Soil-like substrate fertility restoration method in bioregenerative life support systems

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Abstract. The research focused on chard plants grown in a vegetation chamber under irradiation of 600 μmol/(m²s-1) FAR and elevated CO_2 concentration in the range of 1500-2000 ppm. The objective is to determine the feasibility of incorporating gaseous nitrogen-containing products of physicochemical mineralization of human exometabolites into the cycling process in relation to the conditions of bioregenerative systems of human life support. To replenish the Impoverished soil-like substrate with available forms of nitrogen, we introduced NH_4NO_3 into the irrigation solution. This can be obtained from NH_3, which is a part of gaseous intrasystem mass exchange products. The incorporation of NH_3 into the intrasystem cycle comprised three main steps. 1) Some of the NH_3 was oxidized to nitrogen oxides, which dissolved in water and formed acid solutions; 2) Another part of the NH_3 was barbotaged through a nitric acid solution to obtain NH_4NO_3; 3) The obtained ammonium salts were used as fertilizer and added to the irrigation solution for the cultivation of chard plants, which represent the phototrophic link in the bioregenerative life support system. The results clearly demonstrated that replenishing the available nitrogen forms in the Impoverished soil-like substrate to match the original substrate levels resulted in plant mass comparable to that of plants grown on freshly prepared substrate. The research results prove the potential of the developed technologies for involving dead-end gaseous nitrogen-containing waste from human activity in the intrasystem mass exchange of the life-support system.

1 Introduction

The prospects for successful space exploration are directly related to the development of bioregenerative human life support systems ("BLSS"), which allow regenerating on board an orbital station or planetary base the components of the environment vital for humans: oxygen, water and food. If for short-term space missions it is enough to use BLSS based on physical and chemical cycling of substances and reserves, then for longer missions (more than 2 years) the most promising will be systems based on biological cycling [1-3], in which the main role

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in the regeneration of the environment is assigned to the photosynthetic link of higher plants [4-6]. The concept of creation and functioning of such BLSSs includes recycling of plant and animal wastes to simple compounds [7-9]. This can occur through physicochemical and/or biological mineralization methods that allow the recycled waste to be involved in the intrasystem cycle. Among the possible ways of processing organic wastes within the BLSS, one can highlight the technologies for growing plants on soil-like substrate (SLS) [10-12] and on the mineralization products of human exometabolites [3, 13]. The technology of SLS production is based on the principles of biological conversion of organic matter due to the vital activity of fungi, worms and microflora inhabiting SLS. The main disadvantage of this technology is the rapid depletion of available forms of mineral nitrogen due to unbalanced flows between the release of nitrogen from the applied plant organic matter and its consumption by growing plants [11]. As a result, the productivity of growing plants is reduced. The technology of physical and chemical mineralization of liquid and dense human wastes is based on "wet" burning of organic waste in 36% aqueous solution of H_2O_2 under the action of alternating electric current, where in the process of oxidation liquid mineralization products are formed and a gas mixture with NH_3 vapors is emitted [13]. If the emitted NH_3 is not involved in the intrasystem mass exchange, it will lead to partial removal of nitrogen from the cycling process. Calculations show that such a loss can reach 25% of the total amount of nitrogen contained in human exometabolites, which are mineralized within a day. These technological shortcomings of biological and physical-chemical conversion of recycled organic matter indicate the imperfection of organized intrasystem processes.

It is possible to overcome these difficulties by organizing an additional technological scheme in the Biological Life Support System, which would compensate the disadvantages of both technologies. It is known that NH_3 vapors are easily removed from gas mixtures due to the neutralization reaction using aqueous acid solutions with the formation of ammonium salts Besides, NH_3 vapors can be oxidized in the presence of a catalyst to nitrogen oxides with their subsequent dissolution in water to form acid solutions as is common in industrial chemical technologies [13]. Thus, the following technological approach is proposed to engage nitrogen precipitated from the mass transfer process during oxidation of human exometabolites and improve nitrogen nutrition of plants grown on SLS: 1) NH_3 released during oxidation of human exometabolites is divided into equal parts; 2) one part of the released NH_3 is oxidized to nitrogen oxides with dissolution of the latter in water to form HNO_3 solution; 3) the other part of NH_3 is passed through the previously obtained HNO_3 solution, according to [13], to form ammonium nitrate and 4) the obtained solutions of ammonium salts are used as fertilizer for plant cultivation on SLSs.

The purpose of the work was to assess the possibility of involving nitrogen-containing gaseous products of physicochemical oxidation of human exometabolites under the above technological scheme in mass-exchange processes to increase the fertility of SLS and, ultimately, on the productivity of plants grown on it.

2 Materials and methods

Chard *(Beta vulgaris L.)* variety "Charlie" was selected as a test crop. Plants were grown in a sealed vegetation chamber at elevated CO_2 concentration, which was 1500-2000 ppm. This ensured the removal of photosynthetic rate limitation [14-16]. The chamber was periodically ventilated to prevent accumulation of O_2 in the air. Light sources were LED irradiators model LV-EAGLE x144 ADJ BIO VHP from Ledvizor (Russia). Illumination was round-the-clock, and the light intensity at the level of the upper leaves of plants was 650 ± 50 μmol/(m^2*s) FAR. The air temperature was maintained at 24 ± 1°C. The relative humidity was 60-70%. Plant sowing density was 125 pcs/m2. The duration of vegetation from sowing was 28 days.

Watering was carried out by watering the root zone once a day, after which irrigation solutions with extractive elements from the substrate flowed by gravity into watering tanks. The initial solution was distilled water.

To measure the irradiation intensity in the FAR region, an instrument model Li 250A with a quantum sensor Q-35016 (Li-COR, USA) was used. An infrared gas analyzer LI-COR 840A (USA) was connected for continuous registration and control of CO_2 level in the vegetation chamber. An electronic board was connected to the gas analyzer to control automatic CO2 supply from the gas cylinder.

Two types of SLS were selected for chard cultivation: 1) SLS in which the content of available forms of soil nutrients for plants was maximum, which corresponded to freshly prepared SLS (hereinafter referred to as fresh SLS) and 2) SLS in which the amount of available nutrients was reduced during prolonged (3 months) cultivation of plants on it (hereinafter referred to as Impoverished SLS). Determination of mobile forms (available for plant assimilation) of mineral elements contained in SLS was carried out in aqueous and slightly acidic extracts [11]. The content of mineral elements in the used types of SLS, as well as their availability for plant growth is given in Table 1. The amount of SLS used (fresh or Impoverished) for plant growth was 2000-2500 g crude or 530-620 g dry matter per growing vessel. The methodology for oxidation of human exometabolites was published in [13]. This process resulted in the release of a gas mixture consisting mainly of O_2, H_2, CO_2, NH_3 and CH_4 from the reactor, and a mineral solution was formed in the reactor, which can be used as a mineral fertilizer for plant growth. In [13], it was shown that after oxidation of human exometabolites equivalent to its daily allowance (3 L - a mixture of native waste with 36% aqueous H_2O_2 solution), the NH_3 content in the resulting gas mixture was 1.58-1.61 g per liter of oxidized human exometabolites. The method of oxidation of the released NH_3 to nitrogen oxides with subsequent dissolution of the latter in water to form an acid solution as applied to the conditions of BSJO is described in [13]. The NH_3 neutralization reaction was carried out by bubbling through the nitric acid solution. Calculations showed that this procedure could yield up to 11.3 g of NH_4NO_3 per day, based on the average daily rate of exometabolites of one person. To replenish the total nitrogen content of the Impoverished PPP to the same level as fresh PPP, 3.4±0.6 mg N per 1 g PPP dry matter had to be applied. As a result, the total amount of salt applied to the Impoverished PPP was 5.2 g per experiment.

Table 1. Macronutrient content and availability in SLSs

Concentrations and availability of macronutrients	Ca	K	Mg	P	S	N
Fresh SLS						
Macronutrient concentration, mg/g	73.1±2.9	6.5±0.2	5.9±0.4	4.4±0.3	4.9±0.5	26.6±0.5
in available form, mg/g	67.2±1.0	5.8±0.2	4.4±0.1	3.3±0.2	4.1±0.4	2.1±0.1
accessibility rate, %	95.0±1.5	88.7±3.4	74.7±1.0	80.9±4.5	60.3±6.0	7.9±0.5
Impoverished SLS						
Macronutrient concentration, mg/g	70.8±2.5	5.9±0.2	5.8±0.2	4.1±0.2	4.1±0.2	23.2±0.3
in available form, mg/g	68.5±5.5	5.0±0.5	3.3±0.2	3.0±0.1	2.3±0.1	0.4±0.1
accessibility rate, %	93.7±7.5	84.6±8.9	57.5±4.1	69.4±1.0	45.8±2.8	1.6±0.1

The solution with NH_4NO_3 was added to the irrigation solutions in weekly equal portions to mimic the gradual release of available forms of nitrogen when plants were grown on fresh SLS.

The Ca, K, Mg, P, and S contents of the samples were determined by inductively coupled plasma atomic emission spectrometry using an iCAP 6300 Duo spectrometer (Thermo Scientific, U.K.). The analysis methodology is described in more detail in [11]. Nitrate and

ammonium nitrogen content was determined using a photoelectrocolorimeter (KFK-2MP, Russia). The content of organic nitrogen was determined by the Kjeldahl method by the reaction of ammonium nitrogen with Nessler's reagent with detection at a wavelength of 440 nm. This reaction was also used for the determination of ammonium nitrogen in nutrient solutions. Nitrate nitrogen in solutions was determined by reduction to nitrite on a cadmium column and subsequent interaction with Griess reagent (sulfanilamide and a-naphthylamine) at 540 nm light filter.

Statistical analysis of data was performed using standard methods and Microsoft Excel software package. Mean values and standard errors were found at the chosen confidence probability P = 0.95 using data filtering to remove results with extreme values from the experimental data set. Biological repeatability is fourfold, analytical repeatability is threefold.

3 Results

During the vegetation of plants, periodically samples of SLS and nutrient solutions were taken to analyze the content of available forms of macronutrients. The results are presented in Table 2 and Fig. 1.

Table 2. Concentration of available forms of mineral elements in SLS, mg/g dry matter

Mineral element	Duration of vegetation, days	Fresh SLS	Impoverished SLS + NH_4NO_3	Impoverished SLS
Ca	0	67.2 ± 1.0	68.5 ± 5.5	68.5 ± 5.5
	1 4	64.4 ± 3.0	67.2 ± 3.6	66.2 ± 1.9
	21	67.5 ± 5.3	67.2 ± 2.7	64.9 ± 4.4
	28	65.7 ± 2.1	66.4 ± 2.8	65.7 ± 5.0
K	0	5.8 ± 0.2	5.0 ± 0.5	5.0 ± 0.5
	14	4.6 ± 0.3	4.5 ± 0.1	5.2 ± 0.1
	21	4.6 ± 0.3	4.4 ± 0.3	5.3 ± 0.5
	28	3.7 ± 0.2	3.9 ± 0.2	5.6 ± 0.5
Mg	0	4.4 ± 0.1	3.3 ± 0.2	3.3 ± 0.2
	14	3.1 ± 0.1	3.4 ± 0.1	3.6 ± 0.2
	21	3.2 ± 0.2	3.2 ± 0.3	3.6 ± 0.2
	28	3.2 ± 0.2	3.1 ± 0.2	3.5 ± 0.3
P	0	3.3 ± 0.2	3.0 ± 0.1	3.0 ± 0.1
	14	3.5 ± 0.2	2.9 ± 0.2	3.1 ± 0.2
	21	3.7 ± 0.3	2.8 ± 0.2	3.1 ± 0.1
	28	3.7 ± 0.1	2.7 ± 0.1	3.0 ± 0.1
S	0	4.1 ± 0.4	2.3 ± 0.1	2.3 ± 0.1
	14	2.5 ± 0.1	1.7 ± 0.1	2.0 ± 0.1
	21	2.9 ± 0.1	1.5 ± 0.1	2.9 ± 0.3
	28	2.9 ± 0.2	1.1 ± 0.1	2.7 ± 0.1
N	0	2.1 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
	14	1.9 ± 0.1	1.1 ± 0.1	0.4 ± 0.1
	21	1.2 ± 0.1	2.3 ± 0.2	0.6 ± 0.1
	28	0.9 ± 0.1	3.3 ± 0.3	0.7 ± 0.2

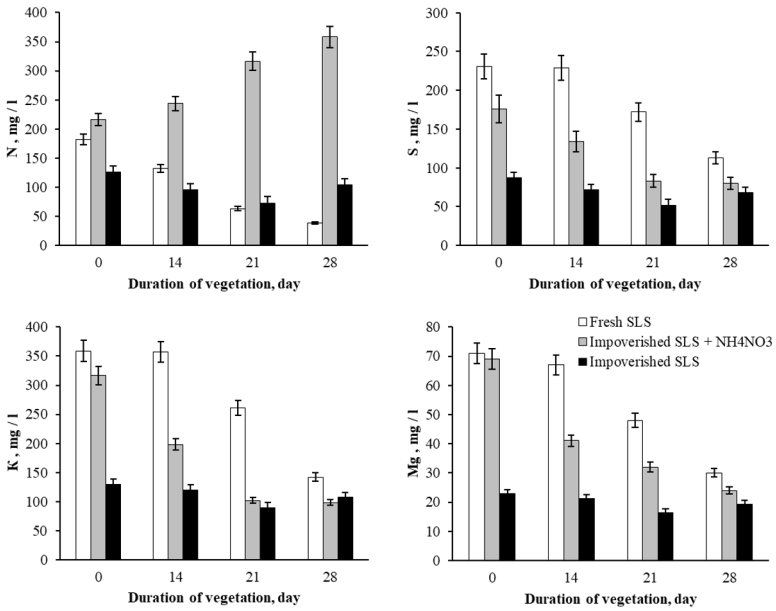

Fig. 1. Concentration of macronutrients in nutrient solutions during vegetation of chard plants grown under different conditions of mineral nutrition.

Cultivation of chard on fresh SLS led to a decrease in the concentration of readily available nutrients in the substrate and solution. At the same time, the availability of macronutrients in SLS remained high, except for N and K (Table 2), the availability of which significantly decreased by the end of vegetation of plants. A different picture was observed in the variant with Impoverished SLS. Thus, the concentration of mineral elements in the nutrient solution was in a rather narrow range relative to their average value (the deviation ranged from 7 to 12%) during the vegetation of plants. At the same time, the availability of most macronutrients in both fresh and Impoverished SLS was comparable. The exceptions were N, which was initially low in the Impoverished PPP (Table 1), and K, the availability of which did not decrease, but had a weak tendency to increase with plant growth. Periodic application of N to the nutrient solution for irrigation of the Impoverished SLS led to a gradual increase in N concentration in the solution (Fig. 1), as well as to an increase in its availability in SLS, with an almost twofold decrease in the availability of S (Table 2) by the end of plant vegetation.

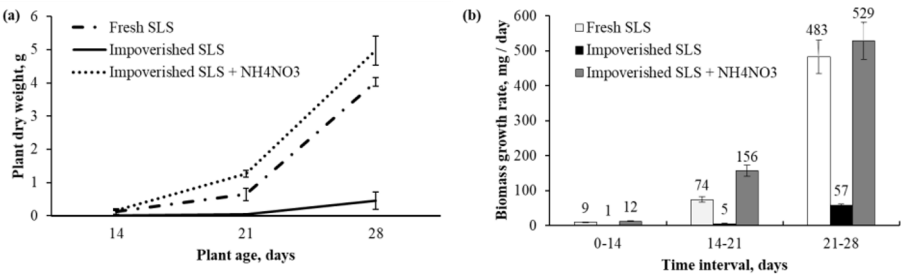

Fig. 2. Weight (a) and biomass growth rate (b) as a function of age of chard plants under different mineral nutrition conditions (per 1 plant).

Growing plants on Impoverished SLS with ammonium nitrate application had a positive effect on the accumulation of dry biomass of chard during the whole vegetation period (Fig. 2a). The biomass growth rate was the lowest in plants grown on Impoverished SLS. The biomass growth rate of plants grown on Impoverished SLS with NH_4NO_3 in the period of 0 to 14 days and 14 to 21 days was 1.3 and 2.1 times higher, respectively, than that of plants grown on fresh SLS, but no significant differences between the biomass growth rates of these variants were observed during the growth period of 14 to 21 days (Fig. 2b). As a result, the final biomass of chard plants grown on SLS with NH_4NO_3 in the solution was 1.2 and 10 times higher compared to the masses of plants grown on fresh and Impoverished SLS, respectively (Fig. 2a).).

4 Conclusions

In this research, we used SLS as a link to accept the gaseous product, NH_3, produced during physicochemical mineralization of human exometabolites as NH_4NO_3 after ammonia binding by nitric acid. We therefore assumed that the nitric acid trapping solution could also be obtained in the BLSS by processes of oxidation of NH_3 to nitrogen oxides with dissolution of the latter in water.

The uniform application of NH_4NO_3 to SLS via irrigation solution definitively restored the nitrogen pool in the plant-SLS system. This was expressed in a clear and gradual increase in the content of available nitrogen forms, not only in solution but also in SLS, due to their uptake by the soil complex. Conversely, this recovery resulted in a reduction in the availability of S and K in SLS compared to the Impoverished SLS (Table 2). However, the large mass and buffering capacity of SLS meant that the plants did not experience a deficiency of these elements. However, there is a risk of such issues occurring if long-term vegetative plants with a higher requirement for these elements than chard are grown. To avoid these negative effects, it is essential to consider the rates of consumption and accumulation of nitrogen forms in the system "plants - SLS" when improving the scientific basis of the proposed technology.

The results clearly demonstrate the feasibility and value of incorporating gaseous nitrogen-containing products of physicochemical mineralization of human exometabolites into irrigation solutions as NH_4NO_3, facilitating rapid and effective SLS exchange. The application of NH_4NO_3 can quickly and effectively compensate for the deficit of available nitrogen forms for plants on SLS, while also increasing their productivity. The considered technological processes undoubtedly offer a way to increase the coefficient of closure of BLSS that include humans, and consequently, extend the system's autonomous mode.

Acknowledgements

The research was carried out within the framework of the State Order FWES-2024-0039 of the Ministry of Education and Science of the Russian Federation.

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