Nitrogen cycling in Bioregenerative Life Support Systems: challenges for waste refinery and food production processes

Peter Clauwaert [a], *Maarten Muys* [b], *Abbas Alloul* [b], *Jolien De Paepe* [a], *Amanda Luther* [a],

Xiaoyan Sun [a], *Chiara Ilgrande* [a], *Marlies E.R. Christiaens* [a], *Xiaona Hu* [a], *Dongdong Zhang* [c],

Ralph E.F. Lindeboom [ad], *Benedikt Sas* [e], *Korneel Rabaey* [a], *Nico Boon* [a], *Frederik Ronsse* [c],

Danny Geelen [f], *Siegfried E. Vlaeminck* [ab]

a Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Gent, Belgium

b Research Group of Sustainable Energy, Air and Water Technology, Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

c Department of Biosystems Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

d Section Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, Stevinweg 1, 2628CN, Delft, The Netherlands

e Department of Food Quality and Food Safety, Ghent University, Coupure links 653, B-9000 Gent, Belgium

f In Vitro Biology and Horticulture lab, Department of Plant Production, Faculty of Bioscience Engineering, Ghent University, 653 Coupure Links, 9000 Gent, Belgium

18   Corresponding author: peter.clauwaert@ugent.be; Tel: +32 9/264 59 78; Fax: +32 9/264 62 48. Coupure

19   Links 653, 9000 Gent, Belgium

20   Abstract

21   In order to sustain human life in an isolated environment, an efficient conversion of wasted

22   nutrients to food might become mandatory. This is particularly the case for space missions where

23   resupply from earth or in-situ resource utilization is not possible or desirable. A combination of

24   different technologies is needed to allow full recycling of e.g. nitrogenous compounds in space.

25   In this review, an overview is given of the different essential processes and technologies that

26   enable closure of the nitrogen cycle in Bioregenerative Life Support Systems (BLSS). Firstly, a

27   set of biological and physicochemical refinery stages ensures efficient conversion of waste

28   products into the building blocks, followed by the production of food with a range of biological

29   methods. For each technology, bottlenecks are identified. Furthermore, challenges and outlooks

30   are presented at the integrated system level. Space adaptation and integration deserve key

31   attention to enable the recovery of nitrogen for the production of nutritional food in space, but

32   also in closed loop systems on earth.

33

34   Keywords

36

37

38

39

1. Introduction

At present, human life in space flights and in the International Space Station (ISS) is guaranteed by a regular resupply of food and water. However, in order to explore deep space with long-term missions and space habitation with increasing crew size, resupply from and return of waste to earth becomes difficult because of the long transport time and high costs associated with mass and volume restrictions for transportation [1-3]. The mass requirements of 5500-12800 kg per crew member per year for Open Life Support Systems (OLSS) without recycling can be lowered to 340-470 kg per crew member per year in a Physicochemical Life Support System (PLSS) (e.g. ISS) by in situ generation of oxygen and water recycling, consuming ~300 W per crew member [1]. The current launch cost advertised by SpaceX service is about $12600 per kilo [1]. In order to further decrease this payload mass and costs for resupply for long-term exploration or permanent habitation in life support systems, in situ food production has been proposed. Such systems are called Bioregenerative Life Support Systems (BLSS) or Closed/Controlled Ecological Life Support Systems (CLSS) [4].

Certain physicochemical methods for recovery of water and air have been developed for use in PLSS and are currently in use at the ISS, but technologies for food production based on nutrient recovery are neither validated nor available for space deployment. On earth, we rely on a vast set of biological production systems to produce food, mainly based on plants and animals, which are in essence based on inorganic nutrients often supplied as fertilizers [5]. In BLSS research, the development of an engineered bio-based system for food production has been investigated by

60 major governmental space research agencies for the past half century [6-9]. The shared focus of

61 these BLSSs has been the integration of different biological and physicochemical technologies

62 for the breakdown and conversion of waste products into useful building blocks for plant food

63 production in a closed material recycle. Nitrogen is a critical nutrient for this cycle, and will be

64 the focus of this review. Processes for nitrogen retention, recovery, and resupply in a closed

65 system will be considered here.

66

2. Refinery stages: converting waste into a fertilizer

68 There is currently no reuse of nitrogen on the International Space Station (ISS). Fecal material is

69 collected, stored and returned to earth without nutrient recovery, while fresh urine is treated by

70 chromium trioxide and sulfuric acid dosing to avoid microbial growth, which would convert urea

71 into volatile and potentially harmful ammonia. Water is recovered from pretreated urine by

72 vapour compression distillation (VCD) through which nitrogen ends up in a brine which becomes

73 a nitrogen dead end as well [10].

74 Human activity and crop production in a BLSS result in the production of different types of

75 organic wastes, all containing nitrogen. A dietary protein intake of 0.8-1.5 g protein kg^{-1} body

76 weight for a crew member with a body weight between 65 and 85 kg is expected to result in a

77 urinary excretion of between 7 and 16 g N d^{-1} (assuming ~16% N in proteins and ~80% N-

78 excretion via urine [7, 11, 12]). Fecal nitrogen excretion is typically in the order of 1-2 g N d^{-1}

79 [13] (Figure 1). Based on the assumptions of Hu *et al.* [8], 5-6 g N d^{-1} per crew member would be

80 collected as inedible biomass (crop residues, kitchen waste) in the proposed BLSS and ~1 g N d^{-1}

81 would be collected as epithelial associated organic waste (hair, nails, saliva solids, dead skins

82   cells, …). In order to make this nitrogen available again for the production of food in a BLSS,

83   these waste streams need to be treated to produce fertilizers adapted to the specific needs of the

84   food production processes. Different technologies have been proposed over the past decades for

85   BLSS (Figure 2) for conversion of organic wastes into carbon dioxide, water and nutrients. For

86   recovery of this nitrogen in a bioavailable form, three main strategies can be distinguished:

87   biological or physicochemical ammonification, and nitrification.

88

2.1   Biological ammonification

90   Most of the nitrogen in the organic waste streams in BLSSs is bound in organic compounds.

91   Although using organic nitrogen (amino acids, urea) for plant production may have

92   biostimulatory effects [14-17], providing inorganic nitrogen (ammonia and nitrate) is often

93   preferred as it allows online monitoring and control of the nitrogen loading and uptake by crops

94   in hydroponic systems and by bacteria in bioreactors for microbial protein production. The first

95   step in converting this organic nitrogen to the desired form for food production is biological

96   ammonification of the organic waste (Figure 2): proteins and peptides are converted into amino

97   acids by proteases produced by living organisms, while amino acids and other amide containing

98   molecules can be hydrolyzed by amidases to form ammonia. Urea ($CO(NH_2)_2$), which contains

99   more than 90% of the nitrogen in fresh urine [18], can be ammonified by the widespread enzyme

100 urease or by urea amidolyase [19].

101 In several concepts of the BLSS, microbial hydrolysis of organic waste occurs in a dedicated

102 aerobic [8, 20-23] or anaerobic [24-26] bioreactor. Besides hydrolysis of organic compounds,

103 biological ammonification and the release of other nutrients from the organic matrix is

104 established with the help of microorganisms. In the current concept of the BLSS of the European

105 Space Agency (ESA), the 'Micro-Ecological Life Support System Alternative' (MELiSSA),

106 organic waste is fermented in a thermophilic anaerobic membrane bioreactor at pH 5.3 to inhibit

107 methanogenesis and to maximize the formation of volatile fatty acids (VFA). Typically, between

108 18 and 71% of the organic nitrogen in the waste (plant residues and fecal material) could be

109 converted into ammonium at a rate between 17 and 30 mg NH_4^+-N L^{-1} d^{-1} in this waste treatment

110 compartment [27-30].

111 The main challenges in aerobic ammonification systems are the difficulty of diffusion driven

112 aeration under microgravity conditions and the high microbial sludge yield growth under aerobic

113 growth. The lower sludge yield under anaerobic conditions is advantageous on one hand, but the

114 separation and downstream processing of the gases that are produced (methane, hydrogen) is

115 challenging on the other hand. Additionally, if nitrate rich organic crop residues are treated in

116 anaerobic conditions, nitrate could be reduced to ammonia via dissimilatory nitrate reduction but

117 denitrification, resulting in gaseous nitrogen losses, is likely to occur.

118 Bioreactors with immobilized urease enzymes have been proposed to convert the urea in human

119 urine into ammonia and carbon dioxide for space applications [31]. Nicolau *et al*. [32] combined

120 this concept with electrochemical oxidation of the ammonia for electrical power production.

121

2.2 Physicochemical ammonification

123 Hydrolysis of nitrogen-containing organic compounds can also be achieved by means of

124 physicochemical processes. Hydrolysis of proteins occurs under acidic and alkaline conditions,

125   but also the use of microwave radiation, high pressure and enzymatic treatment as well as

126   combinations of such treatments have been described [33, 34].

127   Hot water conversion is one commonly tried method to physicochemically hydrolyze nitrogenous

128   compounds. Hydrolysis in hot water is only effective at temperatures above 140-160°C (and at

129   corresponding pressures to keep water in a liquid state), as below these temperatures only

130   denaturation and insolubilisation of proteins occur [35-37]. Hydrolysis of proteins into amino

131   acids has the highest yield in the temperature range of 200 to 290°C [38-40]. When water is

132   heated to just below its critical point (T_c = 374 °C and p_c = 22.1 MPa [41, 42]), its ionic product

133   will rise from 10^{-14} at ambient temperature, to 10^{-11} at near-critical conditions [43]. As a

134   consequence, the higher concentration of protons and hydroxyl ions from the dissociation of

135   water will lead to a higher extent of acid and base catalyzed reactions with nitrogenous organic

136   compounds [44]. Further increasing the temperature in the sub-critical region (i.e. between 250

137   and 374°C) will enhance deamination reactions of the amino acids as intermediates, yielding free

138   NH_4^+ and carboxylic acids [45, 46], although competing polymerisation reactions such as amide

139   formation and Maillard type reactions (i.e. reactions with sugars) [47, 48] could occur.

140   With respect to BLSS, Lissens *et al.* [27] reported that 95-100% of all nitrogen present in the

141   biosolids from an anaerobic digester could be converted into water-soluble components through

142   hydrothermal degradation (~350°C and ~240 bar). The anaerobic digester was fed with a mixed

143   organic stream (food crops, fecal material, algae) resembling a concentrated organic waste stream

144   produced by humans. About 60% of the nitrogen in the effluent of the hydrothermal unit could be

145   identified as NH_4^+-N and NO_3^--N, while the remaining fraction was assumed to be other oxidized

146   and solubilized nitrogen species.

147   The decomposition of the organic compounds contained in the waste streams may be enhanced in

148   hydrothermal conversion processes by the addition of oxidizing agents such as air, pure O_2 or

149   hydrogen peroxide. Below the critical point of water with air as an oxidizing agent, the process is

150   termed 'wet air oxidation' and can result in 90% conversion of organic nitrogen into mainly

151   ammonia and N_2 (at ~275°C and ~103 bar) [49, 50]. Johnson and Wydeven [51] reported an

152   increase in the formation of N_2 gas with increasing temperature (224°C to 300°C). Catalysts may

153   be added to increase selectivity towards N_2-gas formation. Thu and Michele [49] used Ru/TiO_2 to

154   achieve a selectivity to N_2 and nitrate of 85% and 15%, respectively after 22 h, while in the

155   presence of Pt/TiO_2 the selectivity to N_2 was 89% after 8 h from model distillery wastewater

156   feed. For BLSS, the Kudenko process has been developed which is an electrochemical wet

157   oxidation process, using hydrogen peroxide as oxidizer, to treat organic waste (inedible crop

158   residue, urine, feces and grey water) at 90°C and ambient pressure [23, 52]. When treating a

159   mixture of crop residues and urine, about 53% of the nitrogen could be recovered in the effluent

160   as soluble nitrogen, mainly under the form of ammonium but also nitrate and nitrite were

161   generated [52].

162   Finally, the oxidation of wet organic residues can also be carried out in supercritical water, the

163   so-called supercritical water oxidation (SCWO) process. Therein, nitrogen-containing organic

164   compounds are converted to CO_2, ammonia, nitrate, nitrite, N_2 and N_2O [53, 54]. In SCWO, the

165   oxidation of the ammonia as an intermediate is a rate-limiting step, hence ammonia is considered

166   recalcitrant. Temperatures above 500°C are needed to initiate the oxidation of NH_4^+ [55] in the

167   presence of excess oxidizer [56, 57]. Up to temperatures of 650°C, ammonia is still being formed

168   in SCWO effluents despite its oxidation. Compared to (non)catalytic wet air oxidation, SCWO

169   reactions are typically completed in less than 1 min.

170

2.3   Nitrification

Although in some cases urea and ammonia can be taken up directly by plants and microorganisms grown for food production, it can be preferable to convert urea and ammonia, at least partially, to nitrate in a BLSS [25]. In confined spaces, the occurrence of liquid streams with high ammonia concentrations is considered a hazard as ammonia volatilization increases with an increasing pH and temperature, and can accumulate to toxic levels in the atmospheric compartment. Moreover, high ammonia concentrations resulting from failing or inadequate dosage can become toxic as well to the plants and microorganisms, even when the pH is controlled [26, 58] and high levels of ammonium can inhibit the uptake of key minerals in hydroponic solutions [25]. Nitrate, on the other hand, is not volatile and is not considered to be toxic in the concentrations that are expected and can accumulate in the leaves in case of a high nitrate supply [25], although this oxidized form of nitrogen is energetically less favorable for edible protein production.

The biological process in which ammonia is aerobically oxidized to nitrite or nitrate is called nitrification. Full nitrification to nitrate requires a significant amount of oxygen (theoretically 4.57 mg O_2 mg^{-1} N) and its effective distribution to the nitrifying microorganisms (e.g. > 1 mg O_2 L^{-1}) is crucial for minimizing the formation of unwanted byproducts such as nitrite, free nitrous acid (HNO$_2$), nitrous oxide (N$_2$O) and dinitrogen gas (N$_2$).

The first step (nitritation) consists of the oxidation of ammonia over hydroxylamine (NH$_2$OH) to nitrite (NO$_2^-$) and is catalyzed by sequential action of ammonium mono-oxygenase (AMO) and hydroxylamine   oxidoreductase   (HAO).   Nitritation   is   typically   performed   by

192 chemolithoautotrophic ammonia oxidizing bacteria (AOB) and archaea (AOA). Some

193 heterotrophic organisms are also able to oxidize ammonium to nitrite, but without energy

194 conservation [59]. The second step (nitratation) is the oxidation of nitrite to nitrate (NO_3^-) by

195 nitrite oxidizing bacteria (NOB), catalyzed by nitrite oxidoreductase (NXR). Complete ammonia

196 oxidation (comammox) to nitrate in one organism has only recently been described [60] but it is

197 not clear yet whether it will be applicable for a BLSS where a nitrification system with a high

198 volumetric conversion rate is recommended.

199 Proton production during nitritation requires a buffering capacity of 71 meq (mg N)$^{-1}$ to maintain

200 the pH. If the buffer capacity of the medium is not sufficient, only partial nitrification can be

201 expected [61, 62], unless pH control with base addition is foreseen [63]. Biological nitrification

202 activity is typically not observed if the pH drops below 5.5-6.0, although specific AOB adapted

203 to low pH have been described to oxidize ammonium down to pH 2.6 [64].

204 For BLSS, nitrification systems with an open, mixed microbial community are typically proposed

205 [62, 63, 65-68]. On one hand, these self-organizing microbial communities can evolve and adapt

206 to changing conditions and microbial invasions, which increases the robustness of the system. On

207 the other hand, such complex microbial communities and interactions are difficult to capture in a

208 mechanistic mathematical model, which might be required for space application where a high

209 level of predictability is desired. Also, the use of a microbial community with unknown species,

210 which might be pathogenic, is highly undesired in confined spaces as it presents health hazard to

211 the crew members of a BLSS.

212 For this reason, in the MELiSSA loop, nitrification of inorganic streams is envisaged to be

213 carried out by an 'axenic' synthetic co-culture of the AOB *Nitrosomonas europaea* ATCC 19718

214   and of the NOB *Nitrobacter winogradskyi* ATCC 25391 [69]. Given the slow growth of these

215   bacteria (maximum specific growth rate is about 1.6×10^{-5} s^{-1} for *Nitrosomonas* and 1×10^{-5} s^{-1}

216   for *Nitrobacter*), the biomass is immobilized on carriers to minimize washout. The complexity of

217   the reactor construction and operation increases when such synthetic co-cultures are used, but the

218   specific conversion rates obtained (1.7-1.9 g N m^{-2} d^{-1} or 0.55-0.59 g N L^{-1} d^{-1}[69]) are in the

219   range of terrestrial biofilm-based wastewater nitrification systems using an open, mixed

220   microbial community.

221   As a significant fraction of the nitrogen in the waste of a life support system will be present in the

222   human urine, recovery of this nitrogen is a prerequisite. Besides organic nitrogen (~5-8 g N L^{-1})

223   and inorganic nitrogen (~0.4 g N L^{-1}), fresh urine contains organic compounds (~9 g COD L^{-1})

224   and elevated levels of salts (~21 mS cm^{-1}). As the uncharged urea gets hydrolyzed, ammonium

225   and bicarbonate ions are being formed which further give rise to very high electrical conductivity

226   levels (> 70 mS cm^{-1}), especially if base (e.g. NaOH) is added to stabilize the pH. It has recently

227   been shown that complete nitrification can occur in a nitrification reactor fed with undiluted urine

228   at this level of electrical conductivity [63]. In case a synthetic co-culture is used for urine

229   nitrification, specific heterotrophic strains will have to be introduced as well to oxidize the

230   organic compounds.

231   One of the challenges of applying nitrification in BLSS in space, is the survival and storage of the

232   strains during launch and space flight. Two recent experiments demonstrated that nitrifying pure

233   strains, synthetic co-cultures as well as mixed nitrifying microbial communities could

234   successfully be reactivated after spaceflight in low earth orbit (Ilgrande *et al*. in preparation).

235   Reactivation of denitrification and anaerobic ammonium oxidation activity could also be

236   demonstrated after a space flight in orbit. A nitrification biofilter on lava grains was incorporated

237   in the C.E.B.A.S. mini module in which an animal compartment with fishes and snails was

238   combined with a plant compartment with rootless water plants [70]. During the STS-89 flight

239   experiment, which lasted 9 days, the chemical water parameters remained within the limits of

240   what was considered to be a good water quality [71]. During other short term experiments (up to

241   17 days) flown on IML-2 (STS-65 – AAEU facility) and on the Neurolab missions STS-90 and

242   STS-95 (VFEU for marine fish), nitrification could be demonstrated in the bacterial filter of a

243   space aquarium to convert the ammonia excreted by fish into nitrate [72]. To enable long-term

244   experiments (up to 90 days) in the ISS, the 'Aquatic Habitat' (AQH) is being developed to

245   combine ammonia oxidation with heterotrophic denitrification to maintain both ammonium and

246   nitrate concentrations low for an optimal water quality in the aquarium.

247   Another challenge for the application of nitrification in microgravity conditions, which

248   significantly affects fluid dynamics [73-75], is the reduced convection and strong cohesion forces

249   in space which make efficient gas-liquid interactions no longer possible with major consequences

250   for aeration [73, 75, 76]. Due to the lower diffusion rate of oxygen in water, the oxygen transfer

251   rate might become problematic. Therefore, an aerated rotating membrane bioreactor system

252   (ARMS), has been developed at Kennedy Space Center. It contains rotating hollow fiber

253   membranes for the enhanced diffusional transfer of oxygen, which would avoid gas-liquid

254   interactions under microgravity conditions [76, 77].

255   Besides biological nitrification, chemical nitrification is also possible by combining pure

256   ammonia with oxygen at 700-800°C in the presence of a catalyst (Platinum with 10% Rhodium;

257   Ostwald process) to produce nitric acid (HNO_3) [78]. Also, ammoniumnitrite which can be

258   biologically produced at low pH values, spontaneously decomposes into N_2 and H_2O, while nitrite

259   can also be chemically oxidized, e.g. nitrous acid (HNO_2) self-decomposition into nitrate [64].

260

3. Production stages: from fertilizer to protein

Human daily dietary requirements have been estimated several times leading to varying dietary recommendations. Most of the differences in food requirements are attributed to the parameter settings regarding initial body weight, age, gender, physical activity etc. The major part of the energy supply in the food is typically derived from carbohydrate sources. The requirement for moderately active females (19–30 years old) is 1500–2500 kcal, while males of the same age need 2500–3300 kcal. Energy is also extracted from protein and lipids but this should be limited (for protein about 10%) as these are less favorable energy sources and are mainly necessary for replacing metabolic building blocks that cannot be autonomously synthesized. Indeed, nine amino acids found in human tissues must be supplied by dietary sources that need to be digested in our intestinal tract. It is therefore not recommended to target a certain daily protein intake as such, but instead focus on the quality of the protein. The US Food and Drug Administration and the Food and Agricultural Organization of the United Nations (FAO/WHO) have adopted the Protein Digestibility Corrected Amino Acids Score (PDCAAS) to assess this [79]. Based on this method, there is strong variability between different protein sources. As astronauts will have to adopt a rather monotonous diet based on non-animal sources, special care must be taken to ensure certain limiting essential amino acids and vitamins are provided. However, Young and Pellett [80] demonstrated that a mixture of plant proteins can serve as a well-balanced and complete source of amino acids to effectively meet human dietary requirements. As such, it can be hypothesized that a clever blend of plant, fungal and microbial proteins could meet all nutritional requirements for crew members in space.

282 As a result, the production of animal protein is not strictly necessary in a BLSS and a vegan diet

283 is most likely to be adopted in an eventual self-sustaining BLSS in space. Moreover, the low

284 conversion efficiency from feed to meat would entail an excessive need for feed production

285 whereas the requirements for volume, mass, nutrients, energy and water demand in a BLSS

286 should be minimized. Furthermore, the difficulties with waste management (e.g. defecation,

287 slaughter, …) in reduced gravity for typical tetrapod meat sources (chicken, goat, pig, …) might

288 be impossible to solve [70]. There might also be ethical objections against husbandry of sentient

289 animals in confined spaces, but this discussion is outside the scope of this review.

290 Breeding fish as a source of animal protein for human consumption in combination with the

291 cultivation of edible higher aquatic plants which float or buoy (e.g. hornweeds and duckweeds)

292 and waste degrading microorganisms has been proposed by Blum *et al*. [70, 81] since

293 conceptually oxygen transfer and partial nutrient recovery can occur without phase state changes

294 (gas-liquid).

295 The conversion of inedible organic waste residues by edible worms (e.g. mealworms, silkworms,

296 earthworms) has not only been proposed to generate 'soil-like substrates' (SLS) or 'biohumus'

297 for plant production [82], but also as a source of animal protein in BLSS [8, 14, 23, 24, 83]. In

298 this review we will focus only on the protein production through plants, microorganisms, and

299 fungi.

300

301 ## 3.1   Food production based on plants

302   Researchers and space agencies have historically focused on the cultivation of higher plants to

303   produce food for crew members in future BLSS [8, 23, 84, 85]. On a physiological level,

304   ammonium is required for synthesis of amino acids and proteins as it is directly incorporated into

305   the glutamate – so whatever the source, the plant must be able to ultimately convert it to

306   ammonia. Nitrogen is typically taken up from the environment as nitrate, ammonium or urea by

307   plants, but alternative forms of nitrogen can be used that include mostly organic forms [86].

308   Preference towards either form may exist for specific plants, but usually ammonium and urea are

309   used during early crop development and nitrate during later stages. In soil, microorganisms

310   rapidly mineralize the organic matter and thereby release nitrate and ammonium. When applied

311   in conventional hydroponic systems, organic fertilizers are known to cause growth inhibition and

312   have phytotoxic effects. Plant growth chambers for life support systems use conventional

313   hydroponic cultivation systems and are, therefore, not suitable for the direct use of organic

314   fertilizers. Another complication associated with organic fertilizers is that the dosing cannot be

315   controlled to accommodate the nitrogen requirement of crop plants throughout the growth cycle.

316   Usually high nitrogen levels are required during the early growth phase while lower levels near

317   the harvesting time promotes greater yields [87].

318   Direct addition of human urine, which harbors the majority of nitrogen in a BLSS (Figure 1), to

319   hydroponic systems has been proposed and evaluated [84, 88-90]. The major problems with

320   direct urine addition include phytotoxic effects of high ammonium, problems relating to organic

321   compounds, and stress caused by the high salt concentration in urine. Several strategies have

322   been proposed to cope with the high salinity. Dilution can be used to maintain the salt level

323   below a specific threshold, but on the longer term salt accumulation might still occur in

324   hydroponic systems if the plants don't take up these salts at the same rate as other nutrients in the

325   urine. Other strategies that have been proposed include using halophytes [91], or maintaining a

326   low salt diet for the crew members to reduce the salinity in the urine in combination with plants

327   that can accumulate salt in their leaves [92]. Physicochemical techniques that have been proposed

328   for salt removal include hydrothermal treatment of the urine [93] and electrodialysis of irrigation

329   water [91, 94].

330   Given these issues, to reach optimal crop growth, hydroponic systems should be supplied with

331   fully synthetic mineral mixtures which can be monitored and adjusted in accordance to the

332   system requirements. Ammonium and nitrate are usually supplied as a mixture since ammonium

333   uptake acidifies while nitrate basifies the nutrient solution. The amount of ammonium added to

334   the nutrient solution should not exceed the absorption and storage capacities of the plant because

335   excess ammonium causes cell damage. Most plants can directly take up urea but metabolize it

336   with varying efficiencies, depending on the species. Under field conditions, urea is converted into

337   ammonium by urease, a common enzyme in soil microorganisms, while under hydroponic

338   conditions this does not occur and urea has been shown to be a very poor nitrogen fertilizer [95].

339   The total amount of nitrogen taken up by a plant depends on various environmental factors, two

340   of the most important being concentration and dose of nitrogen that is supplied. Indeed, shoot

341   biomass strongly increases with a generous dose of nitrogen. Under field conditions, the nitrogen

342   cycle is strongly influenced by ammonium evaporation and leaching and to some extent to biotic

343   nitrogen fixation and atmospheric deposition. There are ample studies on nitrogen use efficiency

344   (ratio of grain yield over available N) in wheat under field conditions as it is an important

345   economic factor and determines end-use quality. In a recycling hydroponic system, volatilization

346   of ammonium can be kept to a minimum either by closing the nutrient solution circuitry or by

347   supplying nitrogen as nitrate. Beyond nitrogen, different plant species show variation in mineral

348 uptake efficiency, which may cause salinity issues in hydroponic systems. To compensate for

349 this, a life support food production system should include a variety of crops, collectively

350 cultivated with the same hydroponic solution such that optimal mineral use efficiency is attained.

351 Next to the cultivation requirements imposed by the choice of crops, food production should

352 fulfill the needs of the crew. Continuous supply of fresh produce is achieved by a staggered

353 cultivation scheme whereby harvesting is possible on a daily basis. Although this approach offers

354 advantages in terms of low storage requirements and preservation of food quality, estimations on

355 crop production requirements usually assume one crop per plant growth unit, and batch culturing

356 [96]. Biomass and food production levels are strongly dependent on the type of crop and,

357 consequently, the cultivation area is largely determined by the food quality requirements. A daily

358 consumption of 3000 kcal for a male astronaut executing moderate physical activities can be

359 provided by a balanced diet of macronutrients of 70% carbohydrates, 10% protein and 20% fat.

360 The majority of the carbohydrates are likely going to come from wheat and potatoes. Wheat

361 grains are high in starch content (60-70% carbohydrate), and, therefore, ideal to provide energy.

362 Potato tubers are also high in starch (85% of the dry matter), but, in addition, are rich in minerals

363 and vitamins and therefore often combined with other carbohydrate sources such as wheat. A

364 suitable crop composition, however, has to provide nutritional complementarity and variety,

365 which can require combining more than 10 crop species [97]. Balancing cultivation requirements

366 and yield potential of different crops with human nutritional requirements is a complex problem

367 for which different outcomes have been calculated, in terms of minimizing cultivation area,

368 optimizing biomass production rates, crop selection, etc. [98]. Typically, the required surface area

369 for higher plant production for one crew member has been assumed to be in the order of 40-50 m²

370 [8]. However, Do *et al*. [99] have postulated that, depending on the desired variety of crops, 46-

371   117 m² would be a more realistic estimate for a 3040 kcal person^{-1} d^{-1} plant based diet. As a

372   comparison, Cassidy *et al*. [100], estimated that approximately 1000 m² would be necessary to

373   provide one person with a plant based diet on earth (2700 kcal^{-1} person d^{-1}, 41 different crops),

374   not accounting for animal feed, biofuels, nor other non-food products. It can be assumed that the

375   scale of a higher plant compartment will have a great impact on the feasibility of realizing a

376   future closed loop BLSS for the first time. Therefore, first plant production systems for human

377   consumption in the near future will most likely cover only a small fraction of the diet,

378   supplemented with terrestrial resupply.

379   With these different opinions on the required cultivation area in mind, a general estimation of the

380   nitrogen consumption by the cultivated crops is equally difficult to assess with current

381   information. Nonetheless a rough estimation can be made. Considering an average productivity

382   between 10 and 200 g fresh weight m^{-2} day^{-1} [8, 101], and assuming that about 0,5% of fresh

383   green parts of the plant consists of N, the daily consumption of nitrogen per m^2 is estimated to be

384   5 to 40 grams. In case a LED based artificial lighting system is used (~2200 kWh electricity kg^{-1}

385   dry weight, calculated from Do *et al*. [99]), several layers of crops can be stacked in a higher

386   plant growth chamber, so an average specific protein production rate would be, depending on the

387   crop, in the order of 0-4 kg protein m^{-3} year^{-1} (calculated from Do *et al*. [99]).

388

3.2   Food production based on microorganisms and fungi

390   The term 'single cell protein' (SCP) has been coined to describe whole cells of microalgae,

391   bacteria and unicellular fungi (such as yeast) intended for consumption for nutritional purposes

392   [102, 103]. Next to SCP, multicellular fungi are an interesting source of protein due to their

393   versatility in substrate usage. This feature makes fungi applicable in recycling the inedible lignin

394   rich biomass of higher plants in BLSS [14, 82, 104].

3.2.1   Bacterial and microalgal single cell protein production

396   SCPs are highly attractive for protein production on earth as well as in space because they can be

397   produced with highly efficient use of resources. Indeed, microorganisms can be highly efficient

398   in their nutrient conversions, and thus near full recycling of the nitrogen and phosphorus from a

399   recovered stream is feasible, depending on their ratio. One important feature of SCPs is their high

400   volume or surface specific productivity rates, as high as 1.5 kg dry weight m^{-3} d^{-1} for microalgae

401   cultivation in indoor photobioreactors [105] and 1.45 kg dry weight m^{-3} d^{-1} for purple non-sulfur

402   bacteria (PNSB) cultivated in outdoor photobioreactors [106], resulting in a compact type of

403   engineered food production system. In case artificial light is necessary (e.g. LED), the electrical

404   energy demand for lighting can be in the order of 180 kWh kg^{-1} dry weight (assuming 135 μmol

405   m^{-2} s^{-1}; 1.1×10^{-2} kg dry weight m^{-3} h^{-1}; 25 m^{-1} [107] and 1.7 μmol J^{-1} [108]). Finally, given their

406   wide range of metabolic capacities, they are able to use various sources of carbon, energy and

407   electrons, offering multiple options to plug them into resource recovery loops. Microbial biomass

408   has the highest protein content among organisms (50-70% DW) and offers additional functional

409   benefits in terms of vitamins, pigments and potentially prebiotic compounds [102, 103].

410   SCP is facing three important challenges that must be overcome to further improve its cultivation,

411   nutritional value and dietary consumption. Firstly, producing a semi-solid food ingredient from

412   microbial biomass requires some high energy processing steps. In photobioreactors for instance,

413   biomass levels are around 1 g dry weight L^{-1} [109], hence 99.9% of the reactor content consists of

414   water. Typical techniques to concentrate the biomass are energy intensive, including filtration,

415 centrifugation (~2 kWh kg^{-1} dry biomass [110]) and drying [111]. Secondly, microorganisms

416 contain the highest levels of nucleic acids (DNA+RNA) among all organisms, about 15-16% of

417 the dry weight [103], mostly in the form of RNA. For humans, consumption above 2 g per person

418 per day can lead to gout and kidney stone [112]. Due to these health-related issues result in the

419 fact that only 40-50% of the protein intake can be substituted by microbial protein [113, 114].

420 Although this is already a considerable portion, various physical and chemical post-treatment

421 techniques can lower the SCP RNA levels increasing their usage potential in a healthy diet. A last

422 challenge, and often overlooked challenge is that of human acceptance of microbial food

423 products. Humans already consume items produced by microorganisms that often also contain

424 residual microorganisms (e.g. beer, cheese, yoghurt…), yet microorganisms as a main food

425 ingredient remains a novel idea, and one that consumers may find repugnant. Unappealing flavor

426 and texture of food containing microorganisms are likely causes of this distaste. In long-term

427 spaceflight, it is known that the psychological well-being of the astronauts is partially depending

428 on food flavor and variety [115]. In addition, possible rejection and concerns about product safety

429 can be linked with psychological aspects of consuming a novel food that is produced with

430 innovative technologies.

431 The microorganisms *Arthrospira platensis*, also known as *"Spirulina"*, and *Rhodospirillum*

432 *rubrum* have been studied in the MELiSSA concept as an alternative source of proteins [116]. *A.*

433 *platensis* biomass contains as much as 46-71% protein on a dry weight basis [117-119] and the

434 biomass is rich in vitamins, minerals, β-carotene, essential fatty acids, such as γ-linolenic acid

435 [120], and antioxidants [121]. The true protein digestibility of this microorganism is 75.5% [122],

436 which is fairly high. In terms of essential amino acids, cultivation can lead to a nutritionally

437 appealing composition for crew members, yet the opposite is also possible. Figure 3 displays the

438   variability of levels of individual essential amino acids, with a factor seven difference between

439   the minimum and maximum values, depending on cultivation conditions. Further optimization in

440   this area should be able to guarantee the protein quality. A practical advantage of *A. platensis* is

441   its relatively large size, as multiple cells (3-16 μm [121]) form spiral-shaped filaments (100-200

442   μm [123]) which can more easily be separated, for instance through filtration. Furthermore, this

443   cyanobacterium is one of the rare bacteria containing a relatively low nucleic acid concentration

444   (4-6% of dry weight [114]). For nutritional purposes, *A. platensis* is being cultivated at full-scale

445   on synthetic chemicals, while other applications have also explored the use of waste streams to

446   support growth [109, 124].

447   *A. platensis* fulfills a vital role in the photosynthetic compartment (IVa) of the MELiSSA loop

448   [116]. In this loop, *A. platensis* consumes nitrate in nitrified effluents, consumes CO_2 produced

449   by by the crew and in the other compartments, produces O_2 and provides a dietary protein source

450   for the crew members. Previous studies towards the growth of *A. platensis* on urine reported

451   success using highly diluted human urine [125, 126] and nitrified urine [127]. The high dilutions

452   used (10-30% dilution) however, indicates that those systems were operated at low salinities.

453   Coppens *et al*. [63] observed that relevant salt levels had no significant effect on its growth and

454   nitrate resulted in the most optimal growth compared to ammonium and urea as the sole nitrogen

455   source, provided that the nitrate concentrations did not exceed 1 g N L^{-1}. When growing a food

456   product on urine, particular attention needs to be directed towards safety with regards to

457   pathogens and micropollutants such as pharmaceutical residues [128].

458   Next to *A. platensis*, the cyanobacterium *Aphanizomenon flos-aquae* is grown commercially as a

459   food supplement [129]. This species, however, was never considered for use in a BLSS. Another

460   cyanobacterium, which lives in symbiosis with the edible water-dwelling fern Azolla, is

461 *Anabaena azollae*. Liu *et al*. [90] studied this organism as part of a BLSS that also included

462 biological purification of a urine solution using Azolla and a UV photocatalytic oxidation by

463 TiO_2. According to the authors, this treatment could produce effluent to meet standards for

464 drinking water quality.

465 Furthermore, the green microalgae *Chlorella vulgaris* is cultivated commercially and sold as a

466 food supplement. Although *Chlorella* contains a high protein content of 51-58% on a dry weight

467 basis, it also possess a robust cellulosic cell wall which makes human digestion difficult [119].

468 *Chlorella* has been proposed for O_2 generation in a BLSS rather than for food production. To

469 prevent accumulation of dead-end products in a BLSS, the biomass could be burned when the

470 CO_2 concentration is low, or fed to mealworms (*Tenebrio molitor* L.) [130]. Another use for

471 *Chlorella* in BLSSs was proposed by Li *et al*. [130] in which *C. vulgaris*, grown on human urine,

472 was used to control the balance of CO_2 and O_2.

473 *Rhodospirillum rubrum*, which has been proposed for compartment II of the MELiSSA loop,

474 belongs to the group of the purple non-sulfur bacteria (PNSB), and grows photoheterotrophically

475 on VFAs. As indicated by Godia *et al*. [116], PNSB represent an interesting yet unexplored

476 source of microbial protein for consumption. The potential for producing these bacteria on

477 fermentation effluent derives from their metabolic and physiologic features. Firstly, they have a

478 near perfect organic carbon uptake efficiency (when grown on volatile fatty acids) in comparison

479 with other organoheterotrophs, yielding a very high protein output per carbon input [131].

480 Secondly, PNSB also have a high growth rate with respect to photoautotrophs like microalgae,

481 leading to a more compact production system [132, 133]. Thirdly, their unique potential to grow

482 under specific infrared wavelengths adds a selectivity tool for non-axenic cultivation applications

483 [134, 135]. In terms of nutritional quality, the PNSB biomass composition has other appealing

484   features. Firstly, its methionine content, usually a limiting essential amino acid, is much higher in

485   comparison with other types of microbial protein. Secondly, the biomass contains a considerable

486   amount of essential vitamins (B12, B2, B6, C, E, D and folic acid) [136]. Finally, compounds

487   with health stimulating benefits are present, such as carotenoid pigments [113, 137, 138] and

488   poly-β-hydroxybutyrate (PHB) [139]. *R. rubrum* has been demonstrated to reduce for instance

489   LDL-cholesterol in mice [140]. Despite of several animal feeding trials, for instance by Banerjee

490   *et al*. [141], Shapawi *et al*. [142] and Kobayashi and Kobayashi [136], PNSB have not yet been

491   tested for human consumption, to the authors' knowledge. It is unclear whether full-scale

492   production facilities are terrestrially in place at this moment.

493   In the MELiSSA loop, the main challenge with the use of *R. rubrum* as a food source for crew

494   members is its proximity to Compartment I. As fecal matter, and the potentially associated

495   pathogens, enter Compartment I directly, it might be a challenge to maintain a sterile

496   fermentation effluent through membrane filtration at all times.

497   Besides the phototrophic protein production by bacteria or microalgae, the dark production of

498   single cell proteins for life support systems by chemolithoautotrophic bacteria has been proposed

499   by Hendrickx and Mergeay [143] and Verstraete *et al*. [144] for BLSS. Methane or hydrogen

500   formed in the BLSS (e.g. in anaerobic fermentor) or from outside the BLSS could be used as

501   energy source for non-phototrophic microbial food production. Hydrogen could be produced

502   from olivine found on Mars [143]. Matassa *et al*. [145] recently reported a hydrogen oxidizing

503   biomass production rate of 0.28 g dry weight g^{-1} COD-H_2 (81% H_2 conversion efficiency, 76%

504   protein content), so even if hydrogen would be generated through water electrolysis (e.g. 53 kWh

505   kg^{-1} H_2 [146]), the electrical energy demand for water electrolysis would be in the order 29 kWh

506 kg^{-1} dry weight, which is lower than the electricity demand for phototrophic growth with artificial

507 lighting systems.

508   3.2.2   Fungal protein production

509 In general, fungi contain a lower protein content (15-45% DW) in comparison with the earlier

510 discussed microalgae and bacteria. However, both unicellular and multicellular fungi are valued

511 for their ability to grow on a variety of lignocellulosic substrates. The exact composition of

512 hemicellulose, cellulose and lignin, will influence which fungi can be cultivated [103].

513 Gitelson *et al*. [147] could convert approximately 10% of the courser part of the inedible

514 biomass, which was ~45% of the total inedible biomass, into edible fungal biomass (*Pleurotis*

515 *florida*; protein content of 16%). When grown for 60-70 days on wheat straw, 6-7% of the

516 substrate could be converted into *Pleurotis florida* Fovose having a protein content of 23% [23].

517 Use of fungi in the biological waste treatment has typically been combined with other biological

518 treatment steps, e.g. organic waste digestion with worms and bacteria to produce 'soil-like

519 substrates' as an organic matrix for subsequent plant production [82]. He *et al*. [14] used

520 aerobically fermented residues of wheat and rice to cultivate oyster mushrooms (*Pleurotus*

521 *ostreatus*) and then earthworms. Although the degradation efficiency of cellulose and lignin

522 slightly increased from 98.6% to 99.5% and from 93.1% to 98.6% respectively by the addition of

523 oyster mushroom to the earthworm treatment, the produced soil-like substrate resulted in a lower

524 lettuce productivity afterwards and the authors did not consider all the oyster mushroom biomass

525 as food as the lignocelluloses in oyster mushroom cannot be digested well by humans.

526 One drawback in the production of fungi is the possible contamination with fungal species that

527 produce mycotoxins. Small amounts of these mycotoxins can potentially cause neurotoxicity,

528   allergies and rashes on the skin. The monitoring of invasive species and their potential toxins is a

529   prerequisite in producing fungal biomass [148].

530   To recycle the inedible biomass of higher plants in a BLSS, Strayer *et al*. [149] investigated the

531   production of the yeast *Candida ingens* on volatile VFAs, which were produced in a first step

532   using anaerobic digestion of potato crop residue. A low pH of 5 was maintained to favor growth

533   of the yeast while slowing bacterial growth. On average 0.4 g yeast biomass was produced per g

534   VFA consumed and no phytotoxic VFA were detected in the effluent, indicating their complete

535   removal.

536

537

538

539   ## 4. Nitrogen loss, fixation and in situ utilization

540   One of the main challenges of the nitrogen recycle in BLSS, is to avoid nitrogen losses. Main

541   points of loss in these systems can be identified. First, if organic waste is disposed or removed

542   from the BLSS after dewatering without further treatment nor recovery, the nitrogen fixed in this

543   organic material is not available anymore [10, 52, 84, 91, 150-155] (Figure 1, c1). Several other

544   nutrients can be recovered after incineration of the dried organic waste, but the nitrogen is

545   typically converted into N_2 and NO_x [22, 84, 151, 154-157] (Figure 1, c2) and lost in the process.

546   Depending on the temperature and pressure, hydrothermal treatment of organic waste also results

547   to some extent in the formation of N_2 and NO_x [50-52, 93, 150, 158] (Figure 1, c3). When nitrate,

548   e.g. from inedible crop residues, is introduced in an anoxic bioreactor in the presence of organic

549   compounds, denitrification is very likely to occur [25, 27, 62, 67, 77, 159] (Figure 1, c4). For

550   specific mission scenarios where complete nutrient recovery is not mandatory, the formation of

551   N_2 might be useful to help pressurizing the cabin [160], but not for a BLSS where a (near)

552   complete recycling of all the elements is envisaged. During nitrification and denitrification,

553   substantial N_2O formation might occur in case of specific process conditions (e.g. oxygen

554   concentration, nitrite concentration, COD/N ratio) [161], which can also become a potential

555   health hazard in a closed system.

556   Other examples of nitrogen losses that can occur in a BLSS include ammonia volatilization (e.g.

557   from urine at high pH), nitrous acid volatilization at low pH values, and struvite formation in

558   vessels or piping e.g. as a result of urea hydrolysis. Nitrogen can also be lost from the concentrate

559   of membrane filtration steps or the biosolids waste from various bioreactors if these streams are

560   not adequately recycled.

561   In case it is not possible or too complex to avoid the formation some of these nitrogen losses and

562   if terrestrial resupply is not possible or desirable, the introduction of a N_2 fixation step can be a

563   solution. Nitrogen fixation in BLSS can be established in a microbial or chemical way.

564   Biological N_2 fixation can occur in the root nodules of specific plants (e.g. legumine family) via a

565   symbiosis between nitrogen fixing bacteria and plants. It might be necessary to select bacterial

566   strains compatible with specific plants may need to be selected and inoculated to achieve a high

567   nitrogen-fixing efficiency [162]. However, biological nitrogen fixation requires a high energy

568   input (15-16 ATP per molecular N_2) and the nitrogen demand of plants cannot be fully satisfied

569   only by biological nitrogen fixation so nitrogen-fixing plants may be less productive compared to

570   those supplied with mineral nitrogen [162, 163]. Besides bacterial symbiosis with plants, free

571 living microorganisms such as *Azotobacter*, *Clostridium* and specific PNSB and cyanobacteria,

572 are capable of biological nitrogen fixation. Cyanobacteria that combine nitrogen fixation with

573 oxygenic photosynthesis in a dedicated bioreactor have been proposed for BLSS [164].

574 Nitrogen can also be fixed as ammonia in a chemical way by combining dinitrogen and hydrogen

575 gas in the presence of a catalyst at high temperature and pressure [93]. Whereas industrial

576 ammonia production typically occurs at 200-300 bar and 400-500°C (Haber-Bosch process) with

577 the energetic cost of 27 GJ t^{-1} NH_3, Sakamoto *et al*. [78] demonstrated a nitrogen fixing unit for a

578 Life Support System that could be operated at 7-8 bar and 300°C at a rate that was in the order of

579 the nitrogen metabolism rate of one crew member.

580 In specific environments where nitrogen may already be present (e.g. Mars), the in situ utilization

581 of nitrogen could be considered to compensate for nitrogen losses in a BLSS. Oxidized nitrogen-

582 bearing compounds were recently discovered in Martian sedimentary deposits, which indicates

583 the theoretical possibility to extract nitrate from Martian surface [165]. However, separating the

584 nitrogen from other, possibly toxic, compounds (e.g. perchlorate) might be challenging. To deal

585 with pressure losses of the habitat atmosphere caused by leakage and extravehicular activity from

586 habitat on Mars, Do *et al*. [99] suggested an atmospheric processor module to capture high purity

587 N_2 from the CO_2-rich Martian atmosphere with the utilization of a zeolite membrane filter and

588 cryocooler.

589 ## 5. Broader challenges for nitrogen cycling in BLSS

590 Besides crew members, a BLSS contains by definition living organisms, at least for food

591 production and, depending on the BLSS outline, possibly also for waste processing. In space,

592 living organisms are subjected to extreme and hostile conditions including broad ranges of

593 gravity and ionizing radiation, both factors known to have a profound effect on the physiology,

594 morphology and functionality [73, 166-168]. Without sufficient protection (shielding), radiation

595 can induce random DNA damage and mutations, possibly causing alterations in gene expression,

596 which can be detrimental for the growth and survival of microorganisms and plants [73, 74, 169,

597 170]. Exposure to variations in gravity, ranging from 'hypergravity' during vehicle launch (3.2 g)

598 and reentry (1.4 g), to reduced gravity at the lunar (0.17 g) and Martian (0.38 g) surface and even

599 microgravity during orbital flight ($\sim 10^{-6}$ g), brings about mainly indirect effects linked to the

600 altered extracellular fluid properties affecting the nutrient acquisition and waste removal [73, 74,

601 166, 167, 170-172]. In case extraterrestrial habitation becomes possible, genetic evolution of

602 terrestrial species into novel extraterrestrial species is likely to occur over time and the different

603 levels of gravity and the specific composition of the radiation penetrating the shielding of a BLSS

604 in space might affect this process in a different way than on earth. As a result, in depth research

605 on the long-term effects of radiation and microgravity on living organisms in a BLSS in general,

606 and on nitrogen metabolism specifically, will be mandatory for permanent extraterrestrial

607 habitation.

608 Besides selecting and optimizing the different subprocesses in a BLSS for nitrogen and other

609 elements, the integration and real time control over the different processes is the final hurdle still

610 to overcome to allow for the realization of a BLSS for reuse of nitrogenous compounds for food

611 production in space. As an example, the integration of the MELiSSA compartments is being

612 thoroughly tested in the MELiSSA Pilot Plant facility, located at the Universitat Autònoma de

613 Barcelona [173, 174]. Moreover, each system developed for space should eventually comply with

614 the basic rules for space design, including limited volume and mass, low consumable and energy

615 input, a high level of automation to limit the workload of the crew and a high level of robustness,

616 reliability and safety.

617

6. Outlooks

619 When does a BLSS, with at least partial food production, become favorable for space missions?

620 Olson *et al*. [175] estimated that the cumulative cost and mass savings for a BLSS with 50-97%

621 food production compared with a PLSS (in situ water recycling and O_2 production, terrestrial

622 food supply) start to pay of between 1 and 12.9 years depending on the mission scenario. Do and

623 Owens [99] assessed the technical feasibility of the 'Mars One' mission plan (missions with 4

624 crew members every 26 months for a one way trip) and evaluated the payload requirements for

625 supplying terrestrial produced food versus in situ food production via BLSS. They concluded that

626 for the first seven missions, the costs and mass to be delivered to Mars were higher for the BLSS

627 with in situ food supply, but that the mass and costs for resupply (food and spare parts) increased

628 faster for the scenario with terrestrial resupply for an increasing Mars population. They also

629 concluded that the assumptions made by 'Mars One' do not lead to a feasible mission plan and

630 that further technological development (e.g. life support reliability) is mandatory to achieve the

631 goal of sustainable habitation on Mars [99]. The BLSS scenario with in situ food production in

632 this assessment did, however, not take the recovery of nutrients from crop residues and human

633 organic waste stream into account for food production. Czupalla *et al*. [152] evaluated different

634 BLSS concepts (MELiSSA, BIOS, ALM and an Equivalent System Mass (ESM) optimized life

635 support system) with partial nutrient recovery for a 780 day mission to Mars based on an ESM

636 analysis. In their ESM optimized life support system, the use of the Kudenko process (see section

637 2.2) would allow the partial reuse of nitrogen for the plant production. Further research is

638 however needed to perform more enhanced assessments since the mass criterion of ESM is rather

639 minimal and the outcome of the assessment might differ significantly depending on the duration

640 (long-term or permanent BLSS) and the destination of the space mission. Furthermore, increasing

641 the fraction of microbial food production in the crew diet might enhance the feasibility of a BLSS

642 with in situ food production. Moreover, hybrid scenarios combining in-situ food production with

643 terrestrial food resupply will most likely be considered for near future long term space missions

644 and space habitation and these scenarios can be stepping stones towards self sustaining

645 colonizations in outer space.

646

647 These scientific and technological endeavors for enhanced nitrogen recovery in BLSS are not

648 only favoring future extraterrestrial civilizations, they also drive technological innovation on

649 earth in areas such as bioprocessing, circular technology, cleantech and life sciences, among

650 many others [73, 176, 177]. On the one hand, studies on the behavior of microorganisms and

651 higher plants in space can provide new insights into more fundamental microbiology and plant

652 biology [73, 178, 179]. On the other hand, spin-off terrestrial technologies can open up

653 opportunities for terrestrial life support in confined spaces or extreme conditions as well as in

654 recycling schemes for sustainability concepts (eco-cities, eco-buildings, …) [74, 176]. Potential

655 fields of application where these nitrogen reuse technologies can help close or balance the

656 nitrogen loop on earth include treatment and valorization of source separated urine, decentralized

657 water treatment, aquaponics, vertical farming and SCP feed or food production.

658

7. Conclusions

In the context of BLSS, the focus for recovery is often mainly on water, oxygen and carbon while other (micro)nutrients receive little attention. In this review, the state-of-the-art and remaining challenges of the different technologies and subsystems with respect to nitrogen is presented and discussed. Efficient nutrient management will become mandatory for a sustainable and quasi-independent extraterrestrial colonization of human beings, as resupply for increasing populations will not be feasible anymore. Therefore, more research and technological BLSS development focus should be oriented to minimize and remediate the losses of nitrogen, along with other essential elements.
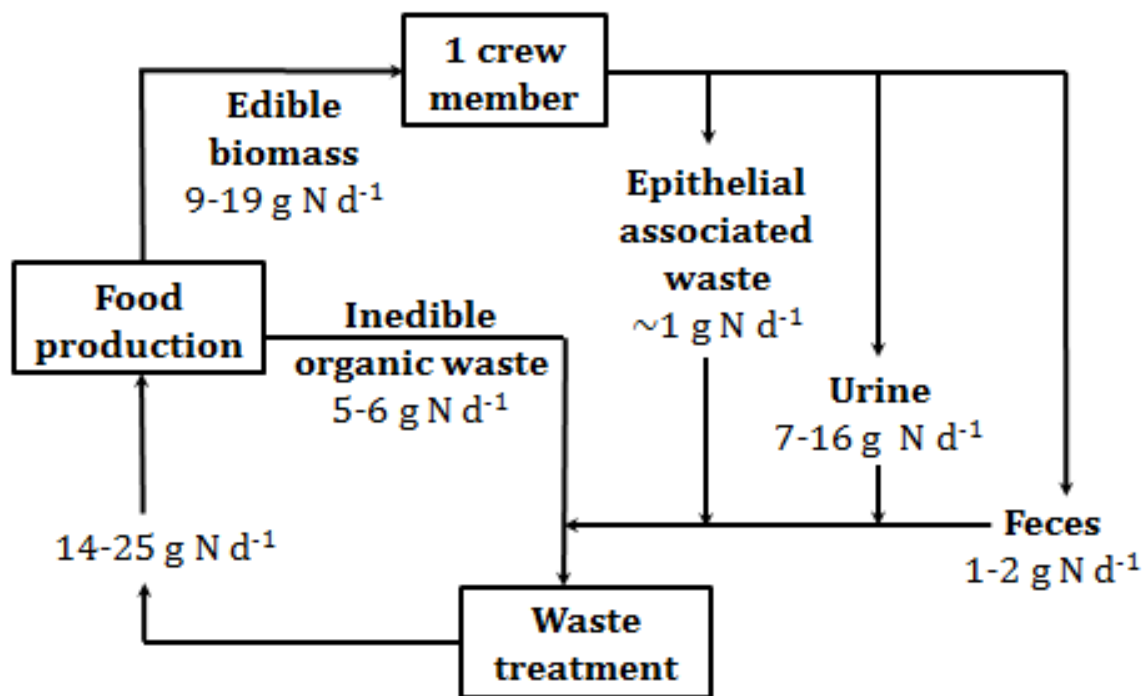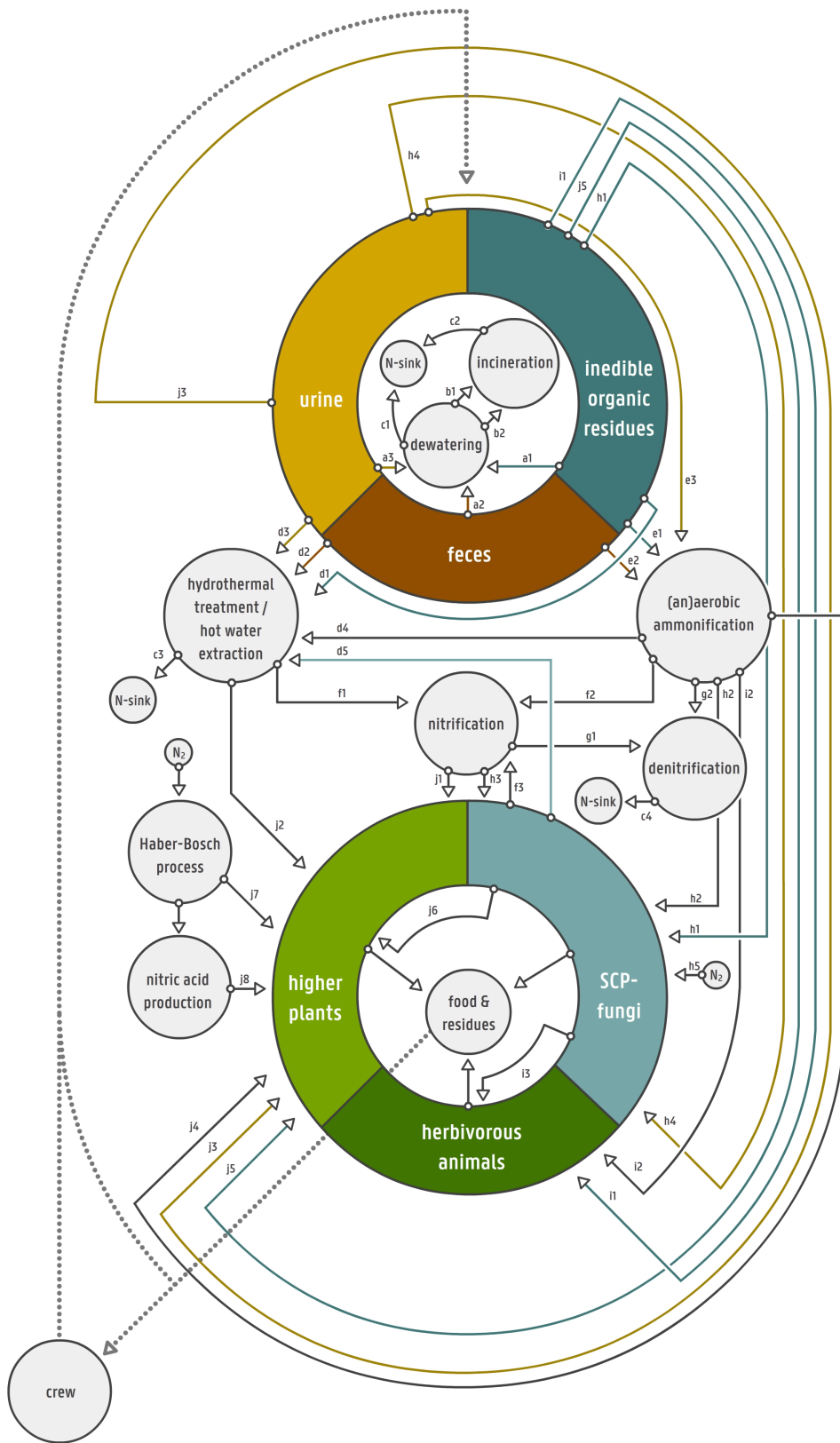
679

680

681

682

683

684 **Figure 1.** Schematic diagram of the theoretically calculated nitrogen balance in a BLSS for one

685 crew member, based on values from [7, 8, 13].

687   **Figure 2.** Schematic overview of the key processes for nitrogen recycle in Bioregenerative Life

688   Support Systems

689   **(a1** [22, 84, 153-157]; **a2** [84, 151, 152, 155, 157, 180]; **a3** [10, 152, 155]; **b1** [22, 84, 153, 154,

690   156, 157]; **b2** [84, 151, 155, 157, 180]; **c1** [10, 52, 84, 91, 150-155]; **c2** [22, 84, 151, 154-157];

691   **c3** [50-52, 93, 150, 158]; **c4** [25, 27, 62, 67, 77, 159]; **d1** [9, 23, 50-52, 93, 152, 155-158, 181];

692   **d2** [52, 91, 93, 150, 152, 155, 182]; **d3** [51, 52, 91, 93, 94, 150, 155, 182]; **d4** [4, 8]; **d5** [181]; **e1**

693   [4, 8, 14, 20, 21, 23-27, 29, 66, 183-188] ; **e2** [4, 8, 29, 66]; **e3** [62, 63, 65-67] ; **f1** [8, 23] ; **f2** [4,

694   8, 62, 63, 65-67, 77, 186] ; **f3** [4, 25]; **g1** [25, 62, 67, 77] ; **g2** [25, 27] ; **h1** [23] ; **h2** [4, 14, 23,

695   25, 26, 63, 66] ; **h3** [4, 63, 66]; **h4** [63, 126] ; **h5** [164]; **i1** [8, 23, 24, 83] ; **i2** [14, 24]; **i3** [14]; **j1**

696   [4, 8, 25, 26, 66, 184-187, 189]; **j2** [50, 91, 150, 151]; **j3** [84, 88, 89, 190]; **j4** [66, 188, 189] ; **j5**

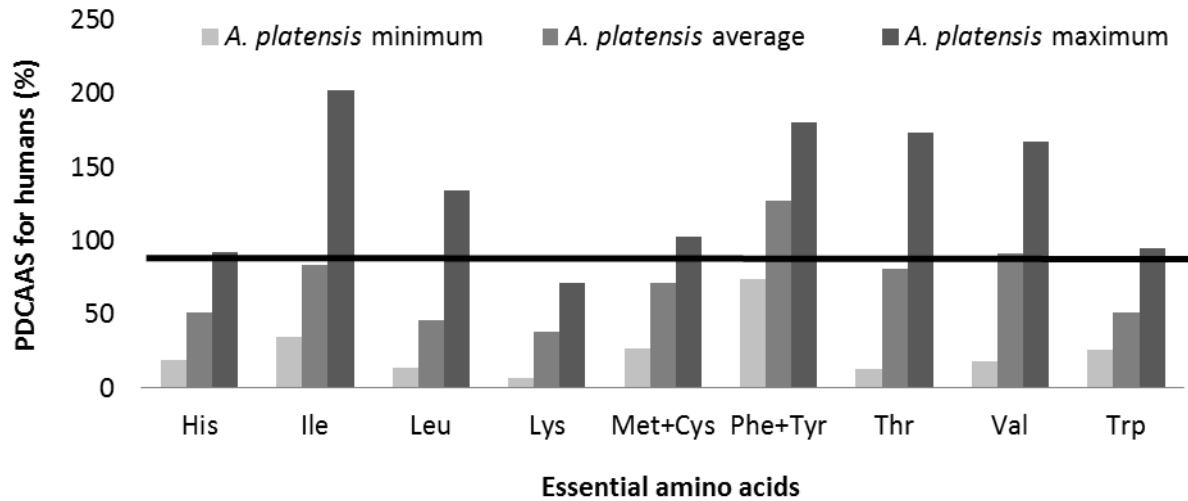697   [21]; **j6** [23, 26]; **j7** [78, 93]; **j8** [78, 93])

698

699

700

701

702

703

704

Figure 3. High variation in protein digestibility corrected amino acid scores (PDCAAS) for *A. platensis*, calculated on available amino acid data from cultivating at different pH and temperature [191], using a digestibility of 75.5% [122]. The line depicts the target threshold to fulfill for every amino acid for a perfect match with the human nutritional requirements.

709

710

711

712

713

714

715

Reference list

[1] Harper LD, Neal CR, Poynter J, Schalkwyk JD, Wingo DR. Life Support for a Low-Cost Lunar Settlement: No Showstoppers. New Space. 2016;4:40-49.

[2] Jones H. Design Rules for Space Life Support Systems. SAE International; 2003.

[3] MacElroy RD, Klein HP, Averner MM. The Evolution of CELSS for Lunar Bases. In: W. MW, editor. Lunar Bases and Space Activities of the 21st Century. Houston1984. p. 623-633.

[4] Godia F, Albiol J, Montesinos JL, Perez J, Creus N, Cabello F, et al. MELISSA: a loop of interconnected bioreactors to develop life support in space. J Biotechnol. 2002;99:319-330.

[5] Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, et al. The nitrogen cascade. Bioscience. 2003;53:341-356.

[6] Hendrickx L, De Wever H, Hermans V, Mastroleo F, Morin N, Wilmotte A, et al. Microbial ecology of the closed artificial ecosystem MELiSSA (Micro-Ecological Life Support System Alternative): Reinventing and compartmentalizing the Earth's food and oxygen regeneration system for long-haul space exploration missions. Res Microbiol. 2006;157:77-86.

[7] Gitelson JI, Lisovsky GM, MacElroy RD. Man-Made Closed Ecological Systems: Taylor & Francis; 2003.

[8] Hu EZ, Bartsev SI, Liu H. Conceptual design of a bioregenerative life support system containing crops and silkworms. Advances in Space Research. 2010;45:929-939.

[9] Nitta K. An overview of Japanese CELSS research activities. Adv Space Res. 1986;7:95-103.

[10] Carter L, Wilson LL, Orozco N. Status of ISS Water Management and Recovery. International Conference on Environmental Systems. Portland, USA2012. p. 12.

[11] Bingham SA. Urine nitrogen as a biomarker for the validation of dietary protein intake. J Nutr. 2003;133:921S-924S.

[12] Larsen TA, Gujer W. Separate management of anthropogenic nutrient solutions (human urine). Water Sci Technol. 1996;34:87-94.

[13] Toscani V, Whedon D. Nitrogen loss in the feces: the variability op excretion in normal subjects on constant dietary intakes. J Nutr. 1951;45:119-130.

[14] He WT, Liu H, Xing YD, Jones SB. Comparison of three soil-like substrate production techniques for a bioregenerative life support system. Advances in Space Research. 2010;46:1156-1161.

[15] Hawkins HJ, Johansen A, George E. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil. 2000;226:275-285.

[16] Costa JAV, Cozza KL, Oliveira L, Magagnin G. Different nitrogen sources and growth responses of Spirulina platensis in microenvironments. World J Microbiol Biotechnol. 2001;17:439-442.

[17] Witte CP. Urea metabolism in plants. Plant Science. 2011;180:431-438.

[18] Udert KM, Larsen TA, Biebow M, Gujer W. Urea hydrolysis and precipitation dynamics in a urine-collecting system. Water Res. 2003;37:2571-2582.

[19] Solomon CM, Collier JL, Berg GM, Glibert PM. Role of urea in microbial metabolism in aquatic systems: a biochemical and molecular review. Aquat Microb Ecol. 2010;59:67-88.

[20] Strayer RF, Finger BW, Alazraki MP. Effects of bioreactor retention time on aerobic microbial decomposition of celss crop residues. In: Wheeler RM, Garland JL, Tibbitts TW, Nielsen SS, Michell CA, editors. Life Sciences: Life Support Systems Studies-I. Oxford: Pergamon Press Ltd; 1997. p. 2023-2028.

760 [21] Mackowiak CL, Garland JL, Strayer RF, Finger BW, Wheeler RM. Comparison of
761 aerobically-treated and untreated crop residue as a source of recycled nutrients in a recirculating
762 hydroponic system. In: Kraft G, Carr KE, Goodwin EH, Ting KC, Finn CK, Tsai KC, et al.,
763 editors. Physical, Chemical, Biochemical and Biological Techniques and Processes. Oxford:
764 Pergamon Press Ltd; 1996. p. 281-287.

765 [22] Wignarajah K, Bubenheim DL. Integration of crop production with CELSS waste
766 management. In: Wheeler RM, Garland JL, Tibbitts TW, Nielsen SS, Michell CA, editors. Life
767 Sciences: Life Support Systems Studies-I. Oxford: Pergamon Press Ltd; 1997. p. 1833-1843.

768 [23] Tikhomirov AA, Ushakova SA, Manukovsky NS, Lisovsky GM, Kudenko YA, Kovalev
769 VS, et al. Mass exchange in an experimental new-generation life support system model based on
770 biological regeneration of environment. In: Nelson M, Pechurkin NS, Dempster WF, Somova
771 LA, Shea MA, editors. Space Life Sciences: Closed Artificial Ecosystems and Life Support
772 Systems. Kidlington: Pergamon-Elsevier Science Ltd; 2003. p. 1711-1720.

773 [24] Li LY, Zhao ZR, Liu H. Feasibility of feeding yellow mealworm (Tenebrio molitor L.) in
774 bioregenerative life support systems as a source of animal protein for humans. Acta Astronautica.
775 2013;92:103-109.

776 [25] Strayer RF, Finger BW, Alazraki MP. Evaluation of an anaerobic digestion system for
777 processing CELSS crop residues for resource recovery. In: Wheeler RM, Garland JL, Tibbitts
778 TW, Nielsen SS, Michell CA, editors. Life Sciences: Life Support Systems Studies-I. Oxford:
779 Pergamon Press Ltd; 1997. p. 2009-2015.

780 [26] Mackowiak CL, Stutte GW, Garland JL, Finger BW, Ruffe LM. Hydroponic potato
781 production on nutrients derived from anaerobically-processed potato plant residues. In: Wheeler
782 RM, Garland JL, Tibbitts TW, Nielsen SS, Michell CA, editors. Life Sciences: Life Support
783 Systems Studies-I. Oxford: Pergamon Press Ltd; 1997. p. 2017-2022.

784 [27] Lissens G, Verstraete W, Albrecht T, Brunner G, Creuly C, Seon J, et al. Advanced
785 anaerobic bioconversion of lignocellulosic waste for bioregenerative life support following
786 thermal water treatment and biodegradation by Fibrobacter succinogenes. Biodegradation.
787 2004;15:173-183.

788 [28] Mansur M, Peiro E. CI test performance: ramp-up (TN 94.71). MELiSSA Pilot Plant Frame
789 Contract 19445/05/NL/CP. 2012.

790 [29] Poughon L, Creuly C, Farges B, Dussap CG, Schiettecatte W, Jovetic S, et al. Test of an
791 aerobic prototype reactor coupled with a filtration unit for production of VFAs. Bioresour
792 Technol. 2013;145:240-247.

793 [30] Jovetic S, Schiettecatte W. Demonstration Test Results Compartment I - (Start-up, Nominal
794 & Steady operation) (TN 80.522). BELISSIMA Contract No. 19297/05/NL/SFe. 2012.

795 [31] Schussel LJ, Atwater JE. A urease bioreactor for water reclamation aboard manned
796 spacecraft. Chemosphere. 1995;30:985-994.

797 [32] Nicolau E, Fonseca JJ, Rodriguez-Martinez JA, Richardson TMJ, Flynn M, Griebenow K, et
798 al. Evaluation of a Urea Bioelectrochemical System for Wastewater Treatment Processes. Acs
799 Sustainable Chemistry & Engineering. 2014;2:749-754.

800 [33] Fountoulakis M, Lahm HW. Hydrolysis and amino acid composition analysis of proteins. J
801 Chromatogr A. 1998;826:109-134.

802 [34] Penas E, Prestamo G, Gomez R. High pressure and the enzymatic hydrolysis of soybean
803 whey proteins. Food Chem. 2004;85:641-648.

804 [35] Jazrawi C, Biller P, He YY, Montoya A, Ross AB, Maschmeyer T, et al. Two-stage
805 hydrothermal liquefaction of a high-protein microalga. Algal Research-Biomass Biofuels and
806 Bioproducts. 2015;8:15-22.
807 [36] Pinkowska H, Wolak P, Oliveros E. Application of Doehlert matrix for determination of the
808 optimal conditions of hydrothermolysis of rapeseed meal in subcritical water. Fuel.
809 2013;106:258-264.
810 [37] Yu G, Zhang YH, Schideman L, Funk T, Wang ZC. Distributions of carbon and nitrogen in
811 the products from hydrothermal liquefaction of low-lipid microalgae. Energy & Environmental
812 Science. 2011;4:4587-4595.
813 [38] Cheng HB, Zhu X, Zhu C, Qian J, Zhu N, Zhao L, et al. Hydrolysis technology of biomass
814 waste to produce amino acids in sub-critical water. Bioresour Technol. 2008;99:3337-3341.
815 [39] Yoshida H, Terashima M, Takahashi Y. Production of organic acids and amino acids from
816 fish meat by sub-critical water hydrolysis. Biotechnol Prog. 1999;15:1090-1094.
817 [40] Rogalinski T, Herrmann S, Brunner G. Production of amino acids from bovine serum
818 albumin by continuous sub-critical water hydrolysis. Journal of Supercritical Fluids. 2005;36:49-
819 58.
820 [41] Brunner G. Gas extraction: an introduction to fundamentals of supercritical fluids and the
821 application to separation processes. 1 ed. New York: Steinkopff-Verlag Heidelberg; 1994.
822 [42] Bermejo MD, Cocero MJ. Supercritical water oxidation: A technical review. Aiche Journal.
823 2006;52:3933-3951.
824 [43] Cantero DA, Bermejo MD, Cocero M. High glucose selectivity in pressurized water
825 hydrolysis of cellulose using ultra-fast reactors. Bioresour Technol. 2013;135:697-703.
826 [44] Kruse A, Dahmen N. Water - A magic solvent for biomass conversion. Journal of
827 Supercritical Fluids. 2015;96:36-45.
828 [45] Barreiro DL, Beck M, Hornung U, Ronsse F, Kruse A, Prins W. Suitability of hydrothermal
829 liquefaction as a conversion route to produce biofuels from macroalgae. Algal Research-Biomass
830 Biofuels and Bioproducts. 2015;11:234-241.
831 [46] Klingler D, Berg J, Vogel H. Hydrothermal reactions of alanine and glycine in sub- and
832 supercritical water. Journal of Supercritical Fluids. 2007;43:112-119.
833 [47] Changi S, Zhu MH, Savage PE. Hydrothermal Reaction Kinetics and Pathways of
834 Phenylalanine Alone and in Binary Mixtures. Chemsuschem. 2012;5:1743-1757.
835 [48] Kruse A, Krupka A, Schwarzkopf V, Gamard C, Henningsen T. Influence of proteins on the
836 hydrothermal gasification and liquefaction of biomass. 1. Comparison of different feedstocks.
837 Industrial & Engineering Chemistry Research. 2005;44:3013-3020.
838 [49] Thu LP, Michele B. Carbon and nitrogen removal from glucose-glycine melanoidins
839 solution as a model of distillery wastewater by catalytic wet air oxidation. J Hazard Mater.
840 2016;310:108-116.
841 [50] Onisko BL, Wydeven T. Phytotoxicity study of the products of wet oxidation of a
842 representative biomass (lettuce), NASA Technical memorandum 84383. Ames Research Center,
843 Moffett Field, California; 1983.
844 [51] Johnson C, Wydeven T. Wet oxidation of a spacecraft model waste, SAE Technical Paper
845 851372. 1985.
846 [52] Kudenko YA, Gribovskaya IV, Pavlenko RA. Mineralization of wastes of human vital
847 activity and plants to be used in a life support system. Acta Astronautica. 1997;41:193-196.

848   [53] Shimoda E, Fujii T, Hayashi R, Oshima Y. Kinetic analysis of the mixture effect in
849   supercritical water oxidation of ammonia/methanol. Journal of Supercritical Fluids.
850   2016;116:232-238.
851   [54] Du X, Zhang R, Gan ZX, Bi JC. Treatment of high strength coking wastewater by
852   supercritical water oxidation. Fuel. 2013;104:77-82.
853   [55] Wang Q, Lv YK, Zhang R, Bi JC. Treatment of cotton printing and dyeing wastewater by
854   supercritical water oxidation. Desalination and Water Treatment. 2013;51:7025-7035.
855   [56] Gong YM, Guo Y, Wang SZ, Song WH. Supercritical water oxidation of Quinazoline:
856   Effects of conversion parameters and reaction mechanism. Water Res. 2016;100:116-125.
857   [57] Garcia-Jarana MB, Kings I, Sanchez-Oneto J, Portela JR, Al-Duri B. Supercritical water
858   oxidation of nitrogen compounds with multi-injection of oxygen. Journal of Supercritical Fluids.
859   2013;80:23-29.
860   [58] Magalhaes JR, Huber DM. Response of ammonium assimilation enzymes to nitrogen form
861   treatments in different plant-species. J Plant Nutr. 1991;14:175-185.
862   [59] Richardson DJ, Wehrfritz JM, Keech A, Crossman LC, Roldan MD, Sears HJ, et al. The
863   diversity of redox proteins involved in bacterial heterotrophic nitrification and aerobic
864   denitrification. Biochem Soc Trans. 1998;26:401-408.
865   [60] van Kessel M, Speth DR, Albertsen M, Nielsen PH, Op den Camp HJM, Kartal B, et al.
866   Complete nitrification by a single microorganism. Nature. 2015;528:555-+.
867   [61] Udert KM, Fux C, Munster M, Larsen TA, Siegrist H, Gujer W. Nitrification and
868   autotrophic denitrification of source-separated urine. Water Sci Technol. 2003;48:119-130.
869   [62] Sakano Y, Pickering KD, Strom PF, Kerkhof LJ. Spatial distribution of total, ammonia-
870   oxidizing, and denitrifying bacteria in biological wastewater treatment reactors for
871   bioregenerative life support. Appl Environ Microbiol. 2002;68:2285-2293.
872   [63] Coppens J, Lindeboom R, Muys M, Coessens W, Alloul A, Meerbergen K, et al.
873   Nitrification and microalgae cultivation for two-stage biological nutrient valorization from source
874   separated urine. Bioresour Technol. 2016;211:41-50.
875   [64] Udert KM, Larsen TA, Gujer W. Chemical nitrite oxidation in acid solutions as a
876   consequence of microbial ammonium oxidation. Environ Sci Technol. 2005;39:4066-4075.
877   [65] Zhang K, Choi H, Dionysiou DD, Oerther DB. Application of Membrane Bioreactors in the
878   Preliminary Treatment of Early Planetary Base Wastewater for Long-Duration Space Missions.
879   Water Environ Res. 2008;80:2209-2218.
880   [66] Nelson M, Finn M, Wilson C, Zabel B, van Thillo M, Hawes P, et al. Bioregenerative
881   recycling of wastewater in Biosphere 2 using a constructed wetland: 2-year results. Ecological
882   Engineering. 1999;13:189-197.
883   [67] Meyer CE, Pensinger S, Pickering KD, Barta D, Shull SA, Vega LM, et al. Rapid start-up
884   and loading of an attached growth, simultaneous nitrification/denitrification membrane aerated
885   bioreactor.   45th International Conference on Environmental Systems. Bellevue,
886   Washington2015.
887   [68] Bornemann G, Wasser K, Tonat T, Moeller R, Bohnmeier M, Hauslage J. Natural microbial
888   populations in a water-based biowaste management system for space life support. Life Sciences
889   in Space Research. 2015;7:39-52.
890   [69] Perez J, Montesinos JL, Albiol J, Godia F. Nitrification by immobilized cells in a micro-
891   ecological life support system using packed-bed bioreactors: an engineering study. J Chem
892   Technol Biotechnol. 2004;79:742-754.

893 [70] Blum V. Aquatic modules for bioregenerative life support systems: Developmental aspects
894 based on the space flight results of the CEBAS mini-module. In: Nelson M, Pechurkin NS,
895 Dempster WF, Somova LA, Shea MA, editors. Space Life Sciences: Closed Artificial
896 Ecosystems and Life Support Systems. Kidlington: Pergamon-Elsevier Science Ltd; 2003. p.
897 1683-1691.
898 [71] Bluem V, Andriske M, Paris F, Voeste D. The CEBAS-Minimodule: Behaviour of an
899 artificial aquatic ecological system during spaceflight. In: Tibbitts TW, Wheeler RM, Mitchell
900 CA, Heidmann J, editors. Life Sciences: Space Life Support Systems and the Lunar Farside
901 Crater Saha Proposal. Oxford: Pergamon Press Ltd; 2000. p. 253-262.
902 [72] Uchida S, Masukawa M, Kamigaichi S. Nasda aquatic animal experiment facilities for Space
903 Shuttle and ISS. In: Ijiri K, Slenzka K, Kronenberg A, editors. Space Life Sciences: Biological
904 Research and Space Radiation. Oxford: Pergamon-Elsevier Science Ltd; 2002. p. 797-802.
905 [73] Clément G, Slenzka K. Fundamentals of Space Biology: Research on Cells, Animals, and
906 Plants in Space: Springer New York; 2006.
907 [74] Eckart E. Spaceflight Life Support and Biospherics. 1 ed. Netherlands: Springer 1996.
908 [75] Council NR. Microgravity Research in Support of Technologies for the Human Exploration
909 and Development of Space and Planetary Bodies. Washington, DC: The National Academies
910 Press; 2000.
911 [76] Rector TJ, Garland JL, Starr SO. Dispersion characteristics of a rotating hollow fiber
912 membrane bioreactor: Effects of module packing density and rotational frequency. Journal of
913 Membrane Science. 2006;278:144-150.
914 [77] Tansel B, Sager J, Rector T, Garland J, Strayer RF, Levine L, et al. Integrated evaluation of
915 a sequential membrane filtration system for recovery of bioreactor effluent during long space
916 missions. Journal of Membrane Science. 2005;255:117-124.
917 [78] Sakamoto T, Eida H, Nitta K, Ashida A. Experimental study on ammonia and ammonia
918 nitrate production system in a closed ecological experiment facility. SAE technical paper 972518.
919 1997.
920 [79] Schaafsma G. The protein digestibility-corrected amino acid score. J Nutr. 2000;130:1865S-
921 1867S.
922 [80] Young VR, Pellett PL. Plant-Proteins in Relation to Human Protein and Amino-Acid
923 Nutrition. Am J Clin Nutr. 1994;59:1203s-1212s.
924 [81] Blum V, Stretzke E, Kreuzberg K. Cebas-aquarack project - the mini-module as tool in
925 artificial ecosystem research. Acta Astronautica. 1994;33:167-177.
926 [82] Manukovsky NS, Kovalev VS, Gribovskaya IV. Two-stage biohumus production from
927 inedible potato biomass. Bioresource Technology. 2001;78:273-275.
928 [83] Tong L, Yu X, Liu H. Insect food for astronauts: gas exchange in silkworms fed on mulberry
929 and lettuce and the nutritional value of these insects for human consumption during deep space
930 flights. Bull Entomol Res. 2011;101:613-622.
931 [84] Salisbury FB, Gitelson JI, Lisovsky GM. Bios-3: Siberian experiments in bioregenerative
932 life support - Attempts to purify air and grow food for space exploration in a sealed environment
933 began in 1972. Bioscience. 1997;47:575-585.
934 [85] Mackowiak CL, Garland JL, Sager JC. Recycling crop residues for use in recirculating
935 hydroponic crop production. In: Kozai T, Kubota C, Fujiwara K, Ibaraki Y, Sase S, editors.
936 International Symposium on Plant Production in Closed Ecosystems - Automation, Culture, and
937 Environment. Leuven 1: International Society Horticultural Science; 1997. p. 19-24.

938 [86] Harper JE. Uptake of organic nitrogen forms by roots and leaves. In: Hauck RD, editor.
939 Nitrogen in crop production. Winsconsin: American Society of Agronomy; 1984. p. 165-170.
940 [87] Liu Q, Chen XB, Wu K, Fu XD. Nitrogen signaling and use efficiency in plants: what's
941 new? Curr Opin Plant Biol. 2015;27:192-198.
942 [88] Lisovsky GM, Gitelson JI, Shilenko MP, Gribovskaya IV, Trubachev IN. Direct utilization
943 of human liquid wastes by plants in a closed ecosystem. Adv Space Res. 1997;20:1801-1804.
944 [89] Qin LF, Guo SS, Ai WD, Tang YK, Cheng QY, Chen G. Effect of salt stress on growth and
945 physiology in amaranth and lettuce: Implications for bioregenerative life support system.
946 Advances in Space Research. 2013;51:476-482.
947 [90] Liu XF, Chen M, Bian ZL, Liu CC. Studies on urine treatment by biological purification
948 using Azolla and UV photocatalytic oxidation. Advances in Space Research. 2008;41:783-786.
949 [91] Ushakova SA, Zolotukhin IG, Tikhomirov AA, Tikhomirova NA, Kudenko YA,
950 Gribovskaya IV, et al. Some Methods for Human Liquid and Solid Waste Utilization in
951 Bioregenerative Life-Support Systems. Appl Biochem Biotechnol. 2008;151:676-685.
952 [92] Polonskiy VI, Gribovskaya IV. A possible NaCl pathway in the bioregenerative human life
953 support system. Acta Astronautica. 2008;63:1031-1036.
954 [93] Nitta K. The Mini-Earth facility and present status of habitation experiment program.
955 Advances in Space Research. 2005;35:1531-1538.
956 [94] Zolotukhin IG, Tikhomirov AA, Kudenko YA, Gribovskaya IV. Biological and
957 physicochemical methods for utilization of plant wastes and human exometabolites for increasing
958 internal cycling and closure of life support systems. In: Nelson M, editor. Space Life Sciences:
959 Closed Ecological Systems: Earth and Space Applications. Kidlington: Pergamon-Elsevier
960 Science Ltd; 2005. p. 1559-1562.
961 [95] Arkoun M, Sarda X, Jannin L, Laine P, Etienne P, Garcia-Mina JM, et al. Hydroponics
962 versus field lysimeter studies of urea, ammonium and nitrate uptake by oilseed rape(Brassica
963 napus L.). J Exp Bot. 2012;63:5245-5258.
964 [96] Aydogan-Cremaschi S, Orcun S, Blau G, Pekny JE, Reklaitis GV. A novel approach for life-
965 support-system design for manned space missions. Acta Astronautica. 2009;65:330-346.
966 [97] Walford RL, Harris SB, Gunion MW. The calorically restricted low-fat nutrient-dense diet
967 in biosphere-2 significantly lowers blood-glucose, total leukocyte count, cholesterol, and blood-
968 pressure in humans. Proc Natl Acad Sci U S A. 1992;89:11533-11537.
969 [98] Nelson M, Pechurkin NS, Allen JP, Somova LA, Gitelson JI. Closed ecological systems,
970 space life support and biospherics. In: Wang LK, Ivanov, V., Tay, J.-H., Hung, Y.-T, editor.
971 Environmental Biotechnology: Humana Press; 2010.
972 [99] Do S, Owens A, Ho K, Schreiner S, de Weck O. An independent assessment of the technical
973 feasibility of the Mars One mission plan - Updated analysis. Acta Astronautica. 2016;120:192-
974 228.
975 [100] Cassidy ES, West PC, Gerber JS, Foley JA. Redefining agricultural yields: from tonnes to
976 people nourished per hectare. Environmental Research Letters. 2013;8:8.
977 [101] Wheeler RM, Mackowiak CL, Stutte GW, Sager JC, Yorio NC, Ruffe LM, et al. NASA's
978 biomass production chamber: A testbed for bioregenerative life support studies. In: MacElroy
979 RD, Kreuzberg K, Nielsen S, Tibbits TW, editors. Natural and Artificial Ecosystems. Oxford:
980 Pergamon Press Ltd; 1996. p. 215-224.
981 [102] Nasseri AT, Rasoul-Amini S, Morowvat MH, Ghasemi Y. Single cell protein: production
982 and process. American Journal of food technology. 2011;6:103-116.

983   [103] Anupama, Ravindra P. Value-added food: Single cell protein. Biotechnology Advances.
984   2000;18:459-479.
985   [104] Tikhomirov AA, Ushakova SA, Manukovsky NS, Lisovsky GM, Kudenko YA, Kovalev
986   VS, et al. Mass exchange in an experimental new-generation life support system model based on
987   biological regeneration of environment. Space Life Sciences: Closed Artificial Ecosystems and
988   Life Support Systems. 2003;31:1711-1720.
989   [105] Silva AG, Carter R, Merss FLM, Correa DO, Vargas JVC, Mariano AB, et al. Life cycle
990   assessment of biomass production in microalgae compact photobioreactors. Gcb Bioenergy.
991   2015;7:184-194.
992   [106] Carlozzi P, Sacchi A. Biomass production and studies on Rhodopseudomonas palustris
993   grown in an outdoor, temperature controlled, underwater tubular photobioreactor. J Biotechnol.
994   2001;88:239-249.
995   [107] Cornet JF, Dussap CG. A Simple and Reliable Formula for Assessment of Maximum
996   Volumetric Productivities in Photobioreactors. Biotechnol Prog. 2009;25:424-435.
997   [108] Nelson JA, Bugbee B. Economic Analysis of Greenhouse Lighting: Light Emitting Diodes
998   vs. High Intensity Discharge Fixtures. Plos One. 2014;9:10.
999   [109] Christenson L, Sims R. Production and harvesting of microalgae for wastewater treatment,
1000   biofuels, and bioproducts. Biotechnol Adv. 2011;29:686-702.
1001   [110] Sevigne-Itoiz E, Fuentes-Grunewald C, Gasol CM, Garces E, Alacid E, Rossi S, et al.
1002   Energy balance and environmental impact analysis of marine microalgal biomass production for
1003   biodiesel generation in a photobioreactor pilot plant. Biomass & Bioenergy. 2012;39:324-335.
1004   [111] Grima EM, Belarbi EH, Fernandez FGA, Medina AR, Chisti Y. Recovery of microalgal
1005   biomass and metabolites: process options and economics. Biotechnology Advances.
1006   2003;20:491-515.
1007   [112] Edozien JC, Udo UU, Young VR, Scrimsha.Ns. Effects of high levels of yeast feeding on
1008   uric acid metabolism of young men. Nature. 1970;228:180-&.
1009   [113] Blankenship RE, Madigan MT, Bauer CE. Anoxygenic photosynthetic bacteria. Dordrecht ;
1010   Boston: Kluwer Academic Publishers; 1995.
1011   [114] Ortega-Calvo JJ, Mazuelos C, Hermosin B, Saizjimenez C. Chemical-Composition of
1012   Spirulina and Eukaryotic Algae Food-Products Marketed in Spain. Journal of Applied Phycology.
1013   1993;5:425-435.
1014   [115] Cooper M, Douglas G, Perchonok M. Developing the NASA Food System for Long-
1015   Duration Missions. J Food Sci. 2011;76:R40-R48.
1016   [116] Godia F, Albiol J, Perez J, Creus N, Cabello F, Montras A, et al. The MELISSA pilot plant
1017   facility as an integration test-bed for advanced life support systems. Space Life Sciences: Life
1018   Support Systems and Biological Systems under Influence of Physical Factors. 2004;34:1483-
1019   1493.
1020   [117] Kay RA. Microalgae as Food and Supplement. Crit Rev Food Sci. 1991;30:555-573.
1021   [118] De Oliveira MACL, Monteiro MPC, Robbs PG, Leite SGF. Growth and chemical
1022   composition of Spirulina maxima and Spirulina platensis biomass at different temperatures.
1023   Aquacult Int. 1999;7:261-275.
1024   [119] Becker EW. Micro-algae as a source of protein. Biotechnology Advances. 2007;25:207-
1025   210.
1026   [120] Belay A, Ota Y, Miyakawa K, Shimamatsu H. Current Knowledge on Potential Health
1027   Benefits of Spirulina. Journal of Applied Phycology. 1993;5:235-241.

1028 [121] Gershwin ME, Belay A. Spirulina in human nutrition and health. Boca Raton: CRC Press;
1029 2008.
1030 [122] Narasimha DLR, Venkataraman GS, Duggal SK, Eggum BO. Nutritional quality of the
1031 blue-green alga *Spirulina platensis* Geitler. J Sci Food Agric. 1982;33:456-460.
1032 [123] Rossi N, Petit I, Jaouen P, Legentilhomme P, Derouiniot M. Harvesting of cyanobacterium
1033 Arthrospira platensis using inorganic filtration membranes. Separ Sci Technol. 2005;40:3033-
1034 3050.
1035 [124] Benemann J. Microalgae for Biofuels and Animal Feeds. Energies. 2013;6:5869-5886.
1036 [125] Chang YY, Wu ZC, Bian L, Feng DL, Leung DYC. Cultivation of Spirulina platensis for
1037 biomass production and nutrient removal from synthetic human urine. Applied Energy.
1038 2013;102:427-431.
1039 [126] Yang CL, Liu H, Li M, Yu CY, Yu G. Treating urine by Spirulina platensis. Acta
1040 Astronautica. 2008;63:1049-1054.
1041 [127] Feng DL, Wu ZC, Wang DH. Effects of N source and nitrification pretreatment on growth
1042 of Arthrospira platensis in human urine. Journal of Zhejiang University-Science A. 2007;8:1846-
1043 1852.
1044 [128] Udert KM, Buckley CA, Wachter M, McArdell CS, Kohn T, Strande L, et al. Technologies
1045 for the treatment of source-separated urine in the eThekwini Municipality. Water Sa.
1046 2015;41:212-221.
1047 [129] Carmichael WW, Drapeau C, Anderson DM. Harvesting of Aphanizomenon flos-aquae
1048 Ralfs ex Born. & Flah. var. flos-aquae (Cyanobacteria) from Klamath Lake for human dietary
1049 use. Journal of Applied Phycology. 2000;12:585-595.
1050 [130] Li M, Hu DW, Liu H, Hu EZ, Xie BZ, Tong L. Chlorella vulgaris culture as a regulator of
1051 CO2 in a bioregenerative life support system. Advances in Space Research. 2013;52:773-779.
1052 [131] Nakajima F, Kamiko N, Yamamoto K. Organic wastewater treatment without greenhouse
1053 gas emission by photosynthetic bacteria. Water Sci Technol. 1997;35:285-291.
1054 [132] Eroglu I, Aslan K, Gunduz U, Yucel M, Turker L. Substrate consumption rates for
1055 hydrogen production by *Rhodobacter sphaeroides* in a column photobioreactor. J Biotechnol.
1056 1999;70:103-113.
1057 [133] Ponsano EHG, Paulino CZ, Pinto MF. Phototrophic growth of Rubrivivax gelatinosus in
1058 poultry slaughterhouse wastewater. Bioresource Technol. 2008;99:3836-3842.
1059 [134] Hulsen T, Batstone DJ, Keller J. Phototrophic bacteria for nutrient recovery from domestic
1060 wastewater. Water Res. 2014;50:18-26.
1061 [135] Batstone DJ, Hulsen T, Mehta CM, Keller J. Platforms for energy and nutrient recovery
1062 from domestic wastewater: A review. Chemosphere. 2015;140:2-11.
1063 [136] Kobayashi M, Kobayashi M. Waste Remediation and Treatment Using Anoxygenic
1064 Phototrophic Bacteria. In: Blankenship RE, Madigan MT, Bauer CE, editors. Anoxygenic
1065 Photosynthetic Bacteria. Dordrecht: Springer Netherlands; 1995. p. 1269-1282.
1066 [137] Martin AM. Bioconversion of waste materials to industrial products. 2nd ed. London ; New
1067 York: Blackie Academic & Professional; 1998.
1068 [138] Dworkin M, Falkow S. The prokaryotes : a handbook on the biology of bacteria. 3rd ed.
1069 New York ; London: Springer; 2006.
1070 [139] Imhoff JF. The phototrophic alpha-proteobacteria. In: Dworkin M, Falkow S, Rosenberg E,
1071 Schleifer K-H, Stackebrandt E, editors. The Prokaryotes. New York: Springer-Verlag; 2006. p.
1072 41-64.

1073 [140] Deng R. Food and food supplements with hypocholesterolemic effects. Recent Pat Food
1074 Nutr Agric. 2009;1:15-24.
1075 [141] Banerjee S, Azad SA, Vikineswary S, Selvaraj OS, Mukherjee TK. Phototrophic bacteria
1076 as fish feed supplement. Asian-Australas J Anim Sci. 2000;13:991-994.
1077 [142] Shapawi R, Ting TE, Al-Azad S. Inclusion of Purple Non-sulfur Bacterial Biomass in
1078 Formulated Feed to Promote Growth, Feed Conversion Ratio and Survival of Asian Seabass
1079 *Lates calcarifer* Juveniles. Journal of Fisheries and Aquatic Science. 2012;7:475-480.
1080 [143] Hendrickx L, Mergeay M. From the deep sea to the stars: human life support through
1081 minimal communities. Curr Opin Microbiol. 2007;10:231-237.
1082 [144] Verstraete W, Clauwaert P, Vlaeminck SE. Used water and nutrients: Recovery
1083 perspectives in a 'panta rhei' context. Bioresour Technol. 2016;215:199-208.
1084 [145] Matassa S, Verstraete W, Pikaar I, Boon N. Autotrophic nitrogen assimilation and carbon
1085 capture for microbial protein production by a novel enrichment of hydrogen-oxidizing bacteria.
1086 Water Res. 2016;101:137-146.
1087 [146] Ivy J. Summary of electrolytic hydrogen production - Milestone completion report. USA:
1088 National Renewable Energy Laboratory (NREL): Golden; 2004. p. 25.
1089 [147] Gitelson JI, Blum V, Grigoriev AI, Lisovsky GM, Manukovsky NS, Sinyak YE, et al.
1090 Biological physical-chemical aspects of a human life-support-system for a lunar base. Acta
1091 Astronautica. 1995;37:385-394.
1092 [148] Nangul A, Bhatia R. Microorganisms: A marvelous source of single cell proteins. Journal
1093 of Microbiology, Biotechnology and Food Sciences. 2013;3:15-18.
1094 [149] Strayer RF, Finger BW, Alazraki MP. Evaluation of an anaerobic digestion system for
1095 processing CELSS crop residues for resource recovery. Life Sciences: Life Support Systems
1096 Studies-I. 1997;20:2009-2015.
1097 [150] Kudenko YA, Gribovskaya IV, Zolotukhin IG. Physical-chemical treatment of wastes: A
1098 way to close turnover of elements in LSS. Acta Astronautica. 2000;46:585-589.
1099 [151] Barta D, Ewert M. Development of Life Support System Technologies for human lunar
1100 missions. SAE Technical Paper 2009-01-24832009.
1101 [152] Czupalla M, Horneck G, Blome HJ. The conceptual design of a hybrid life support system
1102 based on the evaluation and comparison of terrestrial testbeds. In: Nelson M, editor. Space Life
1103 Sciences: Closed Ecological Systems: Earth and Space Applications. Kidlington: Pergamon-
1104 Elsevier Science Ltd; 2005. p. 1609-1620.
1105 [153] Bubenheim DL, Patterson M, Wignarajah K, Flynn M. Incineration of biomass and
1106 utilization of product gas as a CO2 source for crop production in closed systems: Gas quality and
1107 phytotoxicity. In: Wheeler RM, Garland JL, Tibbitts TW, Nielsen SS, Michell CA, editors. Life
1108 Sciences: Life Support Systems Studies-I. Oxford: Pergamon Press Ltd; 1997. p. 1845-1850.
1109 [154] Bubenheim DL, Wignarajah K. Recycling of inorganic nutrients for hydroponic crop
1110 production following incineration of inedible biomass. In: Wheeler RM, Garland JL, Tibbitts
1111 TW, Nielsen SS, Michell CA, editors. Life Sciences: Life Support Systems Studies-I. Oxford:
1112 Pergamon Press Ltd; 1997. p. 2029-2035.
1113 [155] Slavin T, Liening F, Oleson M, Olson RL. Controlled ecological life support systems
1114 (CELSS) physiochemical waste management systems evaluation. NASA CR-177422. Boeing
1115 Aerospace Company; 1986.
1116 [156] Garland JL, Mackowiak CL, Sager JC. Hydroponic Crop Production Using Recycled
1117 Nutrients from Inedible Crop Residues, SAE Technical paper series, 932173. 23rd International
1118 Conference on Environmental Systems. Colorado Springs, Colorado1993.

1119 [157] Gribovskaya IV, Gladchenko IA, Zinenko GK. Extraction of mineral elements from
1120 inedible wastes of biological components of a life-support system and their utilization for plant
1121 nutrition. In: MacElroy RD, Kreuzberg K, Nielsen S, Tibbits TW, editors. Natural and Artificial
1122 Ecosystems. Oxford: Pergamon Press Ltd; 1996. p. 93-97.
1123 [158] Takahahi Y, Nitta K, Ohya H, Oguchi M. The applicability of catalytic wet-oxidation to
1124 CELSS. Adv Space Res. 1987;7:81-84.
1125 [159] Schwingel WR, Sager JC. Anaerobic degradation of inedible crop residues produced in a
1126 controlled ecological life-support-system. In: Kraft G, Carr KE, Goodwin EH, Ting KC, Finn
1127 CK, Tsai KC, et al., editors. Physical, Chemical, Biochemical and Biological Techniques and
1128 Processes. Oxford: Pergamon Press Ltd; 1996. p. 293-297.
1129 [160] Lunn GM. Strategies for Stabilizing Nitrogenous Compounds in ECLSS Wastewater: Top-
1130 Down System Design and Unit Operation Selection with Focus on Bio-Regenerative Processes
1131 for Short and Long Term Scenarios. 42nd International Conference on Environmental Systems
1132 (ICES); 15-19 Jul 2012; . San Diego, CA; United States 2011. p. 14 p.
1133 [161] Kampschreur MJ, Temmink H, Kleerebezem R, Jetten MSM, van Loosdrecht MCM.
1134 Nitrous oxide emission during wastewater treatment. Water Res. 2009;43:4093-4103.
1135 [162] Olivares J, Bedmar EJ, Sanjuan J. Biological Nitrogen Fixation in the Context of Global
1136 Change. Mol Plant Microbe In. 2013;26:486-494.
1137 [163] Gutschick VP. Energetics of microbial fixation of dinitrogen. Microbes and Engineering
1138 Aspects. Berlin, Heidelberg: Springer Berlin Heidelberg; 1982. p. 109-167.
1139 [164] Fry IV, Hrabeta J, D'Souza J, Packer L. Application of photosynthetic N2-fixing
1140 cyanobacteria to the CELSS program. Adv Space Res. 1987;7:39-46.
1141 [165] Stern JC, Sutter B, Freissinet C, Navarro-Gonzalez R, Mckay CP, Archer PD, et al.
1142 Evidence for indigenous nitrogen in sedimentary and aeolian deposits from the Curiosity rover
1143 investigations at Gale crater, Mars (vol 112, pg 4245, 2015). P Natl Acad Sci USA.
1144 2015;112:E3085-E3085.
1145 [166] Benoit MR, Klaus DM. Microgravity, bacteria, and the influence of motility. Advances in
1146 Space Research. 2007;39:1225-1232.
1147 [167] Horneck G, Klaus DM, Mancinelli RL. Space Microbiology. Microbiol Mol Biol Rev.
1148 2010;74:121-+.
1149 [168] Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL. Microbial responses to
1150 microgravity and other low-shear environments. Microbiol Mol Biol Rev. 2004;68:345-+.
1151 [169] Poulet L, Fontaine JP, Dussap CG. Plant's response to space environment: a comprehensive
1152 review including mechanistic modelling for future space gardeners. Acta Bot Gall. 2016;163:337-
1153 347.
1154 [170] Wolff SA, Coelho LH, Karoliussen I, Jost AI. Effects of the Extraterrestrial Environment
1155 on Plants: Recommendations for Future Space Experiments for the MELiSSA Higher Plant
1156 Compartment. Life : Open Access Journal. 2014;4:189-204.
1157 [171] Klaus D, Simske S, Todd P, Stodieck L. Investigation of space flight effects on Escherichia
1158 coli and a proposed model of underlying physical mechanisms. Microbiology-(UK).
1159 1997;143:449-455.
1160 [172] Leys N, Hendrickx L, De Boever P, Baatout S, Mergeay M. Space flight effects on
1161 bacterial physiology. J Biol Regul Homeost Agents. 2004;18:193-199.
1162 [173] Poughon L, Farges B, Dussap CG, Godia F, Lasseur C. Simulation of the MELiSSA closed
1163 loop system as a tool to define its integration strategy. Advances in Space Research.
1164 2009;44:1392-1403.

1165 [174] Godia F, Albiol J, Perez J, Creus N, Cabello F, Montras A, et al. The MELISSA pilot plant
1166 facility as an integration test-bed for advanced life support systems. In: Henninger DL, Drysdale
1167 AE, Kondyurin AV, editors. Space Life Sciences: Life Support Systems and Biological Systems
1168 under Influence of Physical Factors. Kidlington: Pergamon-Elsevier Science Ltd; 2004. p. 1483-
1169 1493.
1170 [175] Olson RL, Gustan EA, Vinopal TJ. CELSS transportation analysis. In: MacElroy RD,
1171 Smernoff DT, Klein HP, editors. XXV Cospar meeting. Graz, Austria: NASA; 1984.
1172 [176] Adams C, Andersson I, Feighery J. Water for Two Worlds: Designing Terrestrial
1173 Applications for Exploration-Class Sanitation Systems. SAE International; 2004.
1174 [177] Menezes AA, Montague MG, Cumbers J, Hogan JA, Arkin AP. Grand challenges in space
1175 synthetic biology. J R Soc Interface. 2015;12:7.
1176 [178] Vandenbrink JP, Kiss JZ. Space, the final frontier: A critical review of recent experiments
1177 performed in microgravity. Plant Sci. 2016;243:115-119.
1178 [179] Kiss JZ. Plant biology in reduced gravity on the Moon and Mars. Plant Biol. 2014;16:12-
1179 17.
1180 [180] Tako Y, Tsuga S, Tani T, Arai R, Komatsubara O, Shinohara M. One-week habitation of
1181 two humans in an airtight facility with two goats and 23 crops - Analysis of carbon, oxygen, and
1182 water circulation. Advances in Space Research. 2008;41:714-724.
1183 [181] Kohlmann KL, Westgate P, Velayudhan A, Weil J, Sarikaya A, Brewer MA, et al. Enzyme
1184 conversion of lignocellulosic plant materials for resource recovery in a controlled ecological life
1185 support system. Adv Space Res. 1996;18:251-265.
1186 [182] Nitta K, Hattori I, Hayashi K, Tora T. Waste management system for the habitat module
1187 for CEEF. SAE Technical paper 972519. 1997.
1188 [183] Strayer RF, Finger BW, Alazraki MP, Cook K, Garland JL. Recovery of resources for
1189 advanced life support space applications: effect of retention time on biodegradation of two crop
1190 residues in a fed-batch, continuous stirred tank reactor. Bioresour Technol. 2002;84:119-127.
1191 [184] Finger B, Strayer R. Development of an intermediate-scale aerobic bioreactor to regenerate
1192 nutrients from inedible crop residues. SAE Technical paper 941501. 1994.
1193 [185] Finger B, Alazraki M. Development and integration of a breadboard-scale aerobic
1194 bioreactor to regenerate nutrients from inedible crop residues. SAE Technical paper 951498.
1195 1995.
1196 [186] Garland JL, Mackowiak CL, Strayer RF, Finger BW. Integration of waste processing and
1197 biomass production systems as part of the KSC breadboard project. In: Wheeler RM, Garland JL,
1198 Tibbitts TW, Nielsen SS, Michell CA, editors. Life Sciences: Life Support Systems Studies-I.
1199 Oxford: Pergamon Press Ltd; 1997. p. 1821-1826.
1200 [187] Trotman AA, David PP, Bonsi CK, Hill WA, Mortley DG, Loretan PA. Integrating
1201 biological treatment of crop residue into a hydroponic sweetpotato culture. Adv Space Res.
1202 1997;20:1805-1813.
1203 [188] Schwartzkopf S, Stroup T, Williams D. Anaerobically-processed waste as a nutrient source
1204 for higher plants in a controlled ecological life support system. SAE Technical Paper 932248.
1205 1993.
1206 [189] Strayer R, Cook K. Recycling Plant Nutrients at NASA's KSC-CELSS Breadboard Project:
1207 Biological Performance of the Breadboard-Scale Aerobic Bioreactor During Two Runs, SAE
1208 Technical paper 951708. 1995.
1209 [190] Katayama N, Yamashita M, Kishida Y, Liu CC, Watanabe I, Wada H, et al. Azolla as a
1210 component of the space diet during habitation on Mars. Acta Astronautica. 2008;63:1093-1099.

1211   [191] Ogbonda KH, Aminigo RE, Abu GO. Influence of temperature and pH on biomass
1212   production and protein biosynthesis in a putative *Spirulina* sp. Bioresour Technol. 2007;98:2207-
1213   2211.

1214