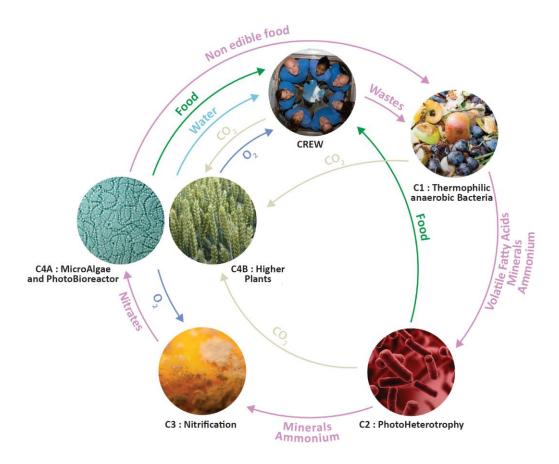
Microbially-based life support systems for interplanetary travel and Space exploration

Mummy, Daddy, do astronauts flush their toilets into Space? And where do they get their water and food?



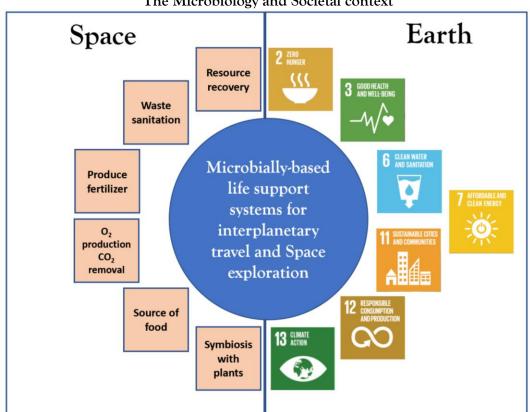
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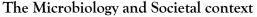
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Microbially-based life support systems

Storyline

Humans are curious beings. For more than 300,000 years we have explored all parts of our planet. Deep space travel and exploration is a long-term dream of humanity that is not far from being technically possible. The first touristic space-flights have already taken dozens of tourists into orbit, and there are plans for future trips to the Moon. While these short trips can be sustained with stocked supplies, this will not be possible in missions far away or in settlements in other planets, including Mars. Humans need food, water and oxygen to survive, and spaceships are too small to transport all these resources on a long trip. And the cost of flying them is extremely high. Space exploration will require the internal recycling of the crew's waste into resources such as oxygen, water or food. And here is where microorganisms can help us in our Space adventures. Just like what is happening on Earth, microorganisms can use our residues in space (e.g. food waste, toilet paper, urine, etc.), degrading pollutants and even transforming them into new resources such as energy, fertilizer, etc. Waste management also ensures that pathogens are killed or cannot develop. Microorganisms can also be grown as sources of food in a more efficient and compact way than agriculture or farming, potentially allowing to feed astronauts "fresh" meals. Last, any long-term space mission will likely rely on plant-based diets. Many plants have beneficial interactions with microorganisms, which can for example help them to fix nitrogen. Among the many microbially-based life support system concepts, the European circular life support system MELiSSA (Micro Ecological Life Support System Alternative) is one of the most advanced.





Microbially-based technologies and processes may be key for future Space exploration, but the knowledge and technological advances that arise from these processes can also provide tangible benefits on Earth, allowing us to address some of the current Sustainable Development Goals (SDGs). Technologies for waste management and recovery of resources developed for closed-loop environments

can significantly contribute to the good health and well-being of developing communities (SDG 3), the availability of clean water and sanitation (SDG 6), the production of affordable and clean energy (e.g. biogas, SDG 7), enabling sustainable cities and communities (SDG 11) and allowing to close resource loops (SDG 12). Additionally, microorganism-based food such as microalgae (as illustrated in this <u>video</u>) can help meet the goal of zero hunger (SDG 2).

Microbially-based life support systems

1. Microorganisms can degrade many types of residues, and can also help convert them to valuable resources. Microorganisms need energy and nutrients such as carbon, nitrogen and phosphorus to live and reproduce and have numerous strategies to do so. Energy is sourced from light (photosynthesis) or organic compounds (and in some cases inorganic materials). The carbon needed to synthesize new microbial cells can for instance be obtained from CO_2 in the atmosphere or from organic compounds such as sugars, proteins or fats, or mixtures thereof. Depending on the environmental conditions, the availability of nutrients, specific stress factors, etc., different groups of microorganisms will thrive and different types of reactions will take place. In the presence of oxygen (aerobic conditions), organic compounds will be oxidized to CO_2 with the release of a lot of energy for growth. In the absence of oxygen (anaerobic conditions), a complex group of microorganisms converts organic compounds to biogas and the energy is mostly channeled into the end product methane. On earth, these activities are for instance exploited in sewage treatment. In the activated sludge process, intensively supplied with air (oxygen), organic compounds in the wastewater are degraded by bacteria to CO_2 . In the anaerobic digestion process, completely different microbial consortia convert mixtures of organic residues in 4 sequential steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) to the final product methane CH₄. This is then burnt for combined heat and power generation.

Also in Space, organic-rich waste streams will be produced, such as food waste and fecal matter, which require treatment. As oxygen is needed to keep the astronauts alive, preference is given to treating the residues in absence of oxygen. Since methane presents potential explosion and safety risks, the stepwise degradation of the organic residues is halted at the second step. As a result, organic acids are produced which can be oxidized to CO_2 through different microbial routes. Interestingly, nitrogen is also released from the waste streams, which can be converted into plant fertilizer (as discussed in Point 3). Rather than simply treating organic wastes, the ultimate goal is to maximally recover and recycle valuable constituents from it. Hence, CO_2 and nitrogen are used to grow micro-algae (or crops) in photosynthetic processes.

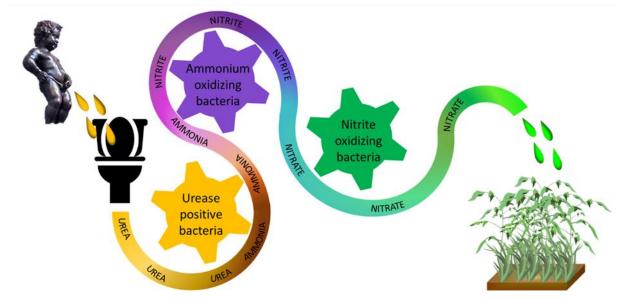
2. Waste management and treatment goes beyond recovering resources, and is important to avoid the development of pathogens. Waste materials, especially those rich in organic materials, can become a breeding ground for pathogens. These pathogens can either come from the astronauts (such as their faeces) or already be present in the environment.

Sanitation on Earth has the objective to kill pathogens to preserve the health of humans, animals, plants, etc. This is accomplished for instance by subjecting the material to high temperatures (> 60 °C) as most pathogens are mesophiles adapted to grow in the temperature range that includes the temperature of the human body, 37°C. For example, composting – the aerobic decomposition of organic materials by microorganisms (bacteria and fungi) – is used to stabilize and sanitize waste, reducing also its mass and volume for easier handling. These aerobic reactions are very exothermic – they release heat – raising the temperature of the materials to 60-70 °C for several days.

In Space, organic waste may also become a potential biohazard, and sanitizing it will be crucial. Microbial stabilization and sanitation is one of the most cost-effective technologies with limited need for additional energy of resources, and hence very suitable for application in life support systems in Space. The same approaches (i.e. aerobic degradation) used on Earth may not be applicable in Space, since oxygen is rather limited. Anaerobic technologies, as those presented in the previous point, can also perform a similar function, although the metabolic processes are slower and heat release is lower, so additional heat may be needed to reach the high temperatures needed for sanitation.

3. From pee to plant fertilizer, and how microorganisms are key to nitrogen (re)cycling in Space. Nitrogen is one of the most important life building blocks, and is present in all organisms, for example in amino acids (and thus proteins) and deoxyribonucleic acid (DNA). Protein (and hence nitrogen) is an essential component of the human diet (15-17 g N/d/person), and nitrogen recycling will be key in long-term human Space missions. For instance, in MELiSSA, vegetable crops (e.g. lettuce, beet) and edible microbes (i.e. Spirulina, now known as Limnospira sp.) will be the crew's source of proteinaceous food, and hence grown from recovered nitrogen.

To produce this biomass, nitrate is needed as fertilizer. In Space, about 85% of the crews' nitrogen intake will end up in pee (urine). When talking about pee one often thinks about smelly waste, but the truth is it is rich in nitrogen that can be easily recovered using microorganisms.



Microbial conversion of nitrogen to fertilizer

Nitrification by microorganisms is the most efficient way to convert pee to plant fertilizer (i.e., nitrate). In this process, urea (a waste product of nitrogen metabolism in our body) is first broken down to ammonia by urease positive bacteria. The resulting ammonia can then be converted to nitrite by ammonia oxidizing microorganisms. Afterwards, nitrite oxidizing bacteria can take this nitrite and convert it to nitrate. These three microbial groups work side by side benefiting from their interactions. To achieve full nitrogen recycling in Space, we can then use the nitrate produced by microorganisms to grow vegetables that can be again consumed by the crew (similarly to how nutrients were traditionally supplied in agriculture).

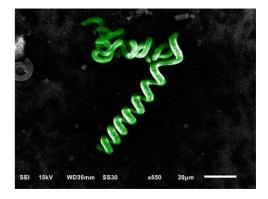
4. *Microorganisms are the food of the future, and may be key to enable fulfilment of our dreams of Space exploration.* The production of microorganisms (yeasts, bacteria or fungi) for feed or food rich in good quality proteins and other nutrients has been widely explored for Earth and also Space applications. Microorganisms already play an important part in many agrifood industries (bread, bear, wine, cheese etc.). Yeasts are certainly the more important and well-known group of microorganisms used by humans and, because of their high content of proteins, vitamins (mainly vitamins of the B group) and minerals, are also used as nutritional additives for food or feed.

Processes to create edible microbial biomass have been developed for waste treatment, animal feed production, food production and microbial ingredients production. In the case of waste treatment, the primary purpose is to eliminate waste but an important secondary goal is to create added value. In other cases, the substrate for cultivation is adapted to the required characteristics of the expected product, for instance fungal protein production or culture of yeast on various sugar syrups. In life-support systems in Space, the aim is to have processes that ultimately recycle waste into food.

When considering microorganisms as a source of nutrition, we can distinguish microorganisms which are produced as food themselves, as well as microorganisms that are grown for specific metabolites that have to be extracted and purified from the microbial biomass (for example essential amino-acids like lysine). Although the production of vitamins or other amino acids or even antibiotics may be very interesting for astronauts, we will further focus here on the potential of whole microorganisms as food that could be directly consumed by astronauts.

The cyanobacterium *Limnospira* used for MELiSSA is a well-known food supplement already consumed by Aztecs hundreds of years ago. A teaspoon of it is full of proteins and antioxidants, while it also stimulates the immune system. Taking advantage of its "microbial features" meaning fast growing with minimal footprint, resistant to ionizing radiation, combined to its "plant features" meaning food and oxygen production, this makes it the ideal candidate for space application.

Left: Scanning electron microscope picture of *Limnospira indica* (artificial color). Right: fresh *L. indica* biomass harvested from a production pond, ready to be directly eaten or oven baked to produce flakes/powder/pills (source: SCK CEN).





5. *Microbes help plants to grow better.* If plants are used to provide nutrients in Space, they will need to be helped with microbes. On Earth, plant growth is usually limited by scant supplies of nitrogen and phosphorus in soil. In farming, these limitations are usually removed by adding fertilizers. However, non-farmed plants usually acquire them as a result of their microbial friends. At the interface of roots and soil there is a layer of a few millimeters – called the rhizosphere – containing a rich diversity of microorganisms. The plant:microbe interaction in the rhizosphere is beneficial for both partners: plants provide the microbes with food, like sugars, and microbes provide the plants with nutrients like nitrate and phosphate. But the rhizosphere also works as a shield against pathogens that potentially could damage the roots: some rhizosphere bacteria secrete chemical metabolites that suppress the growth of pathogens and/or render them avirulent, thereby protecting the host plant.

To the best of our knowledge, there is no soil or microorganisms in other planets. Plant cultivation in Space missions will be soil-less, so will be hydroponic, for example. Plants in these systems will also need similar beneficial interactions with microbes as the ones in conventional terrestrial soils.

Final remarks

Human/Crewed Space exploration is a dream that is only starting. When, What and How are questions that we ask ourselves, and the answers will come in the next decade(s). Regardless, it is clear that microorganisms will play their part (in one way or another) in helping us realize this dream.

The Evidence Base, Further Reading and Teaching Aids

Closed-loop systems that keep astronauts alive in space could inform circular economy strategies

https://phys.org/news/2018-09-closed-loop-astronauts-alive-space-circular.html

Microbes Could Recycle Astronauts' Waste to Make Nutrients and Tools

https://www.scientificamerican.com/article/microbes-could-recycle-astronauts-rsquo-waste-to-make-nutrients-and-tools/

Space farming

https://en.wikipedia.org/wiki/Space farming

Health, food, and climate | We explore. You benefit. https://www.youtube.com/watch?v=9C0rzGO4ENw

MELiSSA Space Research Program, the European Project of Circular Life Support System https://www.youtube.com/watch?v=TEmgWY_4cdY_

How do we feed a crew on a three-year mission to Mars ? https://www.youtube.com/watch?v=sPyupNWIjqk Hendrickx, L., De Wever, H., Hermans, V., Mastroleo, F., Morin, N., Janssen, P., Mergeay, M., Wilmotte, A. 2006. Microbial ecology of the closed artificial ecosystem MELiSSA (Micro-Ecological Life Support System Alternative): Reinventing and compartmentalizing the Earth's food and oxygen regeneration system for long haul space exploration missions. Research in Microbiology 157: 77-86. <u>https://doi.org/10.1016/j.resmic.2005.06.014</u>

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