
Review

Opening Closed Loop Ecological Life Support Systems with In-Situ Resources on the Moon

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Abstract: In this review, we explore a broad-based view of technologies for supporting human activities on the Moon. Primarily, we assess the state of life support systems technology beginning with physicochemical processes, waste processing, bioregenerative methods, food production systems and the robotics and advanced biological technologies that support the latter. We observe that the Moon possesses in-situ resources but that these resources are of limited value in CELSS – indeed, CELSS technology is most mature in recycling water and oxygen, the two resources that are abundant on the Moon. This places a premium on developing CELSS that recycles other elements that are rarified on the Moon including C and N in particular but also other elements such as P, S and K which might be challenging to extract from local resources. Although we focus on closed loop ecological life support systems, we also consider related technologies that involve the application of biological organisms to bioregenerative medical technologies and bioregenerative approaches to industrial activity on the Moon as potential future developments.

Keywords: Bioregenerative life support; closed ecological life support; in-situ resource utilization; lunar industrial ecology; 3D bioprinting; gene editing

1. Introduction

The human body comprises 12 definitive but integrated systems, a highly simplified engineer's schematic of which is shown in Fig 1: (i) integumentary system protects the body from external insults using multilayered skin embedded with sensors; (ii) skeletal system structurally supports the body through bone and cartilage; (iii) muscular system imparts motive power to the body using skeletal, cardiac and smooth muscles and tendons; (iv) nervous system transmits signals from the five special and other senses and controls actions of the body through the brain, spinal cord and nerve networks; (v) respiratory system of airway and lungs absorbs oxygen and expels carbon dioxide; (vi) cardiovascular system transports blood laden with oxygen and nutrients around the body through the arteries, veins and capillaries pumped by the heart; (vii) digestive system ingests and processes food through the alimentary and gastrointestinal tracts using the liver, pancreas and gall bladder; (viii) urinary system expels liquid waste through the urinary tract using the kidneys; (ix) endocrine system comprises glands that produce hormones transmitted via the bloodstream that regulate metabolic activity throughout the body; (x) immune system protects the body against infectious microorganisms; (xi) lymphatic system drives lymphatic fluid through lymph nodes, spleen, thymus and lymphatic vessels for a number of functions; (xii) reproductive system procreates offspring through specialised sex cells.

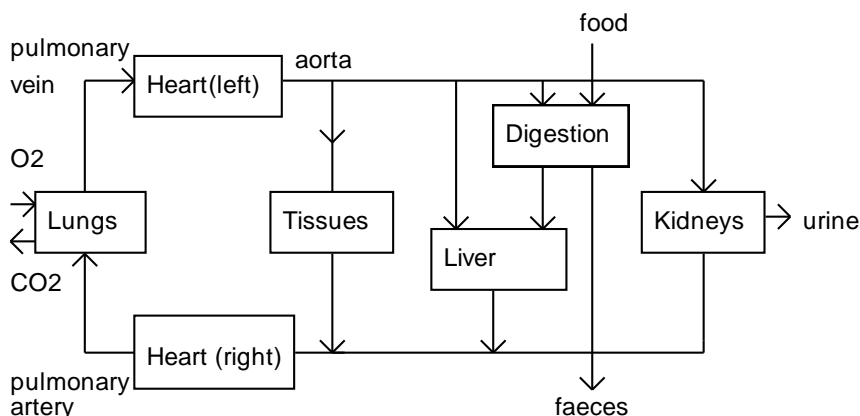


Figure 1. Simplified “engineer’s” sketch of human physiology with input/output interfaces

Space has important implications due to the effects of space on the human body [1]. Of critical importance is the radiation environment. The radiation unit adopted is the rem (Roentgen equivalent man) which normalises the ionization capability of the three different ionizing radiations α , β , and γ into a single number. Terrestrial background radiation exposes people to 0.25 rem/y and it is considered that exposure up to 10 rem is biologically harmless to humans. Exposure to 5 rem (legal limit equivalent to 25 chest X-rays) will yield one death in 10,000 of the exposed population. Although we are focussed on the Moon, a 2.5 y human Mars mission involves a total body dose of 200 rem dominated by the interplanetary transfer periods, particularly due to solar coronal mass ejections and galactic cosmic rays – this is a similar radiation environment and duration to a long-duration Moon habitat mission. If radiation exposure is limited to that that increases the incidence of cancer death by <3%, this favours older astronauts of age 55 or older (300 rem limit) rather than younger at age 25 (40 rem limit) on the assumption of a background adult cancer mortality of 20% [2]. Although the medical effects of partial gravity on planetary surfaces are not expected to be as severe as in microgravity, the effects of microgravity will nevertheless provide a worst-case indication of the effects of partial gravity which will be pervasive. Over the short term, space adaptation syndrome (space sickness) is common during the first 2-3 days until the neurovestibular system has adapted to weightlessness. Over the long term, reduced gravitational loading on the human body causes cardiovascular and musculoskeletal degradation including, most seriously, calcium loss from bones and red blood cell loss in blood circulation. Cardiovascular effects include cardiac deconditioning which reduces stamina and orthostatic intolerance to gravity after microgravity exposure which has implications for human functional performance on planetary surfaces following extended microgravity exposure. Some of these medical problems can be addressed partially through nutrition and/or vitamin supplements. There are also radiation-induced genomic effects, interference with developmental processes and psychological stresses. During planetary missions, it is expected that the risks to human life for 30-60 y olds during any mission is $<2 \times 10^{-3}/y$ by illness, $<4 \times 10^{-4}/y$ by accident and $<3 \times 10^{-2}/y$ by all causes [3]. The primary means for medical mitigation of any medical condition would be countermeasures such as fitness training against spaceflight-induced stresses including treadmills, bicycle machines, spring-based muscular resistance such as rowing machines, pressure suits and/or lower body negative pressure devices [4]. In microgravity, astronauts require a minimum of two hours/day exercise to counter its detrimental effects. It is likely that a similar exercise regimen will have to be maintained on lunar bases so gym facilities will be required. Finally, Biosphere 2 experiments yielded useful psychological lessons for stress reduction including the importance of avenues for creative expression and exposure to the beauty of nature, functions that can be provided through in-situ food production [5].

2. Role of Ecologies

On Earth, human life is supported by a complex and deep biosphere with material recycling including hydrological and biogeochemical processes through the lithosphere, hydrosphere, cryosphere, atmosphere and biosphere. The key features of natural ecosystems are bio-material turnover and energy flows [6]. It is closed to matter permitted by material recycling through biogeochemical (CNP) cycles but open to energy from the Sun. Buckminster Fuller characterised the Earth's biosphere as spaceship Earth in his *Operating Manual for Spaceship Earth* (1968). Artificial life support systems generally lack the large buffering capacity of the Earth's biosphere so they require much higher degrees of precision control. Biosphere 2 was a 12,700 m² glass biospheric enclosure sealed with silicone sealant in the Arizona desert housing a crew of 8 people for two years (1991-1993) with effectively 100% material closure [7]. Energy was input to Biosphere 2 as solar energy and electric generators supplying 700 kW (average) to 1500 kW (peak). The biosphere included 7 modules of 1900 m² tropical rainforest, 1300 m² savanna, 1400 m² desert, 450 m² tidal (freshwater and saltwater) marshes, 850 m² ocean, 2500 m² agricultural system and a 2400 m² human habitat. The habitat comprised a galley, living quarters, an analytic laboratory, computing facilities, machine shop and sickbay facilities. A system of cooling water towers, chilled water and a water boiler-controlled Biosphere 2's air temperature [8]. Biosphere 2 incorporated 6 x 10⁶ litres of water including fish/rice paddies and hosted 3800 different species including three domestic animals (pigmy goat, feral swine and chicken) which consumed inedible crop residue and worms in return for milk, eggs and tilapia meat. Waste was processed through composting and bacterial processing. Food production consumed the majority of the crew's time. The facility incorporated two large expansion chambers ("lungs") to accommodate temperature variations to ensure low gas leakage rates ~10%/year [9]. The most challenging issues were O₂/CO₂ level fluctuations which required periodic intervention and the calorie-restricted diet imposed on the crew. Obviously, the scale of Biosphere 2 renders it impractical for space application (except perhaps O'Neill colonies [10]). Nevertheless, experiments to date suggest that 100% closure is feasible for up to 6 months but the precise means to achieve this has yet to be demonstrated. Crucially, in space or planetary surfaces, we are transplanting the human from the environment in which we have evolved to an entirely alien one in which we have not. In this regard, it is crucial to consider evolutionary medicine as a factor in designing life support systems for long duration missions – diet (with implications for diabetes), microbiome (with implications for autoimmune disease), radiation exposure (with implications for cancer), infectious disease exposure (with implications for virulence), emotional isolation (with implications for mental disorders) [11].

Environmental control and life support systems (ECLSS) involves control of atmospheric pressure, temperature, humidity and composition with most other resources supplied. In the early days of spaceflight, life support systems stored oxygen, water and food for astronaut consumption and returned waste back to Earth. A more comprehensive life support system also requires: (i) air quality including the maintenance of buffering gases, CO₂ removal and O₂ generation; (ii) food production and storage; (iii) water management through waste-water recovery; and (iv) solid waste management through bacterial processing. There are several approaches to such life support: (i) open loop life support systems in which all consumables are supplied and stored as adopted in early space missions of short duration; (ii) physicochemical recycling life support systems that recycle bulk consumables; (iii) bioregenerative life support systems that exploit biological mechanisms; (iv) in-situ resource utilisation supply of consumed material; (v) hybrid life support systems that are combinations thereof. A human being requires 38.5 kg of consumables per day including 24.8 kg of water for showering, toilet flushing, cleaning and clothes washing per day including tankage. Water is the primary human consumption requirement – it is the main constituent of electrolytes of the human body (blood plasma, interstitial fluid and intracellular fluid) regulated by the kidneys through urine production [12]. Grey water is readily recycled so 13.7 kg of water consumables per day is more

realistic. Recycling of water and oxygen may be implemented through physicochemical processes, the two components that can be readily sourced and supplied from lunar resources. Water at $5.6 \pm 2.9\%$ concentration (plus associated vapours of H₂S, ethylene, CO₂ and methanol) was detected by the LCROSS (lunar crater observation and sensing satellite) mission (2009) in an ejecta plume generated by a Centaur rocket stage impacting into the Cabeus crater [13] (Table 1). However, there are considerably greater resources in lunar regolith minerals which may be extracted through a handful of processes (Table 2). Our closed loop lunar industrial ecology system (CLIES) consumes mineral resources only (including impacted asteroidal material). Scarce lunar volatiles require extraction but serve as recycled reagents so are not consumed within CLIES. One notable exception is the carbon resources – this was proposed to manufacture silicone (siloxane) products as elastomeric electrical insulation plastic for wiring harnesses and silicone oils for lubrication [14] but we have re-addressed this issue and concluded that silicone is unnecessary as glass cloth and porcelain may be substituted for this purpose [15] and tungsten disulphide (WS₂) is a high temperature lubricant used in place of MoS₂ which may substitute for silicone oils. Hence, all extracted volatiles are recycled with CLIES. The implication is that, although H₂O resources can be replenished from lunar resources, CNPS elements cannot be supplied from lunar volatile sources but must be recycled through CELSS. We proffer a view that extraction and consumption of water consumables for burning as propellant/oxidiser wantonly wastes finite and valuable resources which would otherwise support human survival on the Moon over future generations.

Table 1. LCROSS ejecta plume show the paucity of volatile species [16]

Volatile Species	% Relative to Water	% by Mass
H ₂ O	100	5.60
H ₂ S	16.75	0.94
NH ₃	6.03	0.34
SO ₂	3.19	0.18
C ₂ H ₄	3.12	0.17
CO ₂	2.17	0.12
CH ₃ OH	1.55	0.09
CH ₄	0.65	0.04
OH	0.03	0.002

Table 2. Near closed loop lunar industrial ecology (emboldened materials are pure metal oxides for direct reduction using the Metalysis FFC process). This summarises the sustainable closed loop lunar industrial ecology system (CLIES) presented in [17]

Lunar Ilmenite

Fe⁰ + H₂O → ferrofluidic sealing

FeTiO₃ + H₂ → **TiO₂** + H₂O + Fe

2H₂O → 2H₂ + O₂

2Fe + 1.5O₂ → Fe₂O₃/Fe₂O₃.CoO - ferrite magnets

3Fe₂O₃ + H₂ ↔ Fe₃O₄ + H₂O) – formation of magnetite at 350-750°C/1-2 kbar

4Fe₂O₃ + Fe ↔ 3Fe₃O₄)

Nickel-Iron Meteorites

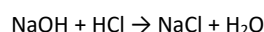
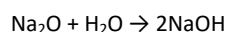
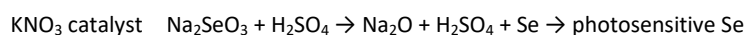
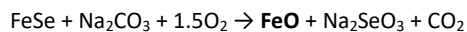
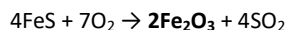
W inclusions – high density of 19.3

Mond process:

→ Thermionic cathodic material

Alloy	Ni	Co	Si	C	W.
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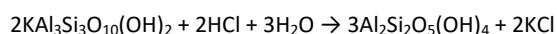
$\text{Fe}(\text{CO})_5 \leftrightarrow 5\text{CO} + \text{Fe}$ (175°C/100 bar) →	Tool steel	2%	9-18%
$\text{Ni}(\text{CO})_4 \leftrightarrow 4\text{CO} + \text{Ni}$ (55°C/1 bar) →	Electrical steel	3%	
$\text{Co}_2(\text{CO})_8 \leftrightarrow 8\text{CO} + 2\text{Co}$ (150°C/35 bar) →	Permalloy	80%	
S catalyst	<u>Kovar</u>	<u>29%</u>	<u>17%</u> <u>0.2%</u> <u>0.01%</u>



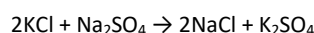
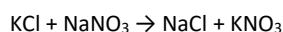
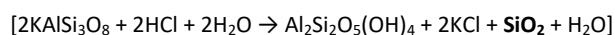
Lunar Orthoclase



orthoclase illite silicic acid (soluble silica)



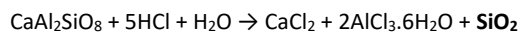
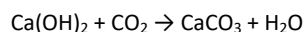
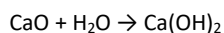
kaolinite



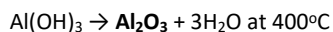
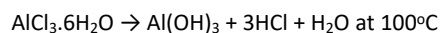
Lunar Anorthite



→ CaO cathode coatings



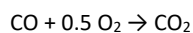
→ fused silica glass + FFC electrolyte



→ $2\text{Al} + \text{Fe}_2\text{O}_3 \rightarrow 2\text{Fe} + \text{Al}_2\text{O}_3$ (thermite)

AlNiCo hard magnets

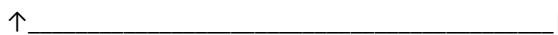
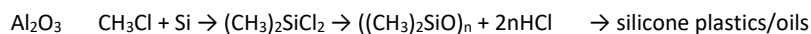
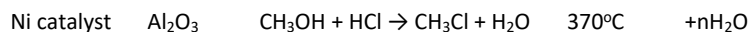
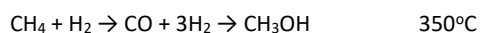
Lunar Volatiles



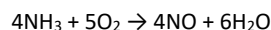
→ for steel/anode regeneration

Ni catalyst

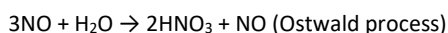
850°C 250°C

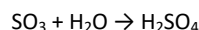
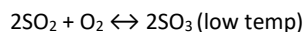


Fe on CaO+SiO₂+Al₂O₃

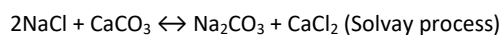


WC on Ni



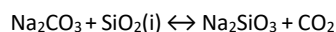


Salt of the Earth



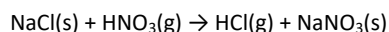
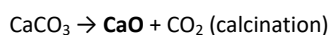
→ FFC electrolyte

350°C/150 MPa

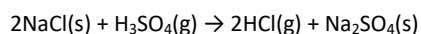


→ piezoelectric quartz crystal growth (40-80 days)

1000-1100°C



→ recycling reagents



Material closure but openness to energy flow are fundamental facets of any closed loop biospheric ecology with the latter driving it to far-from-equilibrium conditions [18]. So it is with life support systems – material closure (with the exception of water/oxygen) will be essential. However, closed loop food production and nutrient recovery from waste can only be provided by biological processes employing living organisms in plant cultivation which is volume intensive. Closed biological regeneration involves the production of food, recycling of waste, recycling of water, and air regeneration.

3. Physicochemical Processes

The modular approach to life support avoids centralisation of life support with ventilation ducting across the modules. Each module operates on a stand-alone basis without intermodule ducting as each has its own independent power and life support systems. This approach also provides inherent safety to astronauts. However, such ducting aids in air circulation. In a lunar base, standard atmospheric pressure of 101 kPa may be reduced as long as the oxygen partial pressure is maintained at 20 kPa but no greater than 48 kPa (beyond which oxygen toxicity is induced) but control of flammability imposes a lower ceiling of no more than 30 kPa. Reducing the proportion of buffer gases increases fire risk (though EVA suits adopt 100% oxygen to reduce internal pressure which retards suit flexibility). Nitrogen is required for atmospheric buffering and agriculture but it is a scarce commodity on the Moon. The major biological elements required to support human life – CHONPSK – must, excepting H and O, be supplied from Earth or recycled efficiently as they are scarce resources on the Moon. Required macronutrients also include metals - 3.5 g/day K, 2.5 g/day Na, 1 g/day Ca, 260 mg/day Mg, 14 mg/day Fe, 7 mg/day Zn and 1.5 mg/day P supplemented by micronutrients Mn, Cu, Zn, Sn, Mo, Pb, Al, Ti, B, Ni, Cr, V and Co though excess Ni, Co and Cr are toxic. Nevertheless, micronutrients are essential [19], e.g. Keshan disease is a juvenile cardiomyopathy common in Se-deficient areas of China; Mo deficiency is apparent only in conjunction with excess W intake; sufficient Cr is necessary for insulin sensitivity. While water is an abundant resource on the Moon, K and P requires complex extraction from KREEP minerals and C, N and S volatile resources are highly rarified. Trace elements may be more problematic though Fe, Ca, Co and Se can be sourced from asteroidal material on the Moon. Other trace elements may be resident in lunar regolith in small quantities but extraction will be challenging. It is essential to minimise the resupply of consumables from Earth implying that extensive recycling will be necessary. A human being in a single day consumes 641 g dry food, 3216 g water (approximately 50% drinking water and 50% water associated with food) and 806 g oxygen while excreting 94 g faeces, 1630 g urine and 943 g carbon dioxide [20]. Water is also required for washing of the body for hygiene (7270 g), dishes (5460 g), clothing (12500 g) and flushing (500 g) [21]. Hence, water is by far the dominant resources consumed. Recycling of oxygen and water permit elimination of 90% of the consumable supply to support human life. To date, most approaches to recycling are physicochemical involving the recycling of air and water with food being re-supplied and waste stored and/or dumped.

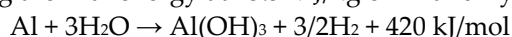
Yet water and oxygen are potentially supplied from in-situ resources on the Moon. On the Moon, oxygen may be extracted from ilmenite using H_2 ($FeTiO_3 + H_2 \rightarrow Fe + TiO_2 + H_2O$ with recycling of H_2 through $H_2O \rightarrow H_2 + \frac{1}{2}O_2$), CO ($FeTiO_3 + CO \rightarrow Fe + TiO_2 + CO_2$ with recycling of CO through $2CO_2 \rightarrow 2CO + O_2$) or CH_4 ($FeTiO_3 + CH_4 \rightarrow Fe + TiO_2 + CO + 2H_2$ with recycling of CH_4 with $2CO + 6H_2 \rightarrow 2CH_4 + 2H_2O$) reducing agents, hydrogen being the obvious choice given the apparent availability of water ice on the Moon. There may be several reservoirs of lunar water [22]: (i) as subsurface regolith ice; (ii) as thin films on minerals; (iii) as water of hydration in minerals; (iv) within mineral inclusions.

The International Space Station (ISS) ECLSS system provided several specific functions integrated in two ECLSS subsystems, the oxygen generation system (OGS) and the water recovery system (WRS) which recycle water and oxygen at 70-80% [23,24,] so requires some resupply:

1. pumping cabin air between modules with motorised fans.
2. maintaining cabin air temperature at 22°C using heat exchangers.
3. monitoring and controlling total atmospheric pressure and the partial pressures of N_2 , O_2 and CO_2 in cabin air.
4. monitoring and controlling the water vapour content of cabin air (humidity at 40%) using dessicants such as silica (which can be sourced on the Moon).
5. mass spectrometer for analysing aerosols, particles, water vapour and gases in the air to provide analytical feedback.
6. fire, smoke and CO detection and suppression.
7. high efficiency particulate air (HEPA) filtering of solid particles from cabin air using replaceable filters impregnated with biocides to prevent microbial infection.
8. airborne contaminant removal such as methane (CH_4) and ethylene (C_2H_4) from cabin air using activated charcoal beds.
9. CO_2 extracted from cabin air using $LiOH$ ($2LiOH + CO_2 \rightarrow Li_2CO_3 + H_2O$) granules in canisters integrated with the charcoal beds. Alternatively, a molecular sieve such as zeolite (sodium, potassium or calcium aluminosilicate) can remove CO_2 in air. Zeolite is manufactured through hydrothermal synthesis – they are formed by slow crystallisation of heated aqueous solutions of SiO_2 and Al_2O_3 (both of which can be sourced from lunar resources) in $NaOH$. A membrane commonly used for recovering CO_2 from the atmosphere is PDMS silicone rubber because of its high permeability to CO_2 relative to other gases (PDMS is manufacturable from syngas through the Rochow process). In these cases, CO_2 is removed without recycling. This may be employed as backup to recycling mechanisms or recycling mechanisms added. The Bosch reaction at 550-700°C catalytically reduces CO_2 : $CO_2 + 2H_2 \rightarrow C + H_2O$. The catalyst is activated steel wool – it is only 10% efficient, far lower in efficiency than the Sabatier reaction. The Sabatier reactor catalytically reduces CO_2 with H_2 from the OGS to generate CH_4 and H_2O at 180-550°C: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$. This is exothermic with 98% efficiency. The catalyst is typically ruthenium-on-alumina and the methane is vented but on the Moon it should be stored as a carbon source. Water electrolysis is required to recycle H_2 and release O_2 as the oxidant to CH_4 fuel or for recycling CO_2 into O_2 as part of ECLSS.
10. OGS generates O_2 into cabin air by electrolysing water from the WRS with H_2 vented or passed to the Sabatier reactor which gives 98% recovery. In both Bosch and Sabatier reactors, water is then electrolysed into its constituents to recycle H_2 for the Bosch or Sabatier reaction and yield O_2 : $2H_2O \rightarrow 2H_2 + O_2$. There are several water electrolysis methods. Suitable solid-state electrolytes include calcia-stabilised zirconia or yttria-doped ceria at 850°C with an electrical energy consumption of 250 W. Static feed water electrolysis electrolyses water using an aqueous KOH electrolyte soaked onto thin asbestos sheets. Solid polymer water electrolysis uses a solid polymer membrane electrolyte of perfluorinated sulfonic acid polymer.

11. WRS reclaims wastewater, urine and condensation through vacuum distillation followed by multifiltration beds giving 80% water recovery – it filters out solid particles initially and then filters out organic contaminants through semipermeable membranes and finally a catalytic oxidation reactor destroys volatile organic material and bacteria.

There are several extensions to such physicochemical processes that may be employed including the incorporation of fuel cells. The main types of fuel cell are polymer electrolyte membrane, alkaline, solid oxide, direct methanol and biological fuel cells. They all operate with hydrogen gas except the methanol fuel cell. Regenerative fuel cells require water electrolysis to split water into hydrogen and oxygen. Hydrogen and oxygen combustion releases an enthalpy of -285.8 kJ/mole H₂/O₂ at STP. Hydrogen and oxygen reactants for fuel cells is usually stored in the cryogenic liquid state. Rather than storing hydrogen cryogenically, it may be used for CO₂ conversion and waste combustion [25]. CO₂ conversion to oxygen uses hydrogen for the Sabatier reaction forming methane – methane may be cracked at high temperature >1000°C to release H₂ on demand and a graphite residue. Wastewater and urine may be recycled into pure water while simultaneously providing both thermal and electrical energy [26]. Aluminium powder (activated with 1-2.5% Li) reacts spontaneously with wastewater and/or urine at room temperature generating thermal energy at 23.5 MJ/kg of Al and hydrogen gas:



The hydrogen may be fed into a fuel cell generating electrical energy and freshwater. Polymer electrolyte membrane fuel cells may accommodate illuminated cultivation chambers supplied with oxygen and nutrients to support microalgae cultivation to recycle air through continuous photosynthesis and which may be harvested as food [27]. Exploitation of biological organisms in fuel cells constitutes microbial fuel cells. By way of illustrative example, microbial fuel cells have been employed as artificial metabolism onboard a small mobile robot (EcoBot) to permit it to engage in pulsed phototactic behaviour [28]. Microbial fuel cells exploit *Escherichia coli* in a bioelectrochemical medium to convert biochemical energy into electrical energy through a proton exchange membrane. The *E coli* is fed with sugar at the anode which transfers electrons to it carried by the coenzyme NADH. The cathode balances the redox reaction. The pulsing behaviour was imposed by the low energy extracted from the microbial fuel cell. Biological fuel cells have very low power densities ~1 mW/cm² compared with the methanol fuel cell at ~60 mW/cm² and polymer electrolyte membrane fuel cell at 300-400 mW/cm² rendering them an inefficient approach to energy storage.

4. Waste Processing

Lack of waste recycling will quickly lead to the depletion of certain elements such as N, K, Na, S, P, etc. On the ISS, urine, wastewater and water condensation is filtered and recycled into potable water. This represents a highly restrictive form of waste processing for the recovery of water only. Waste treatment is commonly conducted in quartz reactors, a quartz tube offering high temperature tolerance by virtue of its very low coefficient of thermal expansion. Organic waste constitutes human waste (urine and faeces) and inedible plant matter (such as cellulose, lignin, etc), the latter being relevant for in-situ food production. Typically, inedible plant matter is produced at 10 times greater dry weight than human faeces production. High-fibre plant waste (which may be as high as 90% of the crop) must be recycled either physicochemically through oxidation to carbon dioxide or biologically to improve processing efficiency. For wheat, there is a 20-40% loss from inedible plant material but inedible plant food can be fed to chickens and fish. Metabolic and plant waste cannot be used directly as manure for plant cultivation but must be composted first. This recycling of waste is a central tenet of permaculture. Recycling of solid and fluid human waste may be implemented through wet oxidative combustion in hydrogen peroxide to which an AC electric field is applied within a ceramic reactor [29]. This approach is rapid and suited to automatic control yielding waste gas and mineralised

waste solution. Complete oxidation of hydrogen into water and hydrocarbons into waste gas requires a Pt catalyst – pressure measurement of waste gas production provides feedback on the state of the process. Mineralisation is regulated by alternating voltage control of the E-field electrodes. Wet oxidation must be coupled with nitrogen fixation. The mineralised waste product may be used as manure supplemented with Knop's solution (for supplementary potassium) to grow crops such as wheat, peas and lettuce [30]. Full mineralisation of human waste is essential to prevent the proliferation of pathogenic bacteria [31]. Traditionally, the Haber-Bosch process has been used to fix nitrogen artificially by reacting nitrogen and hydrogen in the presence of a catalyst to form ammonia – the catalyst comprises a core of magnetite surrounded by a mantle of wustite and a shell of Fe with Al_2O_3 and CaO promoters all of which are derivable from lunar resources. Nitrogen fixation by rhizobium-infected legumes replenishes nitrate in the soil but this requires maintenance of a nitrogen buffer in the atmosphere. Recycling food and waste requires the adoption of bioregenerative methods. Hyperthermophilic aerobic bacteria may be employed for composting of human metabolic waste for use as agricultural fertiliser for forming lunar or Martian soil [32]. Bacterial fermentation generates temperatures up to 80-100°C suitable for aerobic hyperthermophiles for decomposing waste yet sterilising pathogenic bacteria acting as a natural autoclave. The removal of NaCl from waste and back into the human recycling loop may be achieved through the cultivation of the edible salt-concentrating saltwort, *Salicornia europaea* or the alga *Spirulina* [33]. More conventionally, lettuce, celery, Chinese cabbage, Swiss chard, dill and radish accumulate high concentrations of NaCl from NaCl-supplemented Knop's solution (equivalent to that of human urine) sufficient for 30 g of greens to support a low salt diet [34].

Water management systems are prone to biological fouling and mineral scaling in wastewater which can be physically filtered using granular lunar regolith [35]. Although evolutionary emergence and progression of viral disease is almost impossible to predict, epidemiological spread can be well modelled mathematically [36]. Microorganisms exhibit complex sociality in forming biofilms – implicated in up to 80% of human infections - as communal habitats of different microbial species embedded in a nutrient-rich extracellular matrix of DNA, protein and polysaccharides. Biofilms are crucial to the formation of fruiting bodies occurring under starvation conditions mediated by communication through quorum sensing [37]. Quorum sensing is used in bacteria to estimate their population density and regulate their behaviour collectively. They use quorum sensing to communicate and coordinate through extracellular chemical signals (pheromones) that activate the transcription of specific genes. Pheromones diffuse according to bacterial cell density so the production of public goods collectively is determined by the bacterial population. The marine bacterium *Vibrio fischeri* uses the pheromone AHL (N-acyl homoserine lactone) for quorum control of bioluminescence by affecting the transcription of two *lux* genes in neighbouring bacteria [38]. Similarly, both *Streptococcus pneumoniae* and *Staphylococcus aureus* use pheromones to activate genes for toxin production suggesting a means for controlling bacterial infections by inhibiting quorum sensing [39], e.g. degradation of AHL signals (quorum quenching) using lactonases and acylases. By detecting the concentration of specific acyl-homoserine lactone molecules, bacteria form biofilms, become virulent or develop antibiotic resistance. The biofilm provides protection to the microbes permitting communication, feeding and growth. The formation of biofilms – high density, structured colonies of bacteria embedded in an extracellular matrix - represent a bacterial strategy to restrict the invasion of inhibitor chemicals and exhibit enhanced resistance to antibiotics by preventing their infiltration through the extracellular matrix [40]. Similarly, bacterial swarming where bacteria migrate collectively exhibit swarm-specific resistance to antibiotics only while swarming. However, quorum sensing inhibitors degrade quorum sensing molecules to inhibit bacterial pathogenesis [41]. This can block virulence pathways to reduce toxicity of bacteria. Nitric oxide (NO) manufactured through the Ostwald process aids in dispersing bacterial biofilms reducing their pathogenic ability. This can reduce biological fouling and the risk of bacterial or toxic infection.

5. Bioregenerative Methods

The carbon loop, due to the scarcity of carbon on the Moon, cannot be recycled through physicochemical processes but the bioregenerative recycling loop has a long time constant. An agricultural system requires an infrastructure to support the growth of higher plants including providing a nutrient supply to roots, the recovery of water of transpiration (20 litre/day/m²) and provide a photosynthetically active radiation (PAR) lighting system [42,43]. Light may be supplied through solar collector mirrors to provide 0.5-1.0 kW/m² of full spectrum sunlight. Fresnel lenses may also be used as solar concentrators to transmit light energy through optical fibres and distributed in a controlled manner that is independent of direct sunlight. However, during the lunar night, artificial lighting is essential. PAR may be supplied by kW-output lamps – high-pressure sodium lamps or fluorescent xenon lamps have been superseded by LEDs but they are limited in their light intensities for some plants such as spinach, tomato and bell pepper. However, sulphur-microwave lamps offer bright visible light with a near-solar spectrum – it comprises a quartz envelope filled with small amounts of S and Ar ionised by microwaves with high efficiency. Exposure to sunlight is also essential for the production of vitamin D for human health which may require vitamin supplementation during the lunar night. There is other life support hardware required including heat-generating motors, pumps, fans, etc with recirculating hydroponic fluid loops in the case of hydroponic agriculture. Environmental parameters must be monitored reliably and controlled for optimal growth. A system of distributed sensors is required to monitor temperature, fluid pressure, fluid pH, conductive or thermal moisture and electrochemical dissolved oxygen levels, e.g. MELISSA compartments measure temperature, pO₂ and solution pH. The implication is that such autonomous control must be robust to external perturbations, reliable without functional failure and stable to feedback time delays.

Closed ecological life support systems (CELSS) requires agricultural production for food, CO₂ removal, O₂ generation (human respiratory quotient of [CO₂]/[O₂]=0.84-0.87 depending on the percentage formation of carbohydrate, fat and protein in the food consumed) and water recycling with bioreactors for recycling waste. Plants consume CO₂ and H₂O for photosynthesis under the action of sufficient PAR to produce carbohydrate food, regenerate oxygen and filter water through evapotranspiration. There have been several bioregenerative life support system programmes including Biosphere 2 (US), CELSS (NASA), Bios-3 (Roscosmos) and its predecessors and MELISSA (ESA) [44]. CELSS require bioregenerative approaches which are characterised by significantly longer lags in recycling than physicochemical methods [45]. CELSS architectures are hierarchically modular, separating human habitation, plant cultivation, animal husbandry and microbial waste treatment which can be further subdivided [46]. They should be highly functionally redundant with multiple approaches to any specific function, i.e. there should always be physicochemical backup systems as far as possible [47]. A three-tiered architecture with planning–reactive-servo levels is considered suitable for controlling a complex life support system [48]. It is crucial to develop autonomous ecosystem control that includes mass and energy exchange measurements and models [49]. Crop growth rates may be modelled using the S-shaped Lotka-Volterra predator-prey logistics equations but their nonlinearities can give rise the chaotic behaviours [50]:

$$\frac{dm_{in}}{dt} = r_{in}m_{in} \left(1 - \frac{m_{in}}{m_{in}(f)}\right) \quad (1)$$

$$\frac{dm_{ed}}{dt} = r_{ed}m_{in} \left(\frac{m_{ed}(0)+m_{ed}}{m_{ed}(f)}\right) \left(1 - \frac{m_{ed}}{m_{ed}(f)}\right) \quad (2)$$

where m_{ed}=edible biomass, m_{in}=inedible biomass, r=growth rates, m(0)=initial (minimum) biomass, m(f)=final (maximum) biomass. This may be broken down into a mass flow model of growth of edible plant, growth of inedible plant, human consumption of edible plant, waste processing of inedible plant and waste processing of human waste [51].

ESA's micro-ecological life support system alternative (MELISSA) is a microorganism-based artificial ecosystem centred in Barcelona Spain to create a closed loop

bioregenerative system for space application including microbial recycling of human waste. It exploits microbial bioreactors in which bacteria, yeast and algae can recycle all the major biochemical elements and degrade complex organic molecules in waste into usable materials. Microbial bioreactors with bacteria fixed to a filter bed can also act as biofilters to filter air. They are well-suited to carbon recycling in closed life support systems, e.g. cellulase degrades cellulose into its components such as edible glucose. MELiSSA comprises five (of which four are microbial) interconnected functional bioreactor compartments inspired by aquatic ecosystems with closed loop fluid flow [52-54]:

1. a multi-bacterial species anaerobic composter (including species from the complex human microbiome of which many bacterial strains resist culturing) that breaks down human and plant waste; it must also suppress methanogenesis (combusted methane imposes a loss of carbon and methane-consuming sulphate-reducing bacteria are sensitive to environmental conditions), e.g. *Fibrobacter succinogenes* is an anaerobic thermophilic bacteria whose fermentation degrades plant waste into CO₂ and volatile fatty acids;
2. stirred tank bioreactor with photoheterotrophic anaerobic bacteria (purple non-sulphur bacteria *Rhodobacteriaceae rubrum*) that absorbs fatty acid volatiles and converts them into edible biomass;
3. packed bed reactor with immobilised aerobic nitrifying bacteria (involving two bacterial steps by *Nitrosomonas europaea* from NH₄⁺ into NO₂ and *Nitrobacter winogradsky* from NO₂ into NO₃ that oxidises urea-produced ammonium NH₄⁺ into nitrate NO₃ in a culture medium;
4. gas-lift bioreactor with edible higher plant hydroponic system supplemented by edible cyanobacteria (*Arthrospiral/Spirulina platensis*) in a culture medium for photosynthesis to generate food, purify water and recycle air;
5. human habitation compartment.

MELiSSA is designed to produce 1 kg O₂/person/day, 15.8 kg water/person/day, 2.7 kg food/person/day while removing 1.2 kg CO₂/person/day [55]. Constraints on recycling of air, water and food include minimum mass, minimum logistics, minimum energy consumption of 170 W/person, minimum crew time, self-sufficiency and safety. The most challenging aspect has been microbial waste recycling - a six-person crew during a Mars mission of 216 days outbound, a surface sortie of 496 days and 216 days inbound generates waste comprising 5.50 tonnes CO₂, 8.24 tonnes urine, 12.73 tonnes of non-recycled water [56]. The goal is to provide 100% recycling with mass balance for CHONPS cycles in a manner that is safe and robust [57]. The medium of bacterial culture comprises a mixture of variable amounts of components [58]: (i) freshwater algal cultural media comprises - NaNO₃ - MgSO₄.7H₂O - KH₂PO₄ - NaOH - CaCl₂.2H₂O - NaCl - Al₂(SO₄)₃.18H₂O - Na₂SiO₃.9H₂O - FeSO₄.7H₂O - EDTA - H₃BO₃ - ZnSO₄.7H₂O - MnCl₂.4H₂O - Na₂MoO₄.5H₂O - CuSO₄.5H₂O - Co(NO₃)₂.6H₂O; (ii) saltwater algal cultural medium comprises - NaNO₃ - Na₂HPO₄.H₂O - CuSO₄.5H₂O - ZnSO₄.7H₂O - CoCl₂.6H₂O - MnCl₂.4H₂O - Na₂MoO₄.5H₂O. Although some of the metals of the culture medium could be sourced on the Moon, many and the volatiles cannot and require recycling biologically back into cultural media.

6. Food Production Systems

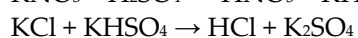
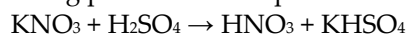
The introduction of food production is a key feature of the bioregenerative system - it also eliminates waste from discarded food packaging. One major consideration that has been exploited by crop breeders is that radiation environments can provide increased genetic mutation to breed hardier crops - the space environment increases mutation rates by 1% (compared with a terrestrial rate of 10⁻⁴%). However, such mutation rates are highly undesirable in a lunar production farm indicating that extensive shielding will be required and that piped sunlight will be necessary. Agriculture utilises natural photosynthesis of plants to convert human metabolic waste (CO₂) combined with wastewater to yield O₂ for human metabolism and renewable food sources: $n\text{CO}_2 + n\text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O})_n + n\text{O}_2$. Only a

small fraction of total water input to growing crops is required for photosynthesis; the vast majority can be recovered via evapotranspiration. Water is filtered through the roots passing up through the xylem within the stem out through the stomata underneath the leaves [59]. They can release 2-10 litres of water vapour/m² of leaf area by transpiration which can be exploited to purify wastewater. Higher plant crop area (m²) is determined by $A = \frac{M}{Y}$ where M=mass of edible crop/day, Y=nominal yield rate/m²/day. It has been suggested that biological recycling to support one human requires 20-40 m² of agricultural land irradiated by 250-300 W/m² of PAR and 10.8 kg/m²/day water to produce 1.25 kg of dried edible vegetation (to supply 3000 kcal/day) and 0.8 kg of inedible plant waste per day [60]. Water recycling driven by evaporation and condensation is crucial in closed ecological systems [61] though lunar water resources relax this requirement. Elevated temperatures, hydroponic nutrient delivery and high CO₂ conditions may increase plant productivity, reduce water transpiration and reduce the required agricultural area to 10 m²/person [62,63]. An agricultural footprint of 10 m² is sufficient to provide full O₂ generation and 200% water requirement per person through photosynthesis but only 50% food requirement (assuming 20 m² required for food production). This of course refers to food-producing land under cultivation. Human habitation requires additional square footage - the 315 m² BIOS-3 facility comprised 4 compartments that sustained a human crew of 3 for 6 months with 100% recycling of air, 95% recycling of water and 50% recycling of food of which 25% was animal products [64].

Lunar regolith can be exploited for multiple roles including as an agricultural soil substrate for plant growth [65,66]. Lunar regolith comprises olivine, pyroxene and plagioclase feldspars with impact glasses and agglutinates. Clay byproducts from the artificial chemical weathering process in our lunar industrial ecology could provide a substrate for a clayey soil substrate (Table 2). These clays may provide sources of Fe, Mg, Ca and K ions though they would be deficient in N, P, etc. On Mars, hydrated clays may have formed early in the Noachian period (4.1-3.7 Gy ago) when the global basaltic crustal magma ocean reacted with the extremely dense outgassed steam [67]. The vast majority of Mars' enormous early water equating to a global depth of 100-1500 m has been sequestered into water of hydration of crustal minerals through the Noachian period which without plate tectonic recycling remains sequestered [68]. Perchlorates in Martian soils are highly toxic but may be removed through heating: $\text{MgClO}_4 \rightarrow \text{MgO} + \text{Cl}_2 + 7/2\text{O}_2$. Martian regolith appears to offer a more favourable soil substrate than lunar regolith even with impregnation with in-situ manufactured clays. However, inedible parts of plants may be recycled as compost for lunar regolith to create the humus component of soil. Metals such as Ni and Cr which occur in lunar regolith are toxic in excess to biology suggesting the use of soil-less hydroponics rather than lunar soils. Other extraterrestrial regoliths may host agricultural soils. The Murchison and Allende carbonaceous chondrites are sources of C, N, S, P, Ca, Mg, Na, K and Fe [69]. They may be subjected to artificial weathering through hydrothermal processing to increase extraction rates of these elements. Mixed microbial cultures (though not higher plants) were successfully grown in Murchison meteorite samples in water [70]. The productivity of soil-based agriculture can approach that of hydroponics by enhancing light intensity with the advantage of simpler nutrient recycling [71]. It is crucial that methods such as no tilling and drip irrigation be adopted to prevent soil erosion and salinisation respectively, two of the central tenets of sustainable permaculture. Small animals may be bred such as worms which can grow on solid wastes within soil. High protein flour can also be obtained from dried worms: a 300 litre soil-bed can yield 60-80 kg of flour per year. Worms can provide food for fish which offer high food value.

Hydroponics and aeroponics offer advantages over soil cultivation with their high nutrient efficiency despite the higher water requirement in the case of the former – given the water resources on the Moon and Mars, this is not considered a major disadvantage. Hydroponics exploits a mineral nutrient solution directly to the exposed root system yielding 25% faster crop growth than soil culture. Seeds must germinate in a growing

medium – the roots are supported by a porous inert material such as rockwool, perlite, vermiculite, arcillite and/or baked clay pellets. All are derivable from lunar resources. Perlite and vermiculite are superheated expanded volcanic glass materials with similar porosity to pumice. Arcillite is a calcined montmorillonite clay that is porous similar to vermiculite. Rockwool is the commonest growth medium comprised of basalt spun into bundles of fibres – a lunar version may be manufactured from lunar fibreglass. Although primarily for supporting seedlings, rockwool can be used throughout the plant lifecycle. The adoption of hydroponics is the default assumption of MELISSA. Hydroponics permit indoor vertically-stacked rack-configured cultivation which may be integrated with structures [72]. Such vertical farming with hydroponics can yield food for a single person within a volume of 10-20 m³ compared with 400 m³ required in field agriculture but at the cost of higher energy consumption from 250 kWh/y/m² to 3500 kWh/y/m² primarily due to artificial lighting. There are several approaches to hydroponics – wick, deep-water culture, ebb-and-flow, drip method, nutrient-film technique and aeroponics [73]. Most suffer from clogging issues which is a significant problem. For minimum maintenance, the pipe-based ebb-and-flow technique involves few mechanical parts. Hydroponics requires aerated nutrient-rich water which must be recirculated. Knop's hydroponic solution comprises the major inorganic components required to grow higher plants including 0.0144 mol calcium nitrate Ca(NO₃)₂, 0.0049 mol potassium nitrate (saltpetre) KNO₃, 0.0145 mol magnesium sulphate MgSO₄, 0.0130 mol potassium dihydrogen phosphate KH₂PO₄ and variable amounts of potassium chloride dissolved in water [74]. Most of these elemental components may be derived from lunar resources except for nitrogen, sulphur and phosphorous (and carbon) which must be recycled through CELSS, i.e. composting with saltpetre (potassium nitrate) as a fertiliser. Saltpetre may be converted to HNO₃ with H₂SO₄ generating potassium bisulphate which decomposes to potassium sulphate at 100-120°C:



K₂SO₄ may be stocked as a fertiliser for a stable source of potassium and sulphur. Generally, inorganic nutrient solutions can be supplemented with organic fertilisers such as processed animal manure, bonemeal, fishmeal, seaweed, dried insect flour, etc dissolved in water. Trees comprise a typical component of the terrestrial biosphere and in permaculture offer different layered niches for a diverse but compact ecological community – canopy (e.g. edibles leaves such as maple and mulberry), dappled layer (e.g. apples), shrub layer (e.g. berry bushes), herb layer (e.g. herbs), soil layer (e.g. wide variety of crops), rhizosphere (e.g. root vegetables), vertical climber layer (e.g. runner beans) and fungus layer (e.g. mushrooms). However, even dwarf varieties of trees are unsuited to CELSS due to their enormous bulk, deep rooting requirements and low yield of edible fruits, favouring bush-grown fruits such as berries.

A nominal complete terrestrial diet might include several basic foodstuffs: fish provide essential fatty acids; spinach provides a wide range of nutrients; carrots provide carotenoids (vitamin A); tomatoes provide lycopene; grapes provide resveratrol and antioxidants. However, fish and grapes present challenges. The salad machine is a conceptual device that produces 600 g of diverse edible produce per week (sufficient for a 50 g salad for a crew of 4 every other day) [75]. Most closed loop higher plant agricultural systems require around 20 crop species [76]. A wide range of plants are required to supply carbohydrates, protein and fats to support human metabolism [77] but dwarf varieties with high harvest index, high light and water efficiency, short growing cycle, high plant density, high nutrition and easy preparation are favoured [78]. While C₄ photosynthesis comprises 2% of plant species, it accounts for 25% of global primary productivity on Earth. Staple crops, high in carbohydrates, include wheat and potato/sweet potato and other root vegetables such as onions, garlic, radish, carrots, beetroot and squash. Potato requires regular dark periods for the growth of tubers so would be unsuitable to 24 hour lighting. Wheat is selected for its versatility and, like most crops, is a C₃ plant but it can grow under continuous light [79] – although less efficient in photosynthesis than C₄ plants like maize, they are more efficient at elevated CO₂ levels. High protein sources include soyabean,

pinto bean and peanut. Vegetables for micronutrients include tomato, bell pepper, chufa, chard, spinach, kale, cabbage, coriander and lettuce. Minerals are provided by bell pepper, lettuce, tomato, cabbage and strawberry. Sprouts such as soyabean and broccoli have high oxygen consumption until leaves sprout but silicate minerals in regolith offer an abundant oxygen source. A core daily diet of 100 g leafy greens (cabbage, spinach, lettuce, chard, etc), 100 g tomato, 70 g carrot and 50 g bell pepper is of particular importance in providing high nutrition [80,81]. Supply of sufficient vitamin D is particularly challenging for astronauts without supplements [82]. Glyphosphate is a common herbicide that may become necessary if weed species infect crops but weeding agribots may be a mechanical solution – based on visual recognition, they either apply herbicides in microdoses, mechanically chop weeds up or electrocute weeds at high voltage.

Animal husbandry requires considerable capital investment with highly variable and marginal return but a culturally familiar diet would comprise 20-30% meat and 70-80% vegetable. The problem of animal husbandry is the vast areas required for grass foraging required of cattle and sheep. It takes 10-20 kg of feed to produce 1 kg of beef or lamb meat. In China, smaller areas of foraging and more limited animal food choices are accommodated by adopting chickens and pigs. Pigs offer better return with 1 kg of pork meat from 5.6 kg of feed. Chickens offer a much higher return with 1 kg of chicken meat from 3.3 kg of feed. This is similar to silkworm. The use of chickens only for eggs still further reduces the farming area required. Insects which can consume vegetable waste represent a low-fat, protein-rich source of human food or as animal feed, e.g. silkworm (*Bombyx mori*), large hawkmoth (*Agrius convolvuli*) and termite (*Macrotermes subhyalinus*) [83]. Insects are biologically similar to common seafoods such as prawns. Silkworm is eaten in China as a delicacy – they are easy to cultivate and demand modest resources while producing little waste. Silkworm larvae exclusively consume (human-inedible) mulberry leaves for 25 days which requires dedicated land area - nevertheless they produce cocoon silk for other purposes. Hawkmoth pupae is much larger but the reproductive adult is airborne imposing complications of containment. Insects may be dried and ground into flour. Termites exploit and consume fungus gardens within termite mounds to indirectly consume inedible plant material – although wingless, kings and queens sprout wings when sexually mature to form new colonies presenting challenges to containment.

Aquaculture combines food production with waste treatment in an already neutral buoyancy environment. Aquatic animals (such as fish and seafood) and plants (such as seaweed) provide a compact form of animal husbandry. Fish require ~5-20 times less energy cost per protein yield than land vertebrates offering higher protein densities per unit volume [84]. Their reproductive and embryonic development appears unaffected by microgravity environments. A commonly proposed fish for aquaculture is *Tilapia* which may be cultivated in subtropical fish tanks but it has difficulties in processing complex polysaccharides. Around 500 kg of catfish can be grown in a 1 m³ tank per year but fish-breeding requires lighting [204]. The freshwater armoured catfish *Hoplosternum* (Hassar) is a bottom-feeding mud-dweller that inhabits low-oxygen pools and can survive drying up of pools. It can gulp air, absorbing oxygen in its gut and expelling exhaled air through its anus. It consumes benthic invertebrates, algae and detritus. They reach sexual maturity in a year and breed by forming bubble nests with serendipitous materials such as plant debris. They lay up to several hundred eggs at a time and their protective behaviour makes it easy to catch. It has a pink salmon-like flesh and is eaten curried in its armour in Guyana which is easily lifted off once cooked. If curried, curry paste possesses high antioxidant ingredients – onion, garlic, salt, chili peppers, turmeric, cumin, coriander, ginger, paprika, garam masala (cloves, cinnamon, nutmeg and anise), thyme and tomato. It is commonly eaten with Guyanese bhaji (sauteed spinach, chili, onions, garlic and tomatoes without the fritters) providing a broad-based nutritional meal. Closed equilibrated biological aquatic system (CEBAS) comprised a four-compartment closed fully-submerged aquatic ecosystem with integrated waste management of fish (Chinese grass carp *Ctenopharyngodon idella*), water snails (*Biomphalaria glabrata*), ammonia-oxidising bacteria biofilter to convert ammonia into nitrate and edible non-gravitropic water plants (hornweed *Ceratophyllum*

demersum fed to the carp) was demonstrated on the STS-89 and STS-90 flights [85]. Algae is more readily processed as waste by fish. Wastewater and air revitalisation may be implemented through algae farming with food production implemented through hydroponics.

In photosynthesis, sunlight invokes the transfer of electrons mediated by photosynthetic complexes through an organic electric circuit - one glucose molecule is synthesised per 48 photos absorbed. Photosynthesis in higher plants in converting CO₂ into O₂ is rather inefficient at ~1-3% but algae offer 10-15% efficiencies. Algae - average composition C_{6.14}H_{10.3}O_{2.24}N - have higher specific photosynthetic productivity ~5-10 times than higher plants and are also more manageable. Green algae are edible with a high nutrient load and protein-rich, e.g. *Chlorella vulgaris* comprises 40-60% protein (all amino acids), 20% carbohydrate, 10-20% fat, 15% water and almost all essential vitamins, minerals and fatty acids. However, algae is unpalatable and it is recommended that it comprise no more than 20-25% of the human diet to prevent excess protein. Seaweed is a fast-growing vegetable grown in ocean waters relieving pressure on arable land and offering high nutrient density food - 3D printing of seaweed allows combination with more palatable flavours for bulk consumption. Seaweed is a high protein alga that is commonly consumed as laverbread in Wales. Populations of photosynthetic cyanobacteria or purple non-sulphur bacteria may be cultivated to produce biosynthesised organic nutrients and medicines from solar energy and waste CO₂ [86] - oxygen, proteins, vitamins, sugars, antibiotics, etc. A single human releases 1 kg CO₂/day. At 1 AU, solar flux is 8.6 x 10²⁵ photons/m²/day with a microbial biomass production rate of 2.64 x 10²⁵ photons/kg glucose, i.e. 1.5 kg glucose/m²/day. Cyanobacteria are particularly suited to Mars [87] but also suited to the Moon. *Arthrospira (Spirulina)* is a filamentous cyanobacteria that is easily cultured at 28-32°C and sun-dried into cakes for its high nutritional value (though insufficient vitamin C) [88] but also offers high photosynthetic efficiency in generating oxygen some 2.5-4.0 times more productively than trees. Spirulina offers many advantages over Chlorella including easier harvesting, ready digestibility and resistance to microbial infection [89]. Spirulina is a rich source of protein (~70% by mass) and photosynthetic oxygen (0.5 kg algae consumes ~2 kg CO₂) [90]. A photo-bioreactor that grew Spirulina has demonstrated the feasibility of algae as part of CELSS [91]. The addition of taste proteins makes nutritional bacteria more palatable, e.g. brazzein (sweetness), miraculin (sweet from sour), valencene (oranges), etc. Synthetic biology may be employed to enhance the productivity of Spirulina by utilising non-CO₂ inputs but any organic can be readily oxidised into CO₂ rendering this option redundant [92].

The provision of living cells and the construction of animal muscle similar to that employed in human organ printing presents possibilities of fresh meat from cell cultures. Recently, beef grown in culture (cultured meat) has been 3D printed into a burger, reportedly slightly less juicy than a conventional burger. The burger was made from cultured stem cells from a cow and took 3 months to grow - a single cow could spawn 175 million burgers. Such cultured meat involves using stem cells extracted from muscle cells to permit them to differentiate and grow into muscle tissue [93]. Culture requires nutrients and growth hormones including bovine fetal serum which must be eliminated to divorce cultured meat from animal husbandry. Once cultured, it is coloured with beetroot and flavoured with blood and fat. An 85g beefburger was showcased - the incorporation of further cultured fat and blood would reduce its dry texture. An alternative is to substitute for meat entirely with other protein sources. Quorn is a mycoprotein-rich high-fibre product grown from the filamentous fungus *Fusarium venenatum* with a similar texture to eggs but no cholesterol as a meat substitute [94]. *Spirulina* and cultured meat offer the lowest land use per edible protein output though the latter requires considerable energy input [95]. Healthier ostrich meat may also be subject to the same technology. 3D printing offers the prospect of portable food with personalised menus while ensuring diet balance [96]. The Foodini 3D printer offers the possibility of 3D printing simple meals. Lasagne may be printed layer-by-layer through nozzles using different cartridges of food (meat sauce, cheese sauce and pasta); spaghetti bolognese may be printed using the same cartridges

but in a different configuration. Food Ink is a London restaurant that 3D prints its food, eating utensils and dishes - cartridges of oil, water and other individual food purees may be mixed and subjected to heat treatment. A food compositor is a 3D printing machine concept that outputs wholesome dishes constructed from basic food "elements" (morsels) and flavours (sweet, salty, sour, bitter and umami) from cartridges selected according to a menu, mixed and rapidly cooked using a pulsed electric field of 1-4 kV/cm [97].

Molecular gastronomy is an approach based on physico-chemical transformation of food ingredients attributed to chef Heston Blumenthal of the Fat Duck restaurant in Berkshire UK. Molecular gastronomy emerged from food pairing in which similar molecules between different ingredients are combined in a single dish often involving flavoured gelatins, e.g. white chocolate with caviar, oysters and fruit caviar, roasted foie gras benzaldehyde, liquorice-gelled salmon with asparagus, bacon-and-egg ice cream, arugula spaghetti, etc. The tools of chemistry are often involved – liquid nitrogen, water baths, syringes, distillation columns, pH meters, and bottles of xanthan, maltodextrin, citric acid, etc (extracted from biological sources). Flavours are bottled – methional tastes like potato, 2-heptanone tastes like gorgonzola cheese, allyl isothiocyanate tastes like Dijon mustard, 2-methyl-3-furanthiol tastes like chicken, verbenone and birneol tastes like rosemary, etc [98]. Agar agar is derived from red algae as a stabiliser and thickening agent – it gels only on cooling after being boiled and can then be extruded and remains solid unlike animal-based gelatin. Sodium alginate is a salt extracted from brown algae that with calcium chloride gels into liquid-containing spheres when cold. From molecular gastronomy developed multisensory cooking in which sights and sounds accompanied the dishes which themselves were presented in an evocative fashion – ocean sounds accompany crab ice cream on crab risotto shaped into a shoreline with "sand" and "sea foam". Cognitive aspects such as nostalgia also enhance taste reminiscent of home comforts and childhood – memories of food smells could reduce isolation felt by lunar astronauts. However, molecular gastronomy requires a complex system of raw ingredients and chemical manufacturing. Nevertheless, a soylent green-type of nutrient biscuit would not be tolerable for long lunar base missions except perhaps for pressurised rover sorties.

7. Bioregenerative Medical Applications

The introduction of engineered food technology introduces the prospect for similar medical technologies. There is a potential medical application of similar 3D printing technology that robustifies the survivability of astronauts against a wide range of medical issues through organ intervention. As mentioned earlier, we expect accidents to be the primary reason for medical intervention on a long-duration lunar base ranging from burns to punctured or otherwise damaged organs. Engineered substitutes to biological organs are typically deficient to the original organ – this may be illustrated by the robo-pancreas [99]. An insulin pump delivers a continuous flow of insulin through a pipette embedded under the skin based on blood sugar measurements by an implanted sensor. The chief difficulty for the control system is time lag between insulin delivery and measured blood sugar response, typically 60 minutes. Such robo-organs cannot replicate the behaviour of biological organs. An interesting technique would be to reprogram some liver tissue to convert into pancreatic tissue as they emerge from adjacent regions of the anterior endoderm during embryonic development – the transcription factor Pdx1 that is expressed in the pancreas but not the liver may be expressed through genetic engineering [100]. This however is likely to be a more distant technology than 3D printing of organs.

Medical applications of 3D printing have grown rapidly for individualised orthopaedic and dental prostheses and implants from CT/MRI scan-derived CAD files [101]. The first medical role of 3D printing has been in stents, bone replacements, cartilage implants, and prosthetics. The main 3D printing techniques involved in 3D biomedical applications include stereolithographic bioprinters, droplet-based bioprinters (inkjet, electrohydrodynamic jetting, acoustic and microvalve), extrusion-based bioprinters and laser-based bioprinters (laser-induced forward transfer and laser direct writing) [102,103]. Laser-based

bioprinters offering 400 μm resolution comprise a laser, transparent print ribbon coated with a cell-laden bioink and a motorised substrate. Inkjet bioprinters emit cell-laden droplets of liquid bioink pressurised through a nozzle. It requires the jetted fluid to solidify on deposition. Extrusion-based bioprinting is the commonest approach in which viscous bioink is extruded through a nozzle under pressure using a mechanical or piezoelectric actuator. Extrusion is used for non-Newtonian fluids with high viscosity while jetting is used for Newtonian fluids with low viscosity. Although stereolithography offers 20 μm resolution, it is limited to UV-activated bioinks such as poly(ethylene glycol) dimethacrylate (PEGDMA) or trimethylene carbonate. Inkjet printing imposes thermal stresses, extrusion imposes mechanical stresses (if extrusion through micro-nozzles) and laser-based printing has poor compatibility with bioink viscosities. 3D bioprinting of cell-laden bioinks have been applied to the construction of 11 of the 12 organ systems of the human body – skeletal, muscular, nervous, lymphatic, endocrine, reproductive, integumentary, respiratory, digestive, urinary and circulatory cells [104] but many tissue systems are interconnected forming a complex of different tissue types, e.g. nerve-skeletal muscle-tendon-ligament-cartilage–bone. This presents significant challenges. Newer techniques include digital light processing and two-photon polymerisation bioprinting for printing of tissue with vascularised networks of microfluidic channels [105].

For 3D printing, the specific tissues must be mapped and modelled with high resolution by CAD/CAM software to ensure biological accuracy [106]. CT imaging is based on differential absorption of transmitted X-rays through different body tissues. The X-ray transmitter head revolves around the body while sensors measure the received beam intensity and angle. It yields high contrast between hard and soft tissue with a resolution of 0.25-0.3 mm though micro-CT offers high resolution of 200 μm . MR imaging adopts nuclear magnetic resonance within tissue induced by a strong magnetic field which aligns hydrogen nuclei to align themselves with the field. This atomic alignment generates radio emissions detected by receiver coils. MRI resolution for imaging soft tissue is lower at 0.5 mm and higher resolution requires high magnetic fields up to 7-9 T. Other imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) are less commonly used. To recover tissue volume, tomographic reconstruction generates 2D cross sectional slices. A series of slices generates a 3D reconstruction in CAD format.

The basic principle of 3D printing tissues (in essence, applied developmental biology) is based on the natural viscoelastic aggregation of adjacent tissue cells [107]. The 3D printed material must be able to endure mechanical and thermal shear stresses introduced by printing as well as emulate biological tissue. Desktop 3D printed silicone provides the basis for manufacturing soft tissue prostheses such as artificial ears, eyes and noses [108]. 3D printing of bio-compatible orthopaedic implants is becoming well established. 3D scanning determines the geometry of the 3D printed ABS plastic mould into which silicone (such as PDMS) is cast and then cured as the prosthetic after the mould has been polished in a controlled acetone vapour environment that removes staircasing. Compliant materials such as polyurethane (cast from a 3D printed silicone mould) offer flexural hinges between rigid members for assembling joints without bearings [109]. Diatoms have cell walls composed of amorphous silica that can be lithographically printed as channels [110]. 3D printed cartilage and bone have been most successful. Biomimetic bone grafts for implants such as craniomaxillofacial surgery should have the appropriate mechanical properties, be porous and permit new bone growth, e.g. polycaprilactone (PCL) is a laser-printable biodegradable polymer for repairing bone and cartilage damage. Natural bone is a hierarchically-structured composite of organic collagen and inorganic apatite which may be approximated by a mixture of polypropylene and tricalcium phosphate. Of particular promise is the use of nanocomposites that can emulate biological materials using extrusion printing, inkjet printing, stereolithographic printing and laser printing. For example, porous silica nanoparticles may be manufactured using the low-temperature sol-gel process with controllable physical properties but this requires prior hydrolysis and condensation of silica alkoxide precursors in a polymer network [111].

3D bioprinting involves 3D printing biological cells in bioinks layer-by-layer to build tissue-like structures for medical uses. Grafts that might be suitable for 3D printing include skin, cartilage, blood vessels, muscle, etc. Natural polymers such as collagen, fibrin and hyaluronic acid have better biocompatibility with human extracellular matrix than synthetic polymers such as polyethylene glycol (PEG) in reducing immunological rejection [112]. Tissue engineering involves artificially growing living cells extracted from healthy biological tissue from either the patient or a donor for transplant to repair physically damaged tissue. These are commonly stem cells, undifferentiated cells that can grow into different types of tissue – adult stem cells are multipotent (capable of limited differentiation capacity), embryonic stem cells are pluripotent (capable of differentiating into all three germ layers but not placenta) while only blastomeres are totipotent (capable of generating the cells of an entire human being including placenta) [113,114]. Adult (mesenchymal) stem cells are typically isolated from adult bone marrow (less commonly from skin, adipose fat or trabecular bone) or from pre-stored umbilical cord blood or placenta. Human embryonic stem cells may be employed for constructing tissues and organs by differentiating into skin, skeletal muscle, cornea, nerves, bone marrow, heart, bone, cartilage, blood vessels, pancreas and liver on scaffolds with appropriate growth factors [115]. 3D printing stem cells permits the construction of engineered organs to compensate for the lack of organ transplant donors. However, fabrication of biological tissue requires the 3D reconstruction of a complex, hierarchical cytoarchitecture of multicellular tissue within a complex microenvironment.

Following 3D printing, the tissue must undergo accelerated maturation in a bioreactor. Biological cells are typically grown in a bioreactor that simulates a specific physiological environment before grafting. The bioreactor attempts to mimic the natural organ environment of high cellular density with access to a blood supply. Tissue is comprised of two major components – cells and extracellular matrix. Cell adhesion to the extracellular matrix is implemented by cell surface receptors – integrins – which activate intracellular signalling pathways determining cytoskeletal morphology. The integrity of 3D printed tissues requires the incorporation of a 3D printed scaffold. Scaffolds must be biocompatible, biodegradable and porous [116]. Biological cells are implanted into the porous artificial scaffold to support tissue growth and provide a template for its topological shape. As the cells extrude their own extracellular matrix, the scaffold should degrade over time. Alginate (similar to that used in molecular gastronomy) or fibrin polymers are used as scaffolds integrated with cell adhesion molecules to encourage cell-to-cell bonding. Hydrogel alginates are one of the commonest scaffolds for their biocompatibility. Common scaffold materials are collagen, chitosan, hyaluronic acid or polylactic acid (PLA), the latter degrading in the human body into lactic acid (but acidification can cause local tissue necrosis). 3D printing has been applied to tissue engineering with printed thicknesses ~20 μm [117]. 3D printing of hydrogels as scaffolds for tissue engineering permits a wide variety of 3D printing techniques – extrusion printing, inkjet printing, bioprinting and 4D printing [118]. Hydrogels form highly hydrated 3D polymer networks mimicking vascularised extracellular matrices of collagen or fibrin as substitutes for soft tissue – hydrogels include polysaccharides linked by glycosidic bonds such as agarose, polyethylene glycol (PEG), polyvinyl alcohol (PVA), etc for bone/cartilage regeneration, spinal cord regeneration, skeletal muscle regeneration [119], etc. PEG is a degradable hydrogel that has been developed to encapsulate living cells [120]. The scaffold mimics the extracellular matrix of tissue to guide its structure. Elastic protein-based polymers such as Gly-Val-Gly-Val-Pro permit emulation of the mechanochemical properties of biological tissue subject to stretching and elastic recoil [121].

3D printing of layers of bio-ink comprising aggregates of cells deposited layer-by-layer permits geometric control of cell distribution to form organs in a manner infeasible with traditional bioreactors. Design of bioinks as mixtures of biological cells and hydrogels is a significant challenge for constructing organs vascularised with blood vessels [122]. Modified inkjets have been used to build prototype heart, bone and blood vessel tissue from different reservoirs of cells. These are based on pre-printed scaffolds

constructed from biodegradable material such as hydrogels. Bio-electrospraying uses an electric field to spray cells into sheets. The chief hindrance has been the required integrated network of blood vessels to nourish the organ (vascularisation) [123]. 3D printing endothelial cells to form layered rings can become channels similar to blood vessels when treated with growth factors. The use of inkjet 3D printing allows the construction of stacked rings of endothelial cells within the tissue matrix to form blood vessels. An integrated tissue-organ printer has been developed that can construct tissue of multiple cell types in any shape with incorporated microchannels for nutrient supply [124]. Most success has been with skin, bladder and bone. Human bladder cells have been grown on biodegradable scaffolds and successfully implanted into children. Biological arteries may be grown through cell cultivation on biodegradable polyglycolic acid (PGA) scaffolds in a bioreactor. Collagen deposition under cyclic radial strain then replaces the biodegrading polymer during maturation to form blood vessels. It is essential to encourage the growth of infusing blood vessels without which the tissue will die for which a vascular-like structure is essential to distribute blood efficiently. 3D printing is particularly valuable for manufacturing 3D scaffolds with complex vascular-like fine-scale geometries [125]. Pore sizes should be around 5-10 times the cell diameter, i.e. 100-300 μm . Protein crystal growth has been demonstrated in microgravity conditions which minimises convective flow, nucleation sites and sedimentation [126]. This could potentially be exploited in 3D printing of biological tissue. Ceramic scaffolds of hydroxyapatite are used as scaffolding for bone regeneration. The feedstock is a mixture of live cells and nutrients and bioink within cartridges that is printed onto the biological scaffold. Alternating patterns of cells and bioink gel are printed layer-by-layer from an extruder nozzle into a 3D organ shape. The living cells gradually fuse together to form the tissue. Once the tissue has formed, the scaffold is washed away leaving only cells. In the future, if the organ is constructed from a recipient's own cells, transplantation could occur without rejection. New methods of 3D printing introduce new possibilities. Direct ink writing of 3D microvascular networks to distribute fluids has applications in self-healing and organ printing [127]. An organic ink is patterned into a microvascular network within photocurable hydrogel and removed by liquefaction to form hollow lattices layer by layer. Electrohydrodynamic jet printing may be employed to construct nanoscale patterns of proteins onto protein microarray substrates with intact functionality [128]. Two-photon polymerisation permits the creation of 3D structures from a photosensitive material using Ti:sapphire femtosecond lasers permit extremely fine morphologies.

To avoid the use of scaffolds altogether and eliminate problems of inflammation or immune response, 3D biological structures can be constructed through bioprinting of a bioink of biological cells by exploiting self-assembly [129]. Self-assembly involves the assembly of constituent components without external intervention – embryonic development is a biological example of self-assembly. This form of self-assembly is an example of morphological self-organisation mediated by cell-to-cell adhesion. In biological development, tissue growth is mediated by regulatory functions and morphogens which control how cells differentiate into different specialised tissue from adult stem cells such as amniotic fluid, bone marrow, joint synovium, adipose fat, dental pulp and blood from the patient thereby alleviating immune response. While adult stem cells are multipotent to a limited number of cell types, only embryonic stem cells or artificially induced pluripotent stem cells are pluripotent including cardiac cells. Sheets $\sim 80 \mu\text{m}$ of muscle cells have been cultured for grafting with self-assembled endothelial vascularisation at multiple scales. Differential adhesion hypothesis is based on different cells having different adhesion strengths with each other as the primary process of cell sorting and assembling tissue. The number of cell adhesion molecules determines the degree of surface tension in aggregates of cell assemblies in cell suspensions. It is this liquidity that is the basis of a bioink. Much development is required to create the technology in which multiple printing head arms can sculpt an organ with multiple cell types. If the cells are pluripotent like embryonic stem cells, they can be used to create any type of artificial organ but organ 3D printing technology has a long way to go.

The goal is to 3D print transplantable liver and kidney organs – these are complex organs with multiple cell types with networks of blood vessels to keep them alive. Since the first heart transplant occurred in 1967, the waiting list for transplant organs such as kidneys is long. In the meantime, 12 hours of haemodialysis per week over 3 shifts recycles blood from the body, filtering out waste and maintaining electrolyte balance. The most favoured but ethically complex source of human organs is the harvesting human organs from non-heart-beating cadavers within three minutes of irreversible cardiopulmonary arrest [130]. Brain death has an injurious effect on human organs, especially the heart and other organs - around 18% of donated kidneys are unused due to poor condition or other reasons of unsuitability. Xenotransplantation has been enabled through immunosuppressive drugs – cyclosporin A and tacrolimus - to reduce xenograft rejection [131]. Organ harvesting from animals for xenotransplantation favours nonhuman primates and pigs for similar sized organs [132]. Ethical considerations and the practical problem of infectious agent transmission (xenozoonoses) disfavors primates, e.g. the devastating HIV virus evolved from a similar harmless virus carried in monkeys (SIV) that may have originated from human consumption. Pigs have the advantage that they are already easily bred as a food source, have short gestation periods, produce large litters and can be bred under non-pathogenic conditions. Pigs currently provide a source for transplanted heart valves but this is not living tissue. The chief constraint is the potential transmission of an extensive range of 62 porcine endogenous retroviruses (PERV) from pigs to humans in a mutational form that is pathogenic. Fortunately, endogenous retroviruses in the porcine genome transferred with the donor tissue into the human appear to be weak viruses. Furthermore, genetically-engineered pig herds can be bred that are free from PERV. Alternatively, eliminating all copies of the gene that integrates retroviral DNA into host DNA achieves much the same effect. Pig vascular endothelium expresses galactose oligosaccharide antigens rather than ABH blood group antigens resulting in anti-Gal antibodies. The primary difficulty lies in the rapid and devastating attacks on such foreign organs by the human immune system (hyperacute rejection – HAR). Humans possess antipig antibodies specific to galactosyl epitopes of pig cells which trigger HAR. One solution is to clone (somatic cell nuclear transfer) genetically engineered pigs that eliminates the gene for the Gal epitope with the addition of human blood group O gene. Genetically modified pigs offer the best solution for human organ supply [133]. This will not address delayed immune response by NK cells and macrophages on foreign organs. In particular, blood vessels that permeate the organ may be rejected. The rearing of pigs for food and/or organs is unlikely to be practical in extraterrestrial environments for the foreseeable future.

Regenerative medicine is being revolutionised by 3D printing of multilayered tissues of skin, cartilage, bone, blood vessels and complex organs for transplant. 3D printing of biological organs for transplants offers a print-on demand facility for lab-grown organs without waste. Furthermore, living 3D bioprinted tissue offers an alternative to animal testing and animal harvesting. 3D printing of organs involves the reverse of tissue imaging technology, the preprocessing step in bioprinting organs. For 3D printing of tissues, 3D model is sectioned into consecutive 2D slices in STL (stereolithography) format. A bioartificial liver comprising viable hepatocytes immobilised in a bioreactor is employed as a temporary substitute until the biological liver can be regenerated [134]. A 3D printed liver has yet to be achieved. A lab-grown kidney has been successfully transplanted into a rat. Next in line will be more complex organs like heart, liver, pancreas and lungs. A donor organ is washed to remove the donor's cells leaving a scaffold of collagen (decellularisation) [135]. The scaffold is seeded with recipient cells from a biopsy which are left to grow around the scaffold (recellularisation). Such an organ should not be rejected by the recipient's immune system but currently the transplanted kidney operates at only 10% functionality. Embryonic organ development is a scaffold-free system of self-assembling cells using a combine Glazier-Graner model with partial differential equations to describe cyclic AMP signalling during morphogenesis. Concomitant advances in 3D printing for edible meat and human organ replacement promises their application in a lunar base context.

8. Bioregenerative Industrial Applications

Microbes energised by widely-available solar energy have several potential applications in space, and planetary surfaces in particular [136]. Phytomining or biomining offers the prospects of leaching of high purity metal from low quality ores using bacteria or plants that preferentially take up metals into their leaves from the soil. Magnetotactic bacteria accumulate 35-120 nm diameter oval-shaped nanoparticles of magnetite (Fe_3O_4) within their cells (magnetosomes) [137]. Could they be exploited to extract nanophase iron from lunar regolith? This is entirely untested. In general, bacteria may be exploited to leach metals from ores, particularly low-grade sulphide ores such as chalcopyrite (CuFeS_2) copper ore, to increase recovery rates of copper which would otherwise be roasted with the emission of SO_2 gas. The most widely used organisms in terrestrial mining for leaching metals such Fe from FeS_2 (iron pyrite) are thermophilic chemolithoautotrophs such as iron-oxidising *Acidithiobacillus ferrooxidans* ($4\text{FeS}_2 + 15\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{Fe}^{3+} + 8\text{SO}_4^{2-} + 4\text{H}^+$) coupled with sulphur-oxidising *Acidithiobacillus caldus* ($\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$) from iron pyrite FeS_2 at $\text{pH} < 2.5$ [138,139]. It is the release of H_2SO_4 ($\text{SO}_4^{2-} + 2\text{H}^+ \rightarrow \text{H}_2\text{SO}_4$) that releases the metal and it is applicable to several metal sulphides such as MoS_2 (molybdenite) and WS_2 (tungstenite). To prevent the acid leachate from contaminating the environment, the process may be reversed using *Desulphovibrio* and/or *Desulphatamaculum* to oxidise H_2 : $5\text{H}_2 + \text{SO}_4^{2-} \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O}$ generating metal sulphide for disposal: $\text{M}^{2+} + \text{S}_2 \rightarrow \text{MS}_x$. *A. ferrooxidans* can also extract other metals from their ores [140]:

1. it attacks copper sulphides such as chalcopyrite (CuFeS_2) ore releasing soluble copper sulphate: $4\text{CuFeS}_2 + 17\text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 4\text{CuSO}_4 + 2\text{Fe}_2(\text{SO}_4)_3 + 2\text{H}_2\text{O}$;
2. it attacks uranium oxide ore in two ways to yield soluble uranium: (a) directly in the presence of oxygen by $2\text{UO}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{UO}_2\text{SO}_4 + 2\text{H}_2\text{O}$; (b) and indirectly in the absence of oxygen via $\text{UO}_2 + \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{UO}_2\text{SO}_4 + 2\text{FeSO}_4$ which may be regenerated in the presence of oxygen by $2\text{FeSO}_4 + \text{H}_2\text{SO}_4 + 0.5\text{O}_2 \rightarrow \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O}$;
3. it decomposes gold-bearing arsenopyrite (FeAsS) ores to expose encased gold permitting 95% recovery: $2\text{FeAsS} + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{FeAsO}_4 + 2\text{H}_2\text{SO}_4$.

There now exist biomining methods using *A. ferrooxidans* beyond sulphide treatment that apply to oxides and silicate ores such as cobalt and nickel from laterites (oxidised nickel ore) in association with ferric host minerals (such as goethite) via or cobalt-nickel-manganese from deep-sea manganese nodules [141]. Electrons are transferred from sulphur to ferric iron: $\text{S}_0 + 6\text{FeO}(\text{OH}) + 10\text{H}^+ \rightarrow \text{SO}_4^{2-} + 6\text{Fe}^{2+} + 8\text{H}_2\text{O}$. The resultant ferrous iron reduces manganese: $\text{Mn}_3(\text{OH})_6 + 6\text{Fe}^{2+} + 12\text{H}^+ \rightarrow 3\text{Mn}^{2+} + 6\text{Fe}^{3+} + 9\text{H}_2\text{O}$. *A. ferrooxidans* can oxidise reduced iron in meteoritic material such as troilite FeS [142]. All these biomining operations are conducted over weeks. Such biomining methods using *Shewanella oneidensis* are directly applicable to the extraction of Fe from iron minerals on the Moon and Mars [143,144]. Many plant species concentrate metals by growing in ultramafic soil enriched in metals. There are around 400 Ni hyperaccumulating species including agricultural species. The kale-related *Alyssum bertolonii* contains 10 mg Ni/g organic tissue. The sap of the shrub *Phyllanthus balgooyi* comprises 9% Ni. The fern *Dicranopteris dichotomata* concentrates a range of rare earth metals but in relatively low concentrations. The chief challenge lies in the extraction of metal from the plant but combustion is the simplest approach. Such plants may be sown to concentrate metals, harvested and burned to recover the metal.

As well as local lunar sources, CELSS generates an excess of water and oxygen under full food recycling production. Biological organisms cultivated through CELSS may be exploited to manufacture useful industrial products. Although not suited to the Moon where carbon is a scarce resource, on Mars, atmospheric CO_2 is abundant as a feedstock (though the discredited deep hydrocarbon gas theory posits planetary mantle methane and other hydrocarbons produced via Fischer-Tropsch reactions [145]). CO_2 reduced to methane can be accomplished by methanogens in a microbial fuel cell with high efficiency [146]. CO_2 may be fixed biologically into energy-laden liquid fuels using H_2 as the energy

source [147]. The anaerobic Wood-Ljungdahl pathway uses ferredoxin as the electron carrier and enzymes CO dehydrogenase/acetyl-CoA synthase and pyruvate synthase which are all oxygen-sensitive – this is unproblematic on Mars for the product of liquid acetate fuel. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the CO₂-fixing enzyme of the globally-dominant Calvin cycle of plants, algae and purple bacteria is highly inefficient at 2.4% for C3 plants and 3.7% for C4 plants. Rubisco is not discriminatory to binding with CO₂ or O₂ contributing to its inefficiency. Cyanobacteria are more efficient at photosynthetic conversion at 3-9% and may be exploited to produce food products (sugar) or biofuels such as ethanol from CO₂ [148,149]. Poly(3-hydroxyalkanoate) thermoplastics, e.g. poly-3-hydroxybutyric acid P(3HB) are a family of biodegradable natural plastics similar to polypropylene that are manufactured biologically by some bacteria (e.g. *Alcaligenes*) to store energy under conditions of carbon excess but nitrogen or phosphorus scarcity [150-152]. However, PHAs are brittle and temperature intolerant. Recently, the biological manufacture of polylactic acid (PLA), derived from corn starch, as a structural plastic has emerged as suitable for extrusion in 3D printing. Ecoflex is a flexible foil plastic produced from corn starch and potato that can be composted. 3D printing has been employed to initiate chemical reactions by directly printing reagents into a 3D reactionware matrix [153]. Printed active reactors with optimal geometries and printed-in catalysts to control reagent flow rates and reaction rates - reactionware - eliminates the need for glassblowing. Silicone paste was used to print the reactionware structure with catalyst-loaded polymer printed-in this reactionware structure. Although this approach is not readily applicable to pyrometallurgy, it may offer application to the synthesis of polymers/silicones or even the synthesis of drugs. Although plastic electronics including photovoltaics promises adaptable electronics, it may contribute to plastic waste on the Moon demanding an alternative biodegradable solution manufacturable by biological organisms [154]. Silk is an insulator composed of fibroin and sericin proteins produced by silkworms onto which electronics can be fabricated. Carotenoids such as β -carotene are resonant π -conjugated polymers – their delocalized π -electrons bonds support semiconductor properties. Such conjugated polymers can potentially operate as LEDs when biased appropriately [155]. Conducting polymers such as chitosan is derived from shrimp. Phytochromes are light sensing proteins employed by plants and cyanobacteria that can be exploited as light sensors [156]. Although Mars offers a plentiful carbon source, this is not the case for the Moon but if sufficient biological sources can be transported to the Moon, these technologies may yet be applicable in assisting industrial-type activities on the Moon.

9. Robotics & Automation

A typical crew 24-hour day might comprise 8.5 hours sleep, 1.5 hours personal and habitat cleaning, 3 hours communal meal breaks, 8 hours work, 2 hours exercise and 1 hour personal time. Given the tight work schedule, it is of high importance to automate as much habitat activities as possible. It is expected that robotics will play a significant role within the lunar base to relieve astronaut workloads. Robotics and automation in agriculture will have the largest impact on astronaut workload as this involves monitoring large-area crop status and the ability to react to enhance crop productivity to yield high quality, healthy crops. This will require large-scale persistent environmental monitoring using sensor networks in a challenging environment [157]. Data muling involves collecting data from fixed sensor nodes as the mobile rover passes within communication range of the node. Environmental variables to be monitored – temperature, pressure, airflow, light intensity, pH and biochemical sensing. Rather than complex molecular analytic instrumentation, biochemical sensing can be implemented using electrochemical cells, turbidity nephelometry, cytometers, and gas microsensors though with diminished capability. The employment of distributed sensing permits the employment of multisensor Kalman filters to robustify estimates. Agricultural measurements [158] include monitoring of soil parameters – moisture (electrical conductivity), pH, compaction (strain gauge-based mechanical impedance) and nitrogen/carbon load (near infrared spectroscopy) – and

monitoring of vegetation status - weed control (visual identification), crop maturity through sugar and acid content (near-infrared imaging) and crop health through normalised difference vegetation index (multispectral imaging). The robotics aspect requires sophisticated autonomous tractors in coordinated multirobot teams capable of following complex paths through a crop field map constructed via visual navigation as well as complex interaction with the crops such as seeding, cutting, grafting, transplanting, weeding, harvesting, etc. Hydroponics eases the complexity of some of these requirements but seeding, transporting, transplanting and harvesting are still complex robotic processes. Bioreactors that are employed extensively in CELSS require autonomous monitoring and control of multiple physical and biochemical parameters – medium temperature, photosynthetic active radiation exposure, pumped fluid flow circulation rate, motorised fluid stirring rate, gas input valve flow rate (NO_x and CO_2), nephelometric cell density (microbial growth), medium pH levels [159]. Inherent time delays and complex dependencies in the bioreactor impose the requirement for intelligent feedback control with feedforward predictive capability. It is plausible that 3D printing of electric motors introduces the possibility of in-situ manufacture of the machines of production, specifically robotic machines including 3D printers [160].

Robots may provide a social psychological function to alleviate psychological stresses. Humans are social beings who employ folk psychology as a theory-of-mind to understand beliefs, goals, motives and affective influences in self and other agents – it implies a shared social environment as well as physical environment through morphological embodiment [161]. Social robots are bio-inspired insofar as they simulate and evoke social behaviour. Social robots may be functional to the task, zoomorphic to imitate domesticated animals or anthropomorphic to permit fully human interactions including language. Language involves turn-taking dialogue but such dialogue must be grounded in the physical environment. Facial and gestural aspects of language including gaze are semantically rich but difficult to emulate naturally. To foster meaningful social interactions with such robots, they should be designed to foster positive anthropomorphic emotions to enhance social evocation [162]. This permits the implementation of personality traits in its five dimensions – extroversion, agreeableness, conscientiousness, neuroticism and openness. However, if they are too life-like, they can invoke the “uncanny valley” in which the robot is perceived negatively as weird or disturbing (such as how some people perceive clowns) [163]. Emotions act as a rapid evaluation mechanism for behaviour selection and are the primary determinant of motivation. Learning through imitation is a means of social learning physical motor movement in robots tempered by understanding of intentionality based on mirror neurons but there are many challenges with this approach [164]. As robotics contributes to a greater range of tasks, human-robot interaction in a social context will become increasingly important.

10. Promise of Advanced Biotechnologies

Plant genome evolution has been dominated by whole genome duplication. Genetically modified (GM) crops have been bred since the dawn of agriculture some 8000 years ago. Many crops are hybrids generated through cross-breeding. The Green Revolution spawned by Norman Borlaug permits the agricultural cultivation of only 38% of the Earth’s land surface to feed the human population but at a cost of consumption of 70% of global freshwater supplies. Today, trial-and-error cross-breeding has been rationalised through biotechnology. Most modern genetically engineered crops have been subject to single gene insertions to provide specific traits for (i) resistance to insect pests; (ii) resistance to viral infection; (iii) tolerance to herbicides deployed to control weeds. This increases yield – insect-resistant corn has 13% higher yield than wild variety corn and an 8% reduction in herbicide application. Public concerns are two fold: (i) effects on spread of GM crops on non-GM varieties; (ii) toxicological effects of human consumption. Transgenic manipulation involves introducing new genes into the germline using a recombinant vector construct (with integration sequences, promoter and regulatory genes) which

are expressed in the developing organism [165]. There is little doubt that transgenes engineered into GM crops could unintentionally migrate into the genomes of non-GM relatives but this would require a complex process involving several hybrid generations with physical proximity and overlapping flowering times [166]. Furthermore, fixation into the wild population requires strong selection pressures which are unlikely. Low risk crops include barley, millet, peanut, potato, corn, rice and cotton; moderate risk includes wheat, sugar beet, canola and sunflower; high risk crops are dominated by sorghum which is a weed. The main approach to mitigation is reducing plant fertility through the prevention of seed germination, reducing pollen fertility or suppression of embryonic development to restrict gene flow. The toxicological effects on human consumption are based on less rigorous studies. GM crop compositional and nutritional content is unchanged from the parent crop. GM foods have been grown for agriculture for decades and do not appear to have any adverse effects following human consumption [167]. Although the most common GM foods (soyabean, corn/maize and rice) are considered safe and as nutritious as their parent varieties, this conclusion is based on a limited set of scientific studies premised on the notion of compositional comparisons with parent varieties conducted by biotechnology companies rather than independent comprehensive scientific studies on long-term hepatic, pancreatic, renal or reproductive effects [168]. Nevertheless, the wider perspective is that rejection of GM crops can be potentially devastating – Golden Rice was a GM rice into which β -carotene genes had been inserted to counteract widespread vitamin A deficiency in the developing world. The developing world with encouragement from GreenPeace rejected this technology and so has ensured widespread blindness in poorer children of the world. Climate change will likely have dramatic effects on future agriculture which must adapt to cope with a more variable environment. It would be highly desirable to genetically incorporate productive C4 photosynthesis into C3 photosynthetic plants which offers higher water and nitrogen use efficiency [169]. C4 photosynthesis occurs in fewer than 2% of Earth's plants (it is extremely rare in trees) yet accounting for 25% of primary productivity due to their 40-50% higher growth rates. C4 photosynthesis evolved from C3 photosynthesis in multiple independent lineages through the evolution of a carbon shuttle that concentrates CO₂ at Rubisco which is an inefficient photosynthetic catalyst because it does not differentiate between O₂ and CO₂ which causes the buildup of oxidative toxicity (photorespiration). C4 photosynthesis avoids photorespiration. Although C3 photosynthesis is enhanced under higher CO₂ concentrations, higher temperatures offset this advantage; C4 photosynthesis is unaffected by CO₂ concentrations but is enhanced at higher temperatures. Of the world's primary crops, only corn, sorghum and sugarcane use C4 photosynthesis but genetically introducing C4 photosynthesis into rice potentially offers 50% higher yield with reduced water. There are new prospects for gene editing of GM crops including reduction of genome complexity (resultant from evolutionary genome duplication) and CRISPR-Cas9 (clustered regularly interspersed short palindromic repeats-CRISPR associated genes) [170]. Prior to CRISPR/Cas9, genetic cutting was performed using restriction enzymes, endonuclease proteins that cut DNA at specific nucleotide sequences of 4 or 6 base pairs by hydrolysing both strand ends. This cuts the DNA strand into random sized fragments that can be passed according to length through agarose gel during electrophoresis.

There are three main genome editing techniques in use currently – zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR-Cas (CRISPR associated) nucleases, the latter proceeding to supplant the first two. CRISPR/Cas9 is a gene editing technique derived from bacterial/archaeal system of adaptive immunity that recognises and destroys viral DNA. CRISPR DNA sequences have short repetitive sequences of 24-48 basepairs in size separated by short similar-sized spacer DNA that appear to be viral DNA that have previously attacked the host. Cas9 is an endonuclease whose genes are located adjacent to CRISPR repeat-spacer sequences. Cas9 recognises specific sequences of viral DNA and slices it apart at these recognition sites into fragments. A helicase scaffold holds the entire assembly securely during cutting. Cas9 possesses two endonuclease domains – HNH and RuvC – each cleaving a single

DNA strand. The DNA fragments are integrated into the CRISPR array as new spacers which recognise and bind with subsequent invader DNA. The guide RNA (gRNA) mirrors the target DNA sequence and attaches to it through base pairing. The gRNA is highly specific to the target DNA sequence. Cutting is accomplished using the bacterial Cas9 enzyme attached to the guide RNA. Cas9 generates the formation of double strand breaks at a DNA site complementary to the guide RNA, i.e. cleaving is RNA programmed by a single guide RNA (sgRNA) transcript for both Cas9 binding (crRNA) and DNA target recognition (tracrRNA) [171]. The Cas9 enzyme performs cutting as directed by the guide RNA, i.e. RNA-programmable genome editing. This bears some resemblance to RNA interference in eukaryotes. Natural DNA repair processes then splice the DNA ends together. Thus, specific sequences of DNA or genes may be deleted from genomes and other sequences or genes inserted into the gaps – mutagenesis if DNA is mutated; cisgenesis if the gene comes from a related species or transgenesis if the gene comes from an unrelated species. There have been a series of further innovations in CRISPR to improve its performance associated with genome targeting expansion, cleaving specificity, RNA editing, etc [172]. CRISPR/Cas9 potentially provide for the recovery of genes lost from previous cross-breeding. Insertion of a gene through CRISPR into the germline of mosquitos can reduce the transmissibility of malaria to humans [173]. CRISPR provides the basis for rational RNA-guided synthetic design of organisms using directed evolution of radical new genomes with novel phenotypes [174,175]. Prior to CRISPR, directed evolution involved sequential rounds of random mutagenesis/recombination using error-prone PCR and DNA shuffling [176] to generate mutant libraries of enzymatic biocatalysts [177-179] or genetic regulatory circuits [180] which are subjected to high-throughput selective screening/assay in suitable hosts. CRISPR/Cas9 has introduced high precision to genetic engineering.

Whereas genetic engineering is a cut-and-paste technology exploiting natural biological processes, synthetic biology is more fundamental. Synthetic biology treats biological pathways and processes as systems of components and circuits that can be created and manipulated within manufactured organisms. It essentially involves constructing intracellular circuits from biological modules – switches, logic gates, oscillators, pulsers, timers, memory elements, etc - to perform specific desired functions. For example, synthetic genetic regulatory networks based on the toggle switch and the repressilator may be constructed to control gene transcription/translation. A toolbox of genetic parts encoding genetic transcription factors may be assembled into genetic circuits in a “biofoundry” connected through polymerase activity [181,182]. There is a diverse range of terrestrial applications of synthetic biology including green fuel production and pharmaceutical production [183]. The heterotroph *E coli* reproduces every 20 minutes – it is suitable for biofuel production such as ethanol. Other suitable synthetic biofuels include the higher performing butanol produced by the conversion of acetyl-coA using three proteins in the *Clostridium acetobutylicum* glycolysis pathway [184]. Synthetic gene regulatory circuits implemented in microalgae such as *Chlorella* may produce biofuels such as biodiesel [185]. Synthetic biology has successfully synthesised artemisinin, the main ingredient of malaria drugs and the hydrocarbon farnesene, an aviation fuel. Synthetically-enhanced cyanobacteria as photosynthetic autotrophs may also be employed for enhanced biofuel production from CO₂ and sunlight [186]. A minimum genome factory *E coli* MGF-01 with a reduced genome potentially offers high growth and protein production rates [187].

Space applications of synthetic biology are broader than terrestrial applications in reducing transported mass for a human Mars (or lunar) mission through propellant production, food production, structural plastic production and pharmaceutical production [188]. Propellant production of CH₄ by the methanogen *Methanobacterium thermoautotrophicum* from CO₂ reduces physicochemical production plant by ~50%. The use of engineered *Spirulina* for rapid food production can reduce the requirement for pre-stored food for six crew by almost 40% for a human Mars mission. Synthesis of polyhydroxybutyrate as regolith binder by *Cupriavidus necator* from CO₂ can reduce the shipped mass of 3D printable polymer for constructing a six-person habitat by 85%. All these approaches are applicable only to Mars with its abundant atmospheric CO₂ as feedstock [203]. The ethical

question over whether it is appropriate to deploy synthetically engineered lifeforms to locations of astrobiological interest remains (though this does not apply to the Moon). The Precautionary Principle imposes restrictions on technological development until environmental and social risks can be assessed, extensive debate involving wider society can be undertaken and mechanisms for governance is established. It is likely that humans will venture to Mars sooner rather than later making these discussions urgent.

11. Discussion

The food and revitalisation module (FARM) greenhouse of 528 m² implementing a range of 21 crops supplies 100% of human nutritional requirements for 18 astronauts [189]. A simulated bioregenerative life support system supplemented by physicochemical methods (for atmospheric recycling, water recycling, waste reclamation and food production) with 15 types of hydroponic crop, silkworm husbandry and both solid and liquid waste recovery yielded 29.7 kg of oxygen, potable and hygiene water and food at 2700 calories/person/day (375.5 g carbohydrate, 99.5 g protein and 91.2 g fat) with 99.4% material closure [190]. However, caloric restriction of energy intake by 20-40% to 1600 calories/day (met with a diet of 3/2 cups of wheat, 1/2 cup of soya beans, 1/2 cup of pinto beans, 1 stalk of broccoli, 1/2 cup of spinach, 1/4 cup of peanuts and a small amount of mushrooms) can potentially reduce the incidence of cancer to offset radiation exposure [191] as long as micronutrients (carotenoids, etc) diet is maintained as antioxidants to DNA damage [192] (evolved as an evolutionary strategy of postponed reproductive investment in favour of temporary somatic maintenance [193]). Although such a draconian diet may foster physiological advantages, it is unlikely to foster psychological well-being.

Flight experiments of cropping in space have generated mixed results including diminutive or abnormal crops, lack of seed production, etc which have yet to be resolved but it is unclear if these problems would persist under partial gravity. Based on experimental and theoretical data from MELISSA and BIOS, near closed loop mass flows were established for a bioregenerative life support system to support a crew of six for a 780 day roundtrip Mars mission [194]. They concluded that the CELSS mass would be 18.09 tonnes (3 tonnes/crew member), some four times higher than an expanded ISS-type physicochemical life support system of 4.83 tonnes (while the latter incorporated double redundancy, the former did not). The system however was oxygen-deficient but this could be supplied from in-situ electrolysis of local water ice. Hence, bioregenerative systems require very high initial mass for plant growth and other supporting equipment and are suitable only for very long duration missions to avoid the cost of launching large amounts of food supplies over multiple years (revised to mass at 53.75 tonne-years per person) [195]. The high system mass and volume of extraterrestrial food production beyond physicochemical methods becomes cost-effective only for missions lasting in excess of 2-3 years. Much of this is attributable to their high power consumption and large buffering volumes to compensate for long response lags. The amount of crop required to feed one person is around 200% that required to supply oxygen and water for one person – hence, growing 50% of the food requirement is considered to be the most efficient approach. Hence, 100% food production would produce 100% excess water beyond requirements which could be diverted to propellant/oxidant production. Staple carbohydrate crops such as wheat, potato and rice is more efficient than growing protein and fat crops such as soyabeans and peanuts. Rice unfortunately requires considerable agricultural area – around 20.1 m²/person compared with 6.6 m²/person for wheat (Table 2 in [196]). However, this analysis is only applicable if all the bioregenerative equipment is launched from Earth – much of it can be built in-situ from in-situ resources [197]. There are sustainability lessons in developing CELSS that can be applied to Earth's biosphere through recycling and regeneration [198,199]. We concur but suggest that it is a two-way process and that extraterrestrial settlement must be sustainable:

1. Maximise exploitation of renewable energy sources (i.e. solar energy) and minimise consumption of non-renewable energy sources (i.e. H₂/O₂ combustion)

2. Minimise the generation of toxic byproducts, e.g. Cr
3. Develop industrial ecosystems of interlocking processes that feed waste of one process into another
4. Exploit feedback loops to recycle scarce resources

It has even been suggested that CELSS technology such as MELiSSA may assist in reclaiming hot desert regions to counter rapid desertification for productive arable farming using closed resource cycles [200]. Such regions offer high intensity PAR (productivity increases linearly with photointensity until saturation at 2.5L₀), high temperature (by 30% from 17°C to 23°C) and high atmospheric CO₂ levels (by 30-40% at 800 ppm) to enhance arable productivity. Experiments in the Laboratory Biosphere and elsewhere suggest that elevated CO₂ concentrations up to 2000 ppm enhance crop productivity proportionally [201,205]. This can be implemented in a lunar CELSS system provided high carbon recycling can be implemented. Nitrogen as a buffer gas is the chief limitation as it is scarce on the Moon and rarified at <5% of atmospheric composition on Mars [202]. Crucial to the realisation of robust human habitation of extraterrestrial environments such as the Moon will be in recycling of scarce nutrients CNPSK etc through CELSS technology supplemented with an industrial ecology that can supply a restricted set of indigenous elements.

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