regenerative Organic-Food Production in Space
PMBR	PhotoMembrane BioReactor
AES	Advanced Exploration System
NASA	National Aeronautics and Space Administration
BIOSYS	Space-Based Biochemical Conversion System
EPSCoR	Established Program to Stimulate Competitive Research
MELiSSA	Micro Ecological Life Support System Alternative project
LSR	Life Support Rack
ESA	European Space Agency
S.A.B.E.R.	Sustainable AI-automated Bioreactor for Environment Remediation
NO ₃ [−]	Nitrate
PO ₄ ^{3−}	Phosphate
ISS	International Space Station
BIOS	Hybrid Biosphere System
CELSS	Controlled ecological life support system

ALSSTB	Advanced Life Support System Test Bed
CEEF	Closed Ecological Experiment Facility
NH ₄ ⁺	Ammonium
C	Carbon
COD	Chemical oxygen demand
DLR	German Aerospace Center
AFC	Algal fuel cell
ISRU	In Situ Resource Utilization
CEBAS	Closed Equilibrated Biological Aquatic System
MABR	Membrane-aerated biofilm reactor
RPM	Random positioning machine
OPA	Organic Processor Assembly ()
SAMBR	Suspended Aerobic Membrane BioReactor
AnMBR	Novel Anaerobic Membrane Bioreactor
HLL	Treatment with high C, low N, and low P
MEC	Microbial electrolysis cell
SAM	Space Analog for Moon and Mars
DMAIC	Define, Measure, Analyze, Improve, and Control
DMADV	Define, Measure, Analyze, Design, and Verify
V-HAB	Virtual Habitat
UPA	Urine Processing Assembly
OGA	Oxygen Generation Assembly
WPS	Water Processing System
AI	Artificial intelligence

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