Experiment design to investigate the possibility of using grey water in aeroponic cultivation of various crops for future long-term space missions

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Abstract. One of the crucial steps in the development of self-sufficient extra-terrestrial human colonies is water and biomass management. One of the attempts to simplify the treatment scheme is to directly implement greywater into soil-less cultivation as no greywater treatment unit would be necessary. In this case, the most essential key factor is the influence of surfactants. In this paper, an analysis of various experiment designs is investigated and shown. While full factorial design of experiment would require more than 500 000 tests, with duration of at least 30 days each, an approach to reduce this number to a total of 5 experiments is shown in this paper for initial experiments.

1 Introduction

Water management and food production are one of the most important key factors for human habitation, either on Earth or beyond. Creating self-sufficient Life Support Systems (LSS) with each subsystem working in cooperation is essential. LSSs include four basic subsystems: biomass production, atmosphere revitalization, as well as waste and water treatment, shown in Figure 1. As it can be seen, each subsystem by-product has to be used to facilitate at least one other subsystem. Thus, recovery of essentials such as water and nutrients is required. It is rather certain, that hybrid (simultaneous use of biological and physiochemical wastewater treatment) wastewater treatment will be considered for future, long-term space missions according to NASA Space Technology Roadmap [1]. It is still undetermined whether it is possible to use untreated waste streams (such as grey water) directly for plant cultivation. Cultivation methods might be divided into two main groups: classical cultivation (in soil) and soilless cultivation. Trends, however, show that the latter type should be considered for spaceflight applications due to the decreased system mass

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[2]. The long-term goal of this project is to close a loop between the water and biomass subsystem, by suppling biomass production with biologically treated wastewater. The first milestone is to study whether plants cultivated with soilless methods (more precisely aeroponics) could be supplied with untreated greywater. Overall assumptions, aims, and beneficial aspects of discussed system design are shown in next chapter.

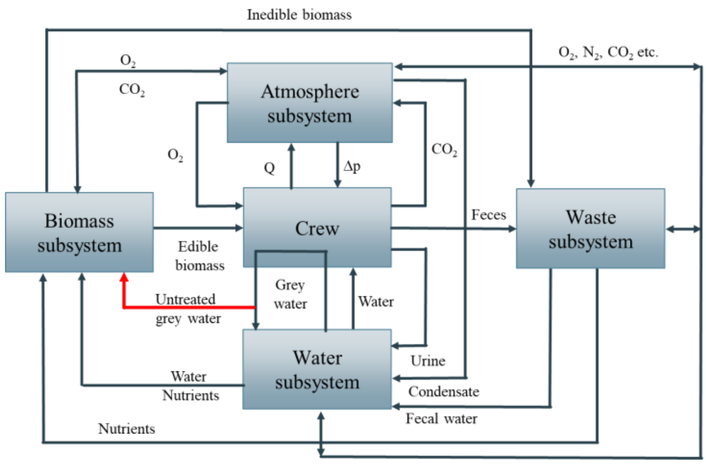

Fig. 1. Self-sufficient Life Support System [own source].

1.1 Why aeroponics?

Typical soil cultivation is not applicable in extraterrestrial conditions as it requires more water and nutrients as well as space than soilless types (aeroponics or hydroponics). In hydroponics, roots are submerged in nutrients solution (water and nutrients), while in aeroponics, roots are suspended in air, and nutrient solution is supplied in form of mist. In both soilless systems, roots have better access to water and nutrients and therefore plant growth is faster. Water that is not taken up by roots returns to the tank, and therefore very limited losses occur [3]. Aeroponics however require less water and have a higher yield. Lower water consumption is due to lower evaporation, while higher yield is due to better oxygenation of roots [4]. Aeroponic systems are therefore intensively studied as part of future life support systems, as this type of cultivation has potential superior parameters over soil and other soilless types of cultivation. With application of aeroponics, future colonies will require less water and nutrients to produce food, as well as less space. These savings in materials will lead to decrease in cost of transport from Earth to further destinations such as Moon or Mars. An example of an aeroponic system is shown in Figure 2. Besides many beneficial features of aeroponics, it has to be noted that a system might be exposed to failures, mainly in regard to the small capillaries and nozzles, which are delivering nutrient solution to the roots and are susceptible to clogging. As grey water is rich in solids and surfactants, enhanced clogging and biofilm forming may occur. While the system does not use any cultivated substrate, the risk of plant withering is greater in the event of a hydraulic system failure. Thus, experiments conducted on grey water contaminated with surfactants might also reveal hydraulic responses that can provide valuable lessons learned for future system designs.

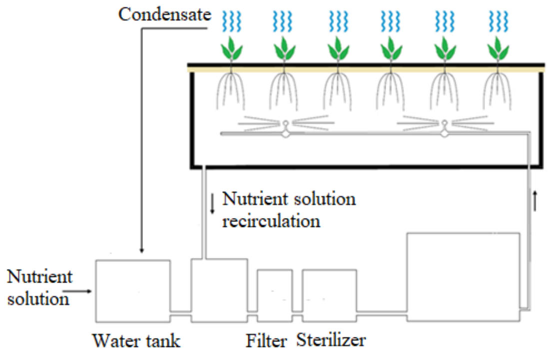

Fig. 2. Aeroponic system scheme [4].

1.2 Why grey water?

Wastewater originating from showers, laundry, bathing, and washing the dishes [5] is called grey water. Grey water or total hygiene water [2] is a main source of water in all waste streams. The composition of grey water is provided for extra-terrestrial condition by Schubert et al. [6], Wydeven and Golub [7] and most important by Anderson et al. [2]. However, it should be noted that data are not obtained directly from space mission conditions. Despite lack of data, hygiene water is a major stream from which water must be recovered and major stream that should be considered as water source in regenerative LSSs.

2 Design of experiment

2.1 Full factorial design

Utilization of untreated grey water for irrigation requires extensive research. List of variables that have influence on results of irrigation with grey water are summarized in Table 1. Grey water contains more contaminants than just surfactants but presence of surfactants is a main obstacle to use grey water. Variable 1 and 2 are connected directly with surfactant presence. Variables 3–10 are variables connected with parameters of aeroponic cultivation or species of plant. While presence of surfactant poses threat to plants, probably some or all negative effects can be diminished by maximization of plant strength with selection of optimal parameters. Therefore, the results of exposure of plants to surfactants will be highly dependent on overall performance of cultivation and must be included in experimental design. Decisive variables can be tested on different levels. In Table 1, data about the number of levels as well as possible ranges of values are obtained from literature review and presented.

Only anionic surfactants (Igepon TC-42: sodium N-coconut acid-N-methyl taurate; Sodium methyl cocoyl taurate (SMCT); Sodium dodecylbenzenesulfonate (SDBS); Sodium laureth sulfate (SLES)) are used during space missions or in research related to space technologies. The detergent concentrations differ, depending on the type of use of water (hygiene water, laundry water, dishwash water). One to three concentrations can be distinguished for each surfactant. In soil cultivation the influence of anionic surfactant was measured and results showed negative effect on wheat growth [27]. It is possible that it was related to the decrease in microbial activity, which in turn reduced solubilizing effects. Studies performed in hydroponic systems while using Igepon TC-42 shows that concentration above 0.25 $g \cdot L^{-1}$ exhibit phytotoxic effects [28]. On board the International

Space Station concentrations obtained in grey water are 55% higher [2]. Additionally, in most space references surfactants concentrations tend to be higher than stated in Bubenheim et al. [28]. Only Wydeven and Golub [7] state lower concentration of SDBS for dishwasher and laundry stream, 0.22 and 0.056 $g \cdot L^{-1}$ respectively. Plant growth stages consist of 4 main stages: sprouting, seedling, vegetative, and reproductive phase [29].

Table 1. List of variables in experiment design process of surfactants impact on aeroponically cultivated plants.

Number	**Variable**	**Number of levels**	**References**
1	Surfactant concentration	1–3 (0.056–0.94 $g \cdot L^{-1}$)	[2, 7–9]
2	Surfactant type	4	
3	Growth stage of plant	2 (seedling stage/vegetative stage)	[10, 11]
4	Duration of sprinkling	3 (1–5min)	[12, 13]
5	Sprinkling interval	3 (5–15min)	
6	Nutrients solution composition	4	[14–17]
7	CO_2 concentration	4 (360–50 000 ppm)	[18]
8	Plant species	14–23	[19, 20]
9	Illumination intensity	6 (150–930 $\mu mol \cdot m^{-2} s^{-1}$)	[21–26]
10	Illumination time	7 (10–24h)	
11	Total number of combinations	677376–1112832	

The age of the plant can affect its response to the stress factor. Lutts et al. [30] compered salt resistance in various development stages of plants. It appeared that the young seedling stage tends to be the most sensitive to NaCl. Impact of two different surfactants in two different concentrations on lettuce (*Lactuca Sativa L.*) in two stages were conducted [11]. Results showed, that for each case the young seedling stage was more susceptible to surfactants.

Sprinkling time and intervals between them depend on the size of the plant roots. The main rule is that the roots should not be allowed to dry between cycles. At the early stage of development of plants, it should be done often, but shorter, and in later stages longer, but less frequent [3]. During each cycle the whole root should be well moisturized and not dry out before the next cycle. The times for sprinkling are from 1 to 3 min, and intervals from 5 to 15 minutes [12, 13].

A nutrient solution for aeroponic systems is an aqueous solution which contains mainly inorganic ions from soluble salts of necessary elements for plant growth. Eventually, some organic compounds such as iron chelates may be present [31]. All of those elements have a clear physiological role and they are necessary to complete a plant's life cycle [32]. 17 elements are considered essential for most plants: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, copper, zinc, manganese, molybdenum, boron, chlorine and nickel [3]. Carbon and oxygen, can be provided from the atmosphere. The rest of the macro and microelements are obtained from the growth medium [33]. The most basic nutrient solutions include nitrogen, phosphorus, potassium, calcium, magnesium and sulphur. The nutrient composition determines electrical conductivity and osmotic potential of the solution. The nutrient solutions must contain the chemical form of ions that can be absorbed by plants, so in aeroponic systems the plant productivity depends on nutrient uptake and pH [33]. Analyzing the impact of macro and micro elements on the growth and development of plants, it can be seen that each nutrient solution must be properly balanced [3, 4]. Nitrogen has a strong influence on the growth and development of plants, but its excess reduces the resistance to damage of the plant and delays blooming. Higher levels of magnesium and calcium cause plants to produce thicker shoots that are more resistant to external factors, however, providing too much of these elements can cause stunted growth. Potassium positively affects the growth of plants and their resistance to external factors, but its excess lowers the content of nutritional elements in the yield. Phosphorus affects the growth rate of the root system and yield. Phosphorus is not a toxic component for plants, but its excess may cause reduced uptake of other elements. Boron has a direct impact on the formation of the cell wall in plants, however, its excess negatively affects the size and quality of the crop.

The lighting is affecting plant growth via three main factors: light cycle, light intensity, and light spectrum. There are 6 most popular configurations for plant illumination cycles (constant light – plants are illuminated for 24 h; 20 h light/4h dark photoperiod; 17 h light/7 h dark; 16 h light/ 8 h dark; 13 h light/11 h dark; 12 h light/ 12 h dark; 10 h light, 14 h dark). An increase in lighting duration might facilitate compensation for low light intensity. Light intensity is responsible for photosynthesis rate. In general, the higher intensity is, more photosynthesis would occur in the plants. In terms of light spectrum, both red and blue spectrum is required for undisturbed growth. However, Sago [26] stated that rapid plant growth under favourable environmental conditions might lead to some adverse effects (*e.g.* decreased calcium concentrations in leaves, tipburns). Thus, selection of appropriate light conditions is essential. Nam et al. [25] studied effects of supplementary lighting intensity and duration on hydroponically grown plants. Five different plants were tested in four configurations (3 h additional lighting with 4000 lux and 8000 lux; 6 h additional lighting with 4000 lux and 8000 lux). Additional lighting did not support plant growth in one case. The height and diameter were smaller than in reference. The best configuration varied for different plants. Thus, it cannot be stated conclusively, which option would provide the most effective growth, as it is strongly dependent on the type of plant.

The number of plant species required to provide a nutritious diet for colonists differs from study to study, however it is certain that many plants have to be cultivated to deliver attractive and sustainable diet for colonists for long time. Therefore, it can be assumed that the number of cultivated plant species will be approximately 20 and every species may have different response to surfactants present in grey water. Increase in CO_2 concentration from ambient concentration to a maximum level of 50 000 ppm results in a higher yield of lettuce. It seems that plants exposed to an abundant CO_2 source prevail better, and that phenomena can be used to limit negative consequences of exposure to surfactants. It is however important to consider that creation of high concentrations of CO_2 require resources and energy, and costs of such modification may outperform costs of surfactant removal from grey water. All mentioned above parameters have influence on plants growth and strength and therefore when applied at proper levels might lead to success in cultivation on water containing surfactants. However, full factorial design in case of that problem is composed of at least 500 000 combinations of tests (calculated in Table 1) with each test requiring time equal to the full growth period of selected plant. Mostly this is due to large number of plant species that has to be cultivated.

2.2 Optimized approach and proposed design

The overall number of possible combinations leads to an immense experiment if full factorial design would be applied. However, the size of the experiment can be diminished for initial pilot studies. From a large number of cultivated plants, mostly consumed crops are those that deliver energy such as rice, wheat, soybean, or peanut Liu et al. [19]. Those crops require most of the cultivation area as well as water. For example, in studies conducted by Tako et al. [20] rice, soybean, and peanut covered 120 m^2 of cultivation area, while all other 19 crops covered only 18 m^2. Therefore, delivery of grey water to dominant crops is the only solution that will provide complete utilization of grey water. The number of tested species can be decreased to 3 or 4 without important loss of quality and usefulness. However, at initial stage of research only one species should be chosen. In this experiment design rice is chosen. It is also necessary to introduce cost analysis into the experiment design to eliminate those combinations that are more expensive, require more mass to be transported to colonies, or are difficult to obtain in extraterrestrial conditions than treatment of grey water. Take high CO_2 concentrations as an example as it requires heavy and high energy consuming equipment such as membranes and pumps. Additionally, high CO_2 concentrations are dangerous for humans. Therefore, even if increase in CO_2 would be beneficial for plants resistance to surfactants, costs of implementation may overcome positive aspects. The CO_2 concentration would be also dependent on chosen location. On Mars for example it might be easier to have high CO_2 concentrations in a green house because there are very high CO_2 concentrations available in the Martian atmosphere. Overall analysis led to the assumption, that first experiment should be conducted in lab scale. Our proposed experiment design includes five separate aeroponic modules, where rice is cultivated. A single module is shown in Figure 3.

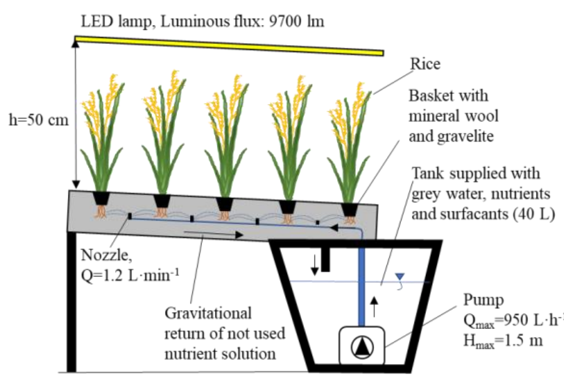

Fig. 3. Aeroponic module scheme [own source].

Four modules would be supplied with different surfactants used for space missions (SMCT, SDBS), as well as SLES, which is used worldwide. The chosen concentrations for SMCT and SDBS are 0.25 $g \cdot L^{-1}$, since this value is reported to be the boundary between affecting plant growth [28]. For SLES two concentrations are considered: 0.78 $g \cdot L^{-1}$ (corresponding average consumption of washing agents at the volume of water consumed at an international space station) and 0.11 $g \cdot L^{-1}$ (corresponding average consumption of washing agents at the volume of consumed terrestrial grey water). The fifth module is used as a reference would be fed with pure diluted nutrient solution, based on Zanão Junior et al. [34]. This solution was formulated with 1.25 $mmol \cdot L^{-1}$ K, 0.25 $mmol \cdot L^{-1}$ P, 3.75 $mmol \cdot L^{-1}$ N, 1.0 $mmol \cdot L^{-1}$ Ca, 0.5 $mmol \cdot L^{-1}$ Mg, 0.5 $mmol \cdot L^{-1}$ S, 20 $mmol \cdot L^{-1}$ Fe, 0.3 $mmol \cdot L^{-1}$ Cu, 0.33 $mol \cdot L^{-1}$ Zn, 11.5 $mmol \cdot L^{-1}$ B and 0.1 $mmol \cdot L^{-1}$ Mo. The duration of the experiment will be determined by the duration of the rice growing cycle (105–150 days), with standard illumination period of 16h.

3 Conclusions and future work

Conclusions can be derived from presented studies:

1. For untreated grey water to be applicable for aeroponics, influence of surfactants on plants growth must be determined.
2. Full factorial design of experiment aimed at determination of influence of surfactants would requires more than 500 000 tests with every test lasting at last 3 months. Large number of tests is due to large number of factors that influence plants response to surfactants.
3. It is advised to optimize approach and to limit test to crops that are cultivated to deliver energy to astronauts as these crops will consume most of the water.
4. It is also advised to develop cost analysis to rule out those combinations of parameters that would be too expensive to implement.
5. First design proposed by the authors consists of five separate modules, where rice is cultivated aeroponically. Four modules would include surfactants (SMCT, SDBS, SLES in different concentrations) and the fifth module would be a reference.

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