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Some Methods for Human Liquid and Solid Waste Utilization in Bioregenerative Life-Support Systems

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Abstract Bioregenerative life-support systems (BLSS) are studied for developing the technology for a future biological life-support system for long-term manned space missions. Ways to utilize human liquid and solid wastes to increase the closure degree of BLSS were investigated. First, urine and faeces underwent oxidation by Kudenko's physicochemical method. The products were then used for root nutrition of wheat grown by the soil-like substrate culture method. Two means of eliminating sodium chloride, introduced into the irrigation solution together with the products of urine oxidation, were investigated. The first was based on routine electrodialysis of irrigation water at the end of wheat vegetation. Dialysis eliminated about 50% of Na from the solution. This desalinization was performed for nine vegetations. The second method was new: after wheat cultivation, the irrigation solution and the solution obtained by washing the substrate containing mineral elements not absorbed by the plants were used to grow salt-tolerant *Salicornia europaea* L. plants (saltwort). The above-ground biomass of this plant can be used as a food, and roots can be added to the soil-like substrate. Four consecutive wheat and *Salicornia* vegetations were

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cultivated. As a result of this wheat and *Salicornia* cultivation process, the soil-like substrate salinization by NaCl were considerably decreased.

Keywords Life-support systems · Closure · Human waste · Sodium chloride · *Salicornia*

Introduction

Bioregenerative life-support systems are studied for understanding the behaviour of artificial ecosystems and developing the technology for a future biological life-support system for long-term manned space missions. The driving element of a BLSS is the recovering of oxygen and edible biomass from waste generated by the crew (CO_2, faeces, urine).

The development of technology for incorporating human exometabolites into matter turnover is one of the most promising current approaches to perfecting mass exchange in BLSS [1, 2]. It will increase the degree of closure of internal mass turnover in BLSS for space applications. The basic problem of human urine introduction is that NaCl is not an important substance necessary for the normal growth and development of plants used traditionally in human diet [3–6]. Also, its concentration in soil-like substrate (obtained from roots), which is close to the concentration of major biogenic elements, subjects plants to stress, significantly reducing productivity. However, considerable progress has been made in overcoming the problems raised by the utilization of NaCl in low concentrations by crop plants representative of a BLSS phototrophic unit [6–10]. One of the possible ways of some elements drop-preventing harmful effect of NaCl on plants was experimentally tested and described by Zolotukhin et al. [11]. The feasibility of using a single substrate for four wheat vegetations was shown in this work. After each vegetation, inedible wheat biomass (straw and roots) was returned to the soil-like substrate (SLS) as a substrate, and urine and faeces mineralized by the method of Kudenko et al. [12] were introduced instead of grain. In each wheat vegetation, the mineralized nutrition solution introduced into one vessel with an area of 0.032 m^2 was equal to 17% of the human daily excretion. This portion of the human daily diet corresponds to one vegetation grain harvest from the area indicated in optimal cultivation conditions in the BIOS-3 life-support system [2, 13]. Irrigation water containing Na^+, Cl^- and other ions not assimilated by plants and not combined by SLS biogenic elements underwent direct-current electrodialysis with neutral membranes at the end of every vegetation. With this process, the Na content in irrigation water was reduced by nearly a factor of 2, which allowed the repeated use of this water for further wheat cultivation. At the same time, the levels of the other biogenic elements in irrigation water were also considerably decreased. The following question thus arose, “How long can one and the same substrate be used with a similar plant-cultivation regime?”

After electrodialysis, some part of Na, K, P, S, Ca, Mg and N elements drop out of matter turnover. It was therefore decided to check the feasibility of desalinizing irrigation water with the help of plant halophytes. *Salicornia europaea* will normally grow under NaCl concentration up to 2% [14, 15] on aqueous media containing basic biogen content in concentrations close to their content in human urine [16–18].

We set out to investigate the feasibility of using mineralized liquid and solid human wastes to grow wheat plants on one and the same substrate. To this end, two means of eliminating sodium chloride from irrigation water after wheat growing were explored. The first method was based on the electrodialysis of irrigation water at the end of wheat vegetation. The second method was to use *S. europaea* plants (saltwort) for the complete

utilization of mineral elements (mainly NaCl) in the nutrient medium together with mineralized human exometabolites not assimilated by wheat plants.

Methods

The general scheme of the experiments is shown in Fig. 1.

For the first method, we used the same technology for plant cultivation, human exometabolite mineralization and irrigation water desalinization as that described by Zolotukhin et al. [11]. During the vegetation period, we introduced mineralized urine (corresponding to 250 ml of natural urine) according to Kudenko's method [12] in 2 l of irrigation water. The quantity of mineralized solution introduced into the nutrient solution depended on the urine proportion in this solution; Na content did not exceed 900 mg. Nine wheat vegetations were cultivated according to the scheme described.

For the second method, spring wheat was cultivated on SLS with 2,880 g total weight of dry matter in four vessels with a total area of 0.13 m^2. The first wheat vegetation was cultivated on the newly made-up SLS [19–22]. Tap water (10 l) was used as irrigation water. After wheat harvesting, straw and roots were returned to SLS as substrate. Instead of grain, 3.2 l of mineralized urine and faeces were gradually introduced into the irrigation solution, equivalent to 80% of their daily rate [12]. After 10 days and before harvesting, irrigation water was replaced by tap water to eliminate NaCl not assimilated by wheat from SLS. Biogenic elements together with NaCl were extracted in the water. After wheat ripening and aboveground biomass harvesting, the SLS was washed with rinsing water. The irrigation water and rinsing water were combined (this mixture is hereafter referred to as the flushing solution). The volume of the solution obtained was 23 l. This solution was used for *S. europaea* cultivation by a hydroponic culture method. For this purpose, 4 l of the solution obtained by the above method was placed in a vegetation vessel of volume 5 l, and six plants were planted and secured with plastic foam. While the water volume in the vessel decreased by evaporation and transpiration, the rest of the flushing solution was added until

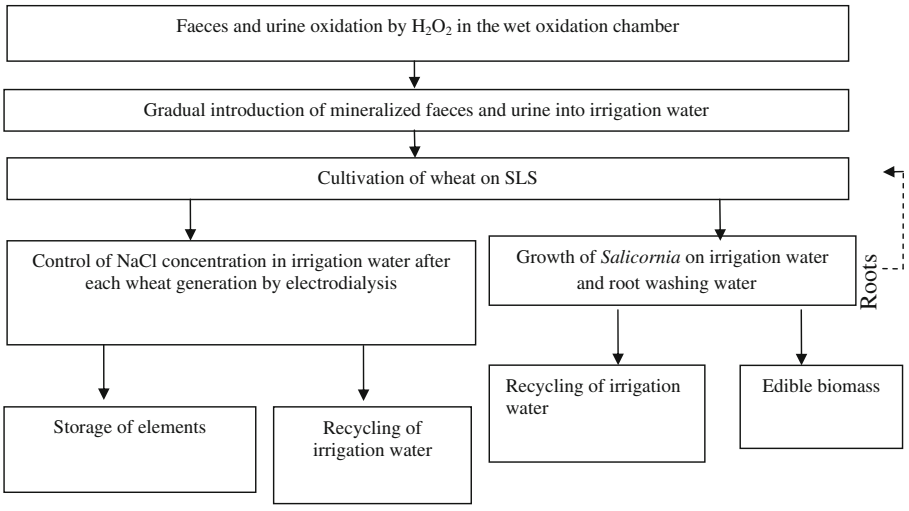

Fig. 1 Scheme of experiments performed with introduction of human mineralized liquid and solid wastes in irrigation solution for wheat watering

it was completely consumed. At the end of the experiment, there was practically no water left in the vessel. The roots were washed with an additional volume of water. This water also left the vessel by evaporation and respiration. The plants were then harvested and analysed. The plant roots were washed beforehand once more with distilled water to separate the salts that had accumulated on the plant roots from those contained in the roots.

Results and Discussion

Grain and straw yields obtained for the nine wheat vegetations, with electrodialysis used for desalinization of irrigation water, are shown in Fig. 2.

The plants grown on a green SLS (first vegetation) had the highest grain and straw mass. During the following two vegetations, considerable inhibition of plant growth and development was observed. As a result after the third vegetation, the wheat productivity was 80% lower for grain and 30% lower for straw than after the first vegetation. During the next vegetations, crop capacity increased, but the yield was not stable from one generation to the next. The average wheat crop capacity for nine vegetations was sufficiently high and compounded slightly more than 2 kg of grain and 2.8 kg of straw calculated for 1 m^2, thus exceeding the wheat crop capacity obtained in BIOS-3 [2, 13]. We note that the SLS mass relative to the root mass amounted 22.2 kg/m^2 and barely differed from the green SLS mass (start) that was 22.5 kg/m^2. We do not associate variations in wheat productivity with Na content in the solution, as from Fig. 3 it is obvious that Na concentration in irrigation water did not exceed the limit of 200–400 mg/l. Here, the lowest wheat yield (two and three vegetations) was obtained with the lowest Na content in the solution. The plant productivity may have been influenced by other factors. We assume that a lower rate of straw organic matter decomposition limited the plant growth process; as a result, the plants suffered a shortage of biogenic elements. Phenol compounds contained in straw may also adversely affect plant productivity [23].

Figure 3 shows the dynamics of Na content in the aboveground wheat organs (straw and grain) and in irrigation water before desalinization. In nine vegetations, Na concentration in straw ranged between 1% and 0.2% (*w*/*w*) and averaged 0.15% (Fig. 2 and Table 1).

Na content was insignificant in grain, and its concentration averaged 0.06% throughout the experimental period. Calculated from average plant productivity, Na absorption by plants was 182 mg. Na mostly remained in irrigation water where its concentration ranged

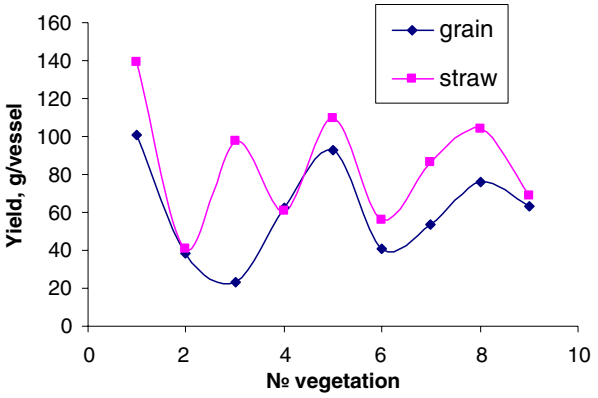

Fig. 2 Yield of wheat grown on SLS with introduction of mineralized human exometabolites and periodical desalinization of irrigation water

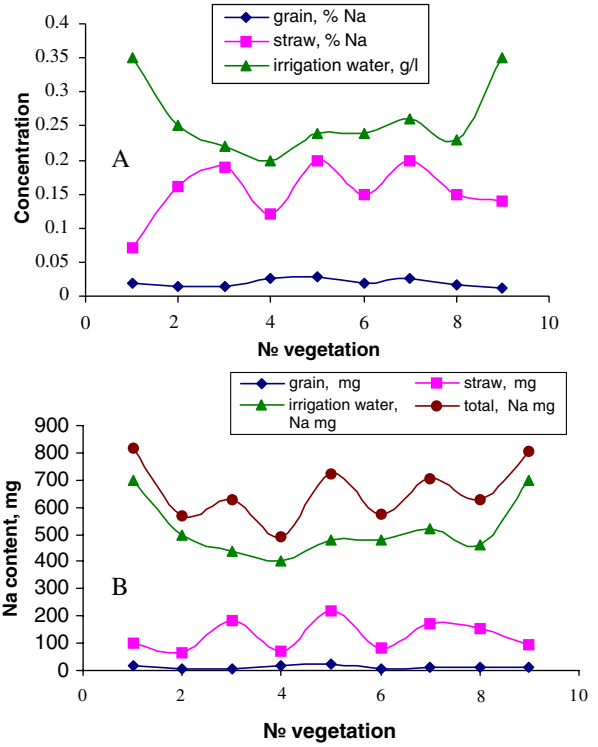

Fig. 3 Dynamics of Na content in the elements of the "wheat cenosis–SLS–irrigation water" system with introduction of 250 ml of mineralized urine in irrigation water during each vegetation period. **a** in percentage; **b** in milligrams per vessel

between 0.2% and 0.35%, i.e. irrigation water could contain from 400 to 700 mg of Na. Thus at the end of vegetation, between 600 and 800 mg of Na was eliminated from SLS. The residual Na was probably in wheat roots. After straw desalinization, Na contained in straw and the Na left in irrigation water after desalinization was returned to the SLS. After nine vegetations, this resulted in an increase in Na content to 900 mg or up to 0.23% Na concentration in the SLS.

It must be noted that besides Na, some K leaves irrigation water during dialysis with a neutral membrane. Given that total K content in the irrigation water can range from 420 to

Table 1 Na and K content in different parts of the "wheat cenosis–SLS–irrigation water" system (average for nine vegetations) obtained on 0.032 m^2.

Sample	Element	% (w/w)	mg
SLS, start	Na	0.05±0.01	360±40
	K	0.95±0.05	6,840±342
SLS with roots	Na	0.24±0.04	1,703±319
	K	0.34±0.09	2,349±614
Straw	Na	0.15±0.02	132±17
	K	2.22±0.23	1,953±204
Grain	Na	0.06±0.002	39.6±1.3
	K	0.56±0.06	369±40
Irrigation water (2 l)	Na	(0.25±0.02) g/l	500±40
	K	(0.39±0.06) g/l	780±120

1,400 mg, this loss can be important. Also, K abundance in grain can reach 0.5% based on dry matter (Fig. 4). On average, during vegetation, about 370 mg of K contained in grain was removed from the system, and 500 mg of K contained in urine was introduced into irrigation water.

In other words, the actual quantity of K introduced with urine was close to that contained in grain. As a result, in nine wheat crops, K content in the "wheat cenosis–SLS–irrigation water" system fell by 64% by the end of the last vegetation.

Thus we see that this technique of desalinization ineluctably leads to SLS salinization. As grains contain very small amounts of Na, and Na return with straw is unavoidable, complete Na extraction from irrigation water is necessary. In this case, the process of SLS salinization will be reduced. At the same time, a technique to decrease K output during dialysis will have to be developed. This will probably be possible using special membranes. Otherwise, a K deficit will occur in the "wheat cenosis–SLS–irrigation water" system and lead to decreased plant productivity.

To prevent loss of K and other biogenic incoming ions eliminated from irrigation water during dialysis and that did not subsequently take part in matter turnover processes, we tested the other scheme for the desalinization of irrigation water (Fig. 1). After wheat vegetation, irrigation water combined with rinsing water was used as an initial solution to cultivate *S. europaea* halophyte plants. The concentrations of mineral elements in rinsing water were fairly close to those of mineral elements in the Knop reference solution except for Na (Table 2). Na content in rinsing water was 1.3 times higher than K content. With our new technique, the quantity of biogenic elements in rinsing water was much closer to

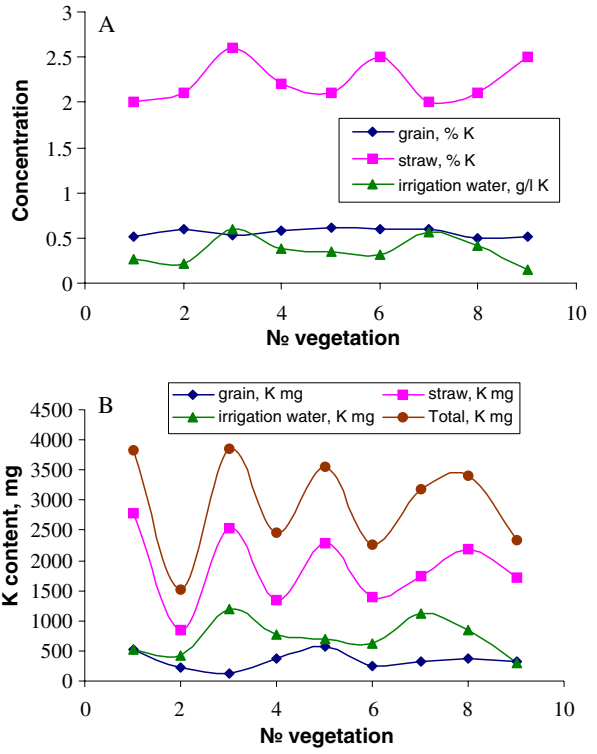

Fig. 4 Dynamics of K content in the elements of the "wheat cenosis–SLS–irrigation water" system with introduction of 250 ml of mineralized urine in irrigation water during one vegetation period. **a** in percentage; **b** in milligrams per vessel

Table 2 Total quantity of macroelements in rinsing water and introduced in the solution simulating urine mineral composition (except for nitrogen) during a vegetation period.

Solution variant	P	S	K	Na	Ca	Mg	N
Rinsing water (mg/l)	116	46	102	136	112	44	127
Rinsing water (mg in 23 l)	2,668	1,058	2,346	3,128	2,576	1,012	2,921
Model solution (mg/l)	1,384	2,358	5,922	7,746	1,915	434	353
Knop solution (mg/l)	57	33	168		170	24	153

concentrations of these elements in urine than in standard mineral solutions for constant volumes of urine and mineral solutions (Table 2). Tikhomirova et al. [16] showed the feasibility of *S. europaea* cultivation on solutions imitating urine in their mineral element content (except for nitrogen) with comparable Na and K quantities. At the same time, it appeared that saltwort was capable of active absorption and accumulation of these elements in the aboveground biomass but could not selectively absorb Na over K. Based on the above, we compared plants cultivated on a model solution simulating one of the possible variants of biogenic element content in natural urine except for nitrogen, the starting content of which was 170 mg/l. As required, we carried out a correction using an initial solution with a Na content of 8 g/l. Mineral element concentrations in the model solution introduced during plant growth are given in Table 2. During cultivation on the model solution, the nutrient solution volume was maintained at 4 l.

From Table 2, the total content of mineral elements is evidently higher in the model solution than in rinsing water, but a fairly high yield of the aboveground biomass of *S. europaea* cultivated on such solutions was obtained (Table 3). This suggests that *S. europaea* cultivation on the solution obtained by the above described technique would be feasible.

Total yield of wheat aboveground biomass obtained by cultivation on an area of 0.13 m^2 with introduction of 3.2 l of mineralized urine in irrigation water was 702 g; 400 g of straw was returned to the SLS for the next vegetation, and mineralized urine was used instead of 302 g of grain. *S. europaea* was then cultivated on the solution left after wheat cultivation (Table 3). Total dry plant mass was 55 g, the edible part was 42.6 g, and the harvest index was 77%. The plant mass was less than the mass of the plants grown on model solutions. Nevertheless, it was sufficient to utilize the Na contained in the rinsing water, as shown previously [16] with comparable Na and K concentrations in solutions, and the aboveground saltwort biomass could accumulate up to 10% of Na based on dry biomass.

K and Na distribution in the system under investigation is given in Table 4. In all, at the end of wheat vegetation, there was 7.3 g of Na and 17.3 g of K in the system, taking into account the SLS starting mineral composition.

After ripening, there was in all 13,489 mg of K and 4,816 mg of Na in the wheat aboveground organs (straw and grains) and in rinsing water. As straw was returned to SLS, the elements introduced with mineralized urine were removed from the “SLS–mineralized

Table 3 Yield of saltwort grown on the solution left after wheat cultivation and on the model solution.

Variant	Mass of aboveground part, g		Dry mass of roots, g
	Raw	Dry	
Rinsing water	334±11	42.6±1.2	11.4±1.2
Model solution	581±27	54±2.4	12.0±1.2

Table 4 Na and K content in the system with wheat and *S. europaea* consecutive cultivations.

Quantity of mineral elements	K, mg	Na, mg
Content in SLS before planting	14,400±900	2,778±300
Content in 3.2 l of mineralized urine and faeces	2,888±280	4,500±300
In straw	9,600±650	1,692±90
In grain	1,523±46	0
In rinsing water	2,366±150	3,124±300
Aboveground biomass of 6 saltwort plants	1,060±100	2,130±210
Roots of 6 saltwort plants	290±30	189±15
Rinsing water after washing of saltwort roots	880±90	640±70

urine" system with grain and rinsing water. The Na accumulated by straw was returned to the SLS, increasing its content in substrate. However, previous experiments suggest that Na content in straw cannot exceed 2% based on straw dry mass (Fig. 2). Excess Na will therefore leave the SLS in rinsing water, and so Na will not accumulate in the SLS from one vegetation to the next. The mass of K exported from the "SLS–mineralized urine" system will accumulate in straw, and after straw return to the SLS, K will be re-incorporated in matter turnover (Table 4). The K contained in grain and carried away in rinsing water exceeds its content in urine by almost 1,000 mg. Additional K exportation is related to its high content in the SLS. There will obviously not be enough to compensate for this exportation and ensure the wheat productivity. So long as the SLS buffering is sufficient, there will be no limitation of wheat cenosis growth and development. Probably at some stage a point will come where a balance will be reached between K incoming with urine and exportation with grain and rinsing water. In addition, K contained in straw will quantitatively regenerate the next straw generation.

Analysis of saltwort mineral composition showed that about 70% of Na contained in rinsing water was fixed in aboveground organs, about 20% remained non-absorbed by roots, and about 10% was fixed directly in roots (Table 4). Thus at any given time, about 30% of Na cannot be used as a nutrient. However, it can apparently be used either further for saltwort cultivation (rinsing water after the root washing) or be introduced into the SLS (roots and rinsing water after the root washing). Introduction of approximately 200 mg of Na contained in roots in 2,880 g of the SLS will increase its concentration by less than 0.01%, and introduction of salts contained in rinsing water after the root washing will increase Na by 0.03%. We assume that after the next cultivation of wheat cenosis on the SLS and the subsequent collection of rinsing water by the technique described above, Na will return to the solution for saltwort cultivation. Na concentration will increase in this solution, causing an increase in Na absorption by saltwort. Tikhomirova et al. [16] and Ushakova et al. [17] showed that when cultivated on model solutions (Table 3) the saltwort aboveground biomass could accumulate up to 10% of Na, and when cultivated on standard solutions with addition of 4 g/l of Na its content in aboveground biomass could increase up to 14% based on dry mass. Thus in saltwort cultivation on rinsing water yielding 42 g of dry aboveground biomass, we can expect accumulation of not less than 4 g of Na in aboveground biomass.

With the solution obtained after root washing and the roots themselves (when there is no need to wash them), slightly more than 1,000 mg of K will be returned to the SLS (Table 4). Thus the loss of K contained in the SLS will be minimized because the quantity of K introduced with urine will be equal to the quantity of K exported by grain and the saltwort aboveground biomass (Table 4).

The above analysed variants of introduction of human liquid and solid wastes into matter turnover and the first desalinization variant with use of irrigation water dialysis help to solve the problem of decreasing Na content in the SLS. The return of biogens removed along with Na in matter turnover can, in principle, be achieved by physicochemical methods. It may be possible to solve this problem by a biological method using the elements eliminated during dialysis as a basis for a nutrient solution for saltwort cultivation.

The second process of desalinization by the use of *S. europaea* allows all the mineral components of urine and faeces to be returned into LSS matter turnover including a human. The aboveground saltwort biomass, containing significant quantities of Na and K, can be used as a foodstuff, and the roots and mineral elements not absorbed by roots can be used for repeated plant cultivation. Further experiments over several cycles of successive wheat and saltwort cultivation, including mineralized human exometabolites according to the scheme described above, will help optimize the plant cultivation technology and provide more precise information on the distribution of mineral elements in BLSS matter turnover.

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