I. TECHNOLOGIES FOR DEPOLUTION

BIOTRANSFORMATION OF USED MEANS OF PERSONAL HYGIENE OF COSMONAUTS AND VEGETABLE WASTE AS APPLIED TO LIFE SUPPORT SYSTEMS FOR SPACE CREWS

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Abstract: In this paper a possibility of applying anaerobic digestion for reduction and stabilization of the organic fraction of solid wastes generated during piloted spacecraft flights is discussed. The culture of *Trihoderma viridae*, capable of utilizing cellulose-containing substrates under mesophilic conditions, was studied. The strain was isolated from the internal environment of the ISS. The main purpose of the work was to test the ability of *Trihoderma viridae* culture to consume the products of anaerobic decomposition of gauze tissue under space flight conditions, i.e. substrate for the cultivation of the fungus served as liquid media, formed after the decomposition of thermophilic clostridia gauze wipes. Under conditions of space flight, the culture of *Trihoderma viridae* reduced the total concentration of volatile organic impurities remaining after the biodegradation of gauze from 34 mg / m³ to 5 mg / m³. The number of detectable volatile compounds decreased almost twice. The carried out researches have shown that the post-cleaning of liquid products of hydrolysis of gauze fabric with the help of cellulolytic fungi can be an effective component of utilization of cosmonaut hygiene items with the help of microbial communities in space flight conditions. Also under terrestrial conditions, the liquid products of biodegradation of plant wastes were further purified with the help of a community of aerobic bacteria. Chromatomass-spectrometric analysis showed that the consortium composed of aerobic mesophilic bacteria actively consumed products present in vegetable homogenates.

Keywords: biodegradation, anaerobic digestion, cellulose containing wastes, *Trihoderma viridae*, piloted spacecraft flights, microgravity conditions

INTRODUCTION

Long terms of the planned space expeditions require the creation of the most closed LSS of crews in spacecraft with cyclic regeneration of substances in an artificial ecosystem [8]. One of the main conditions for increasing the closure factor of LSS is the processing of waste in order to return the maximum proportion of the elements contained in them to the circulation of substances on board the MSC.

Among the wastes that are formed in the conditions of manned space flight, a significant proportion is spent on personal hygiene (gauze napkins). A necessary element of the closed LSS of manned spacecraft are considered greenhouses for the reproduction of the vegetative part of the diet of cosmonauts. Uneaten parts of plants are waste from greenhouses. Used personal hygiene products and greenhouse waste accumulate will occupy significant volumes, which will lead to an increase in the equivalent weight of the system [3, 12]. In addition, the waste contains products of human body secretion, which are dangerous in the sanitary-epidemiological sense. In the perspective of interplanetary flights, based on the requirements of planetary quarantine, it is not possible to carry out the contaminated with microorganisms debris beyond the spacecraft.

Thus, spent solid organic materials must be processed onboard the spacecraft. Among the known methods of degradation of organic materials, the most

preferred should be considered such that the final products of which can somehow be disposed of within the LSS for organizing the circulation of substances. In particular, it would be desirable to use the final liquid waste products as a starting medium for the preparation of nutrient solutions for hydroponic plant cultivation. This would significantly increase the coefficient of closure of the mass flow in the onboard MSC. Similar problems also arise with respect to the physiological waste of the crew, since the decontamination procedures require additional on-board resources, and the waste itself is a growing deadlock in the scheme for cycling substances in the regenerative LSS.

In the case of the application of systems for the disposal of organic waste in space LSSs, in addition to the ecological aspect, that is, the depth and efficiency of processing, technical problems and limitations are also very topical: the technologies used must meet the requirements of low energy consumption, high processing speed, on the area, volume and mass of the processing system.

Such a method as combustion is not optimal, because when burning organic waste, leaches and toxic nitrogen oxides are formed, the utilization of which in the hermetic object is extremely difficult [11].

One of the natural ways of recycling waste is their microbial degradation. In general, microbial degradation is an artificial intensification of

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mineralization processes in natural biocenoses. For example, for the hydrolysis of cellulose-containing materials it is advisable to use anaerobic microorganisms that have a number of important advantages: 1) cellulolytic anaerobes, as well as other fermenting anaerobic crops, have a slight biomass gain - most of the substrate is transformed into various end products such as ethanol, organic acids, CO₂, H₂, etc. [4], which are easier to dispose later than microbial biomass; 2) they have a high specific cellulolytic activity, which depends little on the nature of the substrate; 3) they have relatively high growth rates on cellulose-containing substrates [6]. Since at present one of the constantly accumulating types of airborne waste, which has to be regularly returned from the MSC to the Earth, is the used gauze wipes, in our work the problem of their degradation with the help of anaerobic microorganisms was considered.

One of the significant problems in the way of anaerobic degradation of solid organic waste is that liquid fermentation products contain a significant amount of organic impurities, which often give them a low pH. This circumstance does not allow to use the received liquids as technical water or in any other way without additional processing. It should be noted that the accumulation of liquid organic waste originating from plant residues is also a problem in agriculture, where only 10% of these substrates are somehow disposed of.

Within the framework of the approach we propose, gauze and airborne plant waste are disposed of in two consecutive stages. First, the primary anaerobic biotransformation of the materials is carried out, and the resulting liquid products are used as media for the cultivation of fungi and aerobic bacteria. Anaerobic fermentation of gauze is carried out with the help of the bacterium *Clostridium thermocellum*. The decomposition products of cellulose dissolved in the culture liquid are decomposed in the second stage by microscopic fungi. In the case of biodegradation of plant waste, at the first stage, the associations of anaerobic microorganisms act as primary biodestructures. For the subsequent decomposition of organic substances, either fungi (at acidic pH values) or an association of aerobic bacteria (at neutral pH values) are used.

The **purpose of this work** is to study and develop the processes of the biotransformation of the used personal hygiene products of cosmonauts, as well as vegetable and liquid organic waste, to the life support systems of space crews.

Objectives of work:

1 - isolation, selection and research of microorganisms carrying out fermentation of cellulose-containing waste (gauze and vegetable waste);

2 - evaluation of the efficiency of post-treatment of aerobic bacteria and fungi of solutions formed after fermentation of gauze and vegetable waste: by parameters such as: total concentration, total molar mass and the coefficient of contamination of volatile organic substances;

3 - study of the influence of space flight factors (real and simulated) on the fermentation of vegetable wastes and the post-treatment of hydrolyzed gauze with *Trichoderma viridae* culture.

METHODOLOGY

The following microorganisms were used in the work:

- cultures of anaerobic thermophilic bacteria *Clostridium thermocellum* strains F1, F4, F7, F9, F13, F15, F19, LQRI, LQR9 from the collection of the laboratory of anaerobic metabolism of IBPM RAS named after G.K. Scriabin;

- association of mesophilic anaerobic cultures trophically adapted for various types of plant waste, from the collection of the IBMP RAS;

- aerobic mesophilic cultures of *Pseudomonas esterophilus* VKM-1736D, *Paracoccus denitrificans* VKM B-1324, *Achromobacter parvulus* VKM-1541D and *Stenotrophomonas maltophilia* VKM B-591 from the All-Russian Collection of Microorganisms of the Russian Academy of Sciences;

- cultures of cellulolytic fungi *Chaetomium globosum*, *Trichoderma viride*, *Rhodotorula glutinis*, *Candida sp.* from the collection of IBMP RAS.

Biodegradation substrates were: dry wiping gauze used by cosmonauts as sanitary napkins, homogenates of vegetables: cabbage stalks, potato and carrot peels, liquid products of microbial decomposition of the above materials.

Media and conditions of cultivation of microorganisms

Thermophilic anaerobic bacteria

Gauze, used by cosmonauts as dry sanitary napkins, consists of natural cellulose fibers (70-100%) and inclusions of synthetic polymeric materials (up to 30%). It is known that thermophilic cellulolytic bacteria of the genus *Clostridium* are the most promising agents capable of destroying

cellulose of various degrees of ordering [1]. The advantages of these bacteria are high specific endoglucanase activity that depends little on the nature of the substrate, and relatively high growth rates on cellulose-containing substrates.

For the cultivation of thermophilic anaerobic bacteria, a medium with the following composition (g/l) was used: NH_4Cl - 2.0 (or urea), MgCl_2 - 0.2, CaCl_2 - 0.07, FeSO_4 - 0.0025, K_2HPO_4 - 3.0, KH_2PO_4 - 3.0, yeast extract 6.0, resazurin - 0.0002, cysteine hydrochloride - 0.125, Na_2S - 0.125, cellobiose - 5 (other sources of carbon), prepared under anaerobic conditions. The initial pH of the medium is 6.8.

Thermostable components of the medium were sterilized by autoclaving at 1 atm, 1 hour, 120°C, the yeast extract was autoclaved as a 10% solution at 0.5 atm, 30 min, 120°C. The reducing agent (cysteine sulphide) was prepared separately, autoclaved at 0.5 atm, 15 min, 120°C and added to the medium immediately before use.

Depending on the research objectives, the environment underwent corresponding changes: with gauze biodegradation, it was introduced in the concentrations of 25 and 50 g/l. Cultivation was carried out at a temperature of 55°C, under anaerobic conditions. The medium and solutions were prepared in distilled water.

The identification of cultures was carried out by conventional methods [10]. The medium of the above composition was used for cultivation. The cultivation was carried out in Hangate test tubes with butyl plugs with a total volume of 15 ml, the working volume of the medium was 10 ml. Screening of cultures was carried out by cultivation of strains on microcrystalline cellulose MN-300 (10 g/l). Cultivation was carried out in bottles with butyl plugs with a total volume of 125 ml, the working volume of the medium was 100 ml. The initial pH of the medium was 6.8. Cultivation was carried out at a temperature of 55 °C until the cultures reach a stationary growth phase.

Mesophilic anaerobic microorganisms

As a medium for mesophilic anaerobic associations, substrates were used for biodegradation (plant waste: carrot and potato peelings and cabbage cobs), placed in a 1% solution of peptone. The initial pH was 7.0, the cultivation was performed at a temperature of 37 °C in 500 ml vials. The weight of dry substrate (vegetable waste) was 50 g. The volume of water added was 250 ml. Preliminary sterilization was not carried out.

Mesophilic aerobic microorganisms

The basis of media for aerobic mesophilic cultures was the liquid products of anaerobic biodegradation of plant waste: cabbage stalks, potato and carrot cleanings. The initial pH was neutral or slightly acidic. In the latter case, 10% NaOH was used to correct the pH level. Cultivation was carried out on Petri dishes and in 750 ml flasks at 29 °C.

In the process of cultivation, the liquid part of the substrate was selected and seeded into dense and liquid differential-diagnostic nutrient media.

To study the quantitative composition and the preparation of isolated colonies of microorganisms, nutrient media such as triprazo-soy agar, potato agar, yeast agar, Haektoen agar, Saburo medium, Czapek's medium, Ashby medium, sulfite-iron medium, sterile control medium, Vinogradsky medium, Wednesday Mishustin, Wednesday Imshenetsky, Wednesday Getschinsky.

Identification of the isolated microorganisms was carried out using an automated bacterial analyzer Vitek-60, bioMerieux France.

Cellulolytic fungi were cultivated in 500 ml vials on media that are liquid products of anaerobic biodegradation of gauze and plant waste. The initial pH is 5.5, the temperature is 37 °C. Before introducing the cultures, the liquid substrates were sterilized by autoclaving at 1 atm. 1 hour.

Estimation of biodegradation parameters

The degree of gauze degradation was assessed by the degree of substrate consumption and expressed as a percentage of its original amount contributed to the growth medium. The substrate consumption was assessed by weighing on filters through which the entire volume of the sample (10ml) was filtered after treatment with 25% formic acid to destroy the bacterial cells (1 ml of acid per 2 ml of medium). Growth was monitored by measuring the optical density at $\lambda = 600 \text{ nm}$ on a Specol spectrophotometer (Germany).

The metabolic products were determined chromatographically.

The goals of the studies of the chemical composition of gases formed during the degradation of medical gauze were the qualitative identification and quantification of microimpurities released into the air.

Samples of liquid biodegradation products (culture liquids formed after hydrolysis of gauze (25 and 50 g/l) and vegetable wastes (200 g/l), volume 4 ml were placed in 9-liter desiccators in open Petri dishes and kept for several days at temperature

of 23°C. Analysis of air samples was carried out according to the procedure recommended by GOST R ISO 16000 ("Air of closed rooms - Part 6. Determination of volatile organic compounds in air in enclosed spaces and a test chamber by active sampling of the sorbent Tenax TA, followed by t The air samples were taken to the adsorption tubes "Gerstel" with a layer of sorbent, subjected to thermal desorption at 290°C for 4 minutes with simultaneous cryogenic capture of volatile components at -30°C and subsequent chromatographic separation by HP-5MS capillary column with programming of column temperature from 30 to 280°C and detection by a quadrupole electron impact ion mass analyzer (ionization energy 70 eV) in the range m/z 2-500. Identification of compounds was carried out using the libraries of mass spectra of the National Institute of Standards USA. Calibration of the Agilent GC 5973 MSD with integrated Gerstel desorption and venting systems TDS3 and CIS4 was carried out using solutions of the target compounds manufactured by Sigma-Aldrich (Switzerland). When sampling a volume of 0.5 liters, the detection limit of the identified compounds in the mode of full mass scanning is 5 x 10-12 g/m³.

Microbial aftertreatment of liquid biodegradation products of various organic substrates under orbital flight conditions:

For the aerobic aftertreatment of the substrates remaining after the biodegradation of the gauze tissues, a strain of the fungus *Trihoderma viridae*,

capable of growing on a weakly acidic medium, was selected and its pH adjusted to neutral values.

The study was carried out on board a biosatellite Bion-M1, which was placed bioreactor, designed for fermentation in space flight.

For the experiment, the biocorot BTS.084.011.000, Head # 03 Fragmenter, produced by BioTechSIS, equipped with a gas separation membrane (Fig. 1-1) was used. The working volume of the bioreactor was 40 ml.

Seeding of the bioreactor was carried out according to the following algorithm:

Before planting, the bioreactor was disassembled into components, abundantly sprinkled all the details with a 70% solution of ethyl alcohol, placed in a laminar box and irradiated under a UV lamp for 30-40 minutes. Then thoroughly rinsed all the details of the bioreactor in sterile distilled water, collected the stirrer (Fig. 1 - 5, 6 and 9) and placed in a vessel of the bioreactor (Fig. 1 - 1).

The ready-made bioreactor was filled in succession with the ready-made sterile Czapek medium without the carbon component (volume 15 ml), the inoculum of *Trihoderma viridae* culture (5 ml) and the liquid products of biodegradation of gauze tissue (20 ml).

Then the membrane was placed in the bioreactor (Fig. 1-3, 1-4 and 1-8), the substrate was fixed with bolts and a ring (Fig. 1-3, 1-7) and then the piston cover and the fixing ring were installed (Fig. 1-3, 1-7). In the final, a pressure and temperature sensor was installed and screwed the top cover.

Fig. 1. The bioreactor of the BTS. 084.011.000, Plant No. 03, production of BioTechSis. 1. Bioreactor capacity, 2. Bioreactor lid-piston, 3. Pressure relief connector, 4. Membrane support (metal frame and silicone gasket), 5. Magnetic stirrer (base, metal frame and magnet), 6. Magnetic stirring blade and axis, 7. Shut-off ring, 8. Polymer gas permeable membrane, 9. Stirrer lock, 10. Top cover, 11. Built-in pressure and temperature sensor.

As a control, cultivation of the fungus *Trichoderma viridae* on a liquid medium obtained after the degradation of the gauze with the monoculture *Clostridium thermocellum* was carried out, with the addition of the mineral components of the Czapek medium. Cellulolytic fungi were cultivated in 500 ml flasks at a temperature of 37 °C. Before introducing the cultures, the liquid substrates were sterilized by autoclaving at 1 atmosphere for 1 hour.

The metabolic products were determined chromatographically. The goals of the studies of the chemical composition of gases formed during the degradation of medical gauze were the qualitative identification and quantification of microimpurities released into the air.

Cultivation of the *Trihoderma viridae* strain on the satellite was carried out for 14 days at 37 °C.

RESULTS

Biodegradation of personal care products using *Clostridium thermocellum* culture:

In the present work, the effectiveness of the application of different bacterial strains for the destruction of gauze wipes was determined with a high content of the original ungrounded substrate. The results of a study of the properties of the 5 strains of *Clostridium thermocellum* are presented in Table 1. Based on the results obtained, a choice was made in favor of strain F1, which exhibited the highest specific growth rate on cellobiose and the yield of cells on the used substrate.

Table 1. Growth parameters of strains of *Clostridium thermocellum*

Strain	Conditions for cultivation	OD600	Specific growth rate, h ⁻¹	Cell yield, g/l
F1	Hangate Test tubes, 10 ml of media, 55°C	0.85	0.091	0.55
F4		0.60	0.043	0.39
F7		0.38	0.038	0.37
F13		0.46	0.040	0.28
LQRI		0.65	0.067	0.43

Further, strain F1 *Clostridium thermocellum* was used for biodegradation of gauze with a thermophilic anaerobic culture on an aqueous medium with gauze napkins as a carbon source. The initial weight of napkins in the medium was either 25 or 50 g/l. Cultivation was carried out in bottles with a total volume of 500 ml, working volume of the medium - 200 ml. The initial pH of the medium was 6.8. Seed was obtained by culture culture on cellobiose and added in an amount of 4.5%. The initial density of the medium was 0.009. Cultivation took place for 4 weeks at a temperature of 55°C. Growth was controlled by measuring the optical density by the

above method. The experiment was carried out in three variants: 1-cultivation of the strain on the medium without any additives (control); 2 - cultivation on medium with initial single addition of chalk to neutralize pH (10 g/l); 3 - cultivation on medium with periodic addition of alkali.

Data on the growth of *Clostridium thermocellum* F1 on a medium containing napkins as a substrate are presented in Table 2 and in Fig. 1. The greatest increase in biomass and maximum destruction of the substrate was observed in variants where the medium contained chalk. The presence of chalk in the medium significantly reduced the lag time.

Table 2. Growth of *Clostridium thermocellum* F1 culture in saline medium with gauze

№	The content of gauze, g/l	Time, days																				Substrate consumption, %
		0		2		5			7			14			17			20			29	
		OD	pH	OD	pH	OD	pH	NaOH	OD	pH	NaOH	OD	pH	NaOH	OD	pH	NaOH	OD	pH	NaOH	pH	
1	25	0,09	6,8	-	-	0,09	6,2		0,13	7,1		-	-		-			0,22	5,5		5,6	10,7
2	50	0,09	6,8	-	-	0,15	5,2		0,22	7,1		-	-		-			0,32	5,2		5,2	6,2
3	25	0,09	6,8	-	-	0,34	6,4		-	6,8		0,37	6,5		-			0,59	5,9		6,0	100
4	50	0,09	6,8	0,13	6,8	0,34	5,9		-	6,7		-	-		-			0,90	5,8		5,9	95,6
5	25	0,09	6,8	0,14	6,8	0,16	5,8	2,5	H.o.	5,2	4,0	0,37	6,0	2,0	0,36	5,9	2,0	0,38	6,4	1,0	5,7	17,9
6	50	0,09	6,8	H.o.		0,13	6,6	1,0	H.o.	6,8		0,17	6,7	1,0	0,35	5,2	4,0	0,43	5,5	3,0	5,6	1,0

Fig.2

Data on the fermentation of the tissue material of the tissue culture are shown in Table 3. According to the experimental data obtained earlier [2],

no more than 10% of the mass of chalk introduced into the medium is consumed to neutralize *C.thermocellum* products.

Table 3. Consumption of substrate with *C. thremocellum* F1 growth on medical gauze for the 29th day of fermentation

№ п/п	Initial amount of substrate, mg	Filter weight with substrate, mg	Filter weight, mg	Mean Cell Weight, mg	Residual substrate, mg	Degree of degradation substrate, %
1	5000	5900	1386	48	4466	10.7
2	10000	10838	1386	70	8544	14.6
3	5000	10746	1386	130	0	~ 100
4	10000	12021	1386	198	437	~ 95
5	5000	5577	1386	84	4107	17.9
6	10000	11381	1386	95	9900	1.0

The increase in the substrate load to 25 and 50 g/l led to an elongation of the lag phase to 5 or more days and a rapid acidification of the medium in variants without addition of chalk. The addition of alkali did not significantly affect the extent of degradation of the substrate. In variants with the addition of chalk,

the lag-period value was significantly reduced, the rate of biomass growth was observed, and the degree of substrate degradation reached 25 g/l for 2 weeks. In the variant with the initial content of tissue material 50 g/l, almost complete degradation occurred in 4 weeks (Fig. 2).

It can be assumed that a significant increase in the rate of degradation of the substrate with the addition of chalk is partly due to the stimulating effect of calcium ions formed during the neutralization process on the work of endoglyukinase of strain F1.

The dynamics of the mass of hydrolyzed gauze in solution (y) in variants with addition of chalk to the medium is satisfactorily described by a linear regression equation of the form: $y = 0.021 * x$.

Hence: during the time $x = 1$ day, mass of hydrolyzed gauze $y = 1,7857$ g/l. We round this value to 1.8 g/l. One package of dry personal care products for cosmonauts contains 25 g of gauze with an area of 2 m². To decompose this mass within 24 hours, approximately 14 liters of medium will be required. If you examine the scenario of a manned expedition to Mars with a crew of six people, described in the work [5], then a fermenter with a working volume of 85 liters will be needed to recycle their daily napkins.

Fig. 3

Proceeding from the dependence shown in the form of the graph in Fig. 3, the specific value of the biomass of *Clostridium thermocellum* cells can be calculated. It will be equal to 65 mg of biomass / 1 g of substrate. For 6 recycled napkins (initial weight

150 g), the biomass will be 9.7 g. in biomass passes 6.5% of the original mass of gauze.

Thus, 150 g - 9.7 g = 140.3 g of substrate are converted to biogas and liquid biodegradation products:

$$M(C_6H_{12}O_6) = 190 \text{ g/mol}$$

$$M(CO_2) = 44 \text{ g/mol}$$

$$190 \text{ g/mol} / 140,3 \text{ g} = 44 \text{ g/mol} / m(CO_2)$$

$$m(CO_2) = 140,3 \text{ g} * 44 \text{ g/mol} / 190 \text{ g/mol} = 65 \text{ g}$$

$$n(CO_2) = m(CO_2) / M(CO_2) = 65 \text{ g} / 44 \text{ g/mol} = 1,5 \text{ mol}$$

Under normal conditions $V_m(CO_2) = 22,4 \text{ l/mol}$, from here:

$$V(CO_2) = 22,4 \text{ l/mol} * 1,5 \text{ mol} = 33,6 \text{ l}$$

33.6 liters is theoretically the maximum volume produced from 150 g of CO² gauze, if we assume

that all cellulose is fermented to ethanol, lactic and acetic acids and carbon dioxide.

Data on the growth and consumption of the substrate (Tables 2, 3 and Fig. 2) indicate a positive effect of the addition of chalk on the processes of hydrolysis of gauze with the culture of *Clostridium thermocellum* F1.

Microbial aftertreatment of liquid products of biodegradation of gauze wipes

According to the data of chromatographic mass spectrometry in liquid media formed as a result of anaerobic fermentation of gauze tissue, a significant number of organic impurities were contained.

To purify the substrates obtained, four cultures of fungi and yeast isolated from hermetically sealed objects were selected which grew in a liquid medium where the products of decomposition of

plant waste and gauze (*Chaetomium globosum*, *Trihoderme viride*, *Rhodotorula glutinis*, *Candida sp.*). The ability of these cultures to change the pH of the medium from weakly acidic to neutral weakly alkaline is shown (Table 4) Based on the results of chromatographic mass spectrometry (Fig. 4), a significant decrease in the total molar mass of the detected organic impurities contained in the primary products of gauze destruction, after additional cleaning with the help of mushroom cultures. The average concentration of volatile organic impurities decreased to trace values (Fig. 5). These cultures were able to maintain a steady growth, feeding on the products of the metabolism of bacteria that carry out primary biotransformation.

Table 4. pH value of culture liquids of fungi and yeast

Culture	Initial pH of the medium	pH, 5 day
<i>Candida sp.</i>	5.5	6.96
<i>Saccharomyces cerevisiae</i>	5.5	6.75
<i>Rhodotorula glutini</i>	5.5	7.32
<i>Hypomyces sp.</i>	5.5	7.44
<i>Cladosporium cladosporioides</i>	5.5	6.12
<i>Chaetomium globosum</i>	5.5	7.18
<i>Trihoderme viridae</i>	5.5	7.21

In Fig. 5 shows the ratio of the contamination coefficients (Averyanov's formula) for liquid media before and after post-cleaning of products of bacterial decomposition of gauze napkins with fungi. The diagram shows that, after the use of fungi, the contamination factor decreased by several orders of magnitude. Obviously, the substrate obtained after the

mushroom post-treatment is practically chemically pure (in terms of the content of volatile organic substances) and much more suitable for further use in life support systems for space vehicles. Also, this indirectly indicates the absorption of a significant portion of the carbon contained in the products of gauze hydrolysis in the form of dissolved organic substances.

Fig. 4

Fig. 5

Fig. 6

Biodegradation of plant waste

Anaerobic degradation of cleaning carrots and potatoes, as well as cabbage cob, led to the formation of an opaque liquid phase containing a thick slurry and sediment. Degree of degradation was about 76%. The pH of the resulting substrates ranged from 5.5 to 7.0.

Thus, 50 g of the purifications are hydrolyzed in 7 days in a volume of 250 ml

For 1 day hydrolysis of 50 g in 1.7 liters, hence: 29 g/l day - the specific rate of decomposition.

For aerobic post-treatment, the main end products are NH_3 , H_2O , CO_2 .

In proteins, on average, the C : N ratio is 3.5 : 1, therefore, out of 50 g of plant waste, about 11 grams of nitrogen will be needed, hence:

$$m(\text{NH}_3) = m(\text{N}) + m(\text{H}_3)$$

$$M(N) / M(H_3) = m(N) / m(H_3)$$

$$m(H_3) = M(H_3) * m(N) / M(N)$$

$$m(H_3) = 3 \text{ g/mol} * 11 \text{ g} / 14 \text{ g/mol} = 2.4 \text{ g}$$

$$m(NH_3) = 2.4 \text{ g} + 11 \text{ g} = 13.4 \text{ g with 50 g waste}$$

The concentration of ammonia is $13.4 \text{ g} / 250 \text{ ml} = 53.6 \text{ g/l}$.

Chromatomass-spectrometric analysis showed the presence of a large number of volatile organic compounds contained in the resulting liquids.

The quantitative and qualitative composition of associations of microorganisms carrying out the decomposition of plant wastes was carried out. As a result of the studies it was found that the total amount of strictly anaerobic microorganisms was 1.1×10^{11} CFU per ml of biodegradation fluid, and aerobic 4.3×10^8 CFU.

The results of identification of the above microorganisms are shown in Table 5.

A total of 16 microorganisms were isolated and identified.

Basically, in the biodegradation fluid there were representatives of anaerobic flora - bacteria of the genus *Clostridium* (*C. pasterianum*, *S. cellobioparum*). The quantitative level of bacteria of the genus

Clostridium was 1.1×10^{11} CFU per ml of biodegradation fluid.

It is known that these species are capable of biodegrading starch and, to some extent, cellulose. High titers of these microorganisms attest to their active role in the biodegradation of vegetable waste.

Aerobic microorganisms were represented by bacteria of the genus *Bacillus* (*B. cereus*, *B. Mesentericus*, *B. polymyxa*, *B. subtilis*).

In second place were enterobacteria (*Enterobacter cloacae*, *Hafnia alvei*, *Citrobacter freundii*), which have high hydrolytic and enzymatic activity. Their quantitative level, respectively, was 5.2×10^4 , 1×10^6 , 8×10^7 CFU per ml.

A significant place in this association was occupied by bacteria that break down cellulose - *Cellulosomonas*, *Cytophaga*, *Azomonas*, *Azotobacter*, *Flavobacterium*, *Pseudomonas*.

Representatives of Gram-positive cocci flora were represented only by bacteria of the form *Enterococcus faecalis*, the quantitative level of which reached 2.8×10^8 CFU per ml.

In the association under investigation, no pathogenic species were found, hence, it is relatively safe in the sanitary-hygienic aspect.

Table 5. Quantitative and qualitative composition of associations of microorganisms carrying out the decomposition of plant wastes

Group	Genus	Species	CFU/ml
4	<i>Azomonas</i>	<i>Azomonas agilis</i>	2.5×10^3
	<i>Azotobacter</i>	<i>Azotobacter macrocytogenes</i>	5×10^3
	<i>Flavobacterium</i>	<i>Flavobacterium breve</i>	1.4×10^3
	<i>Pseudomonas</i>	<i>Pseudomonas fluorescens</i>	2×10^3
5	<i>Enterobacter</i>	<i>Enterobacter cloacae</i>	5.2×10^4
	<i>Hafnia</i>	<i>Hafnia alvei</i>	1×10^6
	<i>Citrobacter</i>	<i>Citrobacter freundii</i>	8×10^7
15. Subgroup 1	<i>Cytophaga</i>	<i>Cytophaga diffluens</i>	8×10^1
17	<i>Enterococcus</i>	<i>Enterococcus faecalis</i>	2.8×10^8
18	<i>Clostridium</i>	<i>Clostridium pasteurianum</i>	1×10^{11}
		<i>Clostridium cellobioparum</i>	1×10^{10}
	<i>Bacillus</i>	<i>Bacillus cereus</i>	8×10^5
		<i>Bacillus mesentericus</i>	4.5×10^6
		<i>Bacillus polymyxa</i>	1×10^6
		<i>Bacillus subtilis</i>	4×10^7
20	<i>Cellulmonas</i>	<i>Cellulomonas cellulans</i>	4×10^1

Microbial aftertreatment of liquid products of biodegradation of vegetable wastes

Depending on the pH level of the liquid biotransformation products of plant substrates, different types of microbial post-treatment were used: if the pH remained neutral, the association of aerobic bacteria was used; if the pH was acidic, cellulolytic fungi were used (Table 4).

As a result of the studies, it was possible to compile an association of microorganisms for post-cleaning a mixture of liquid products found in cabbage, potato and carrot homogenates after the anaerobic phase of their decomposition, consisting of well-studied non-pathogenic bacteria.

Chromatomass spectrometric analysis of samples of substrates taken at different stages of fermentation (Fig. 7) showed that the consortium composed: *Pseudomonas esterophilus* VKM-1736D, *Paracoccus denitrificans* VKM B-1324, *Achromobacter parvulus* VKM-1541D and *Stenotrophomonas maltophilia* VKM B-591, active consumed products present in vegetable homogenates.

In the initial substrate, obtained as a result of the decomposition of carrots by mesophilic microorganisms, various volatile organic compounds were present. During the post-treatment, most of the organic impurities were removed from the original substrate.

Fig. 7A

Fig. 7B

Fig. 7C

A similar result was demonstrated by cellulose fungi on substrates that had an acidic pH. However, chromatographic mass spectrometry analysis showed that the total molar mass was contained in

the primary waste products (Fig. 8), and the average concentration of volatile organic impurities on the contrary decreased to the trace values (Fig.9).

Fig. 8

Fig.9

Fig. 10

In Fig. 10 shows the ratio of contamination coefficients of liquid media before and after post-cleaning of products of bacterial decomposition of vegetable waste with fungi. The diagram shows that, after the use of fungi, the contamination factor has decreased to trace values. Obviously, the substrate obtained after the mushroom post-treatment is practically chemically pure (in terms of the content of volatile organic substances) and much more suitable for further use in life support systems for space vehicles. As in the case of additional purification of biotransformation products of gauze, this indirectly indicates that the fungi are absorbed by a significant fraction of the carbon contained in the products of fermentation of plant substrates in the form of dissolved organic substances.

Based on the above data (chromatography-mass spectrometric analysis, pH fluctuations), it can be stated that aerobic bacterial cultures and fungi can absorb organic substances that remain in the culture media after anaerobic biodegradation of gauze and vegetable wastes.

Additional purification of gauze tissue biodegradation products with *Trichoderma viridae* culture in space flight conditions

Cultivation of the strain *Trichoderma viridae* on the satellite was carried out in the bioreactor BTS.084.011.000, No. 03 (Fig. 1) for 14 days at 37°C. The strain of the fungus *Trichoderma viridae*, isolated from the flight sample showed stable growth under conditions identical to those of pre-flight cultivation. Analysis of the composition of the gas phase by chromatography-mass spectrometry showed that the total concentration of volatile organic compounds in the products of biodegradation of

gauze decreased by about 7 times (Fig. 11) after post-treatment in space flight conditions.

The total molar mass of volatile organic substances has not decreased significantly (Fig. 12).

The pollution factor of the environment has decreased more than 2 times (Fig. 13).

Despite the limited oxygen resource associated with the lack of aeration system in the Fragmenter bioreactor, the *Trichoderma viridae* strain demonstrated the ability to produce an aerobic post-treatment of the substrate obtained after the anaerobic biodegradation of gauze tissue under orbital flight conditions.

DISCUSSION

Studies of biodegradation of personal care products have shown high efficiency of using the *Clostridium thermocellum* culture under a number of conditions that impose some limitations on the application of this technique to LSS spacecraft. Compliance with the temperature regime is a certain difficulty, since the maximum activity of the enzymes of the cellulase complex is attainable at a temperature of 55 °C. On the other hand, when cultivated in relatively large volumes (in fermenters), the bacteria warm up the medium because of the high intensity of the metabolic processes. This fact must be taken into account when scaling up microbial degradation methods.

In addition, an important condition is preliminary sterilization of the substrate.

In addition to the carbon substrate, *Clostridium thermocellum*, as a thermophilic anaerobic, needs a complex composition including mineral components,

a stock that will need to be carried on board. But their concentration of degradable personal hygiene concentration does not exceed 3 g/l, while the products can reach 50 g/l.

Fig. 11

Fig. 12

Fig. 13

The most significant problem is the formation during the hydrolysis of cellulose acetate and other biodegradation products, which cause significant acidification of the medium and, consequently, inhibition of the growth of the culture. Maintaining the desired pH level can be achieved by adding various substances (alkali, chalk) or by the withdrawal of the resulting fermentation products.

The use of culturing with continuous supply of substrate, mixing, maintaining pH at the optimum level and removing the resulting fermentation products can significantly improve the degree and rate of degradation of the initial substrate.

However, liquid products formed after biodegradation of gauze substrates are not suitable for use in LSS space vehicles. In the composition of biotransformation products, acids, alcohols, aldehydes, ethers, etc. volatile organic substances are found which give the medium an acidic pH. Correction of the pH level by adding alkali or other reagents is applicable only at the stage of primary biodegradation of personal care products, as neutralization of acids leads to the formation of salts, including organic. In addition, oligosaccharides are present in the culture liquid, which are a by-product of hydrolysis of cellulose.

The presence of organic compounds in solution interferes with its direct use in LSS. Anaerobic biotransformation basically only transfers wastes from solid to liquid form.

One of the possible ways of utilization of liquid substrates is their post-cleaning with the use of acidophilic cultures. According to the experiments presented in this paper, four types of cellulolytic fungi, represented by strains isolated from hermetically sealed objects, were able to maintain stable growth by feeding on the products of the metabolism of the *Clostridium thermocellum* culture, which carries out the primary biotransformation of the gauze substrates. The after-treatment of the medium contained a significantly smaller number of organic impurities than the products of biotransformation of the gauze and had a slightly alkaline pH. In microaerophilic conditions in the metabolism of fungi, fermentation processes prevailed over respiration. Fungi consume significantly more carbon in the course of constructive metabolism compared to anaerobic bacteria and increase their biomass faster, perhaps for this reason in the substrates obtained after post-cleaning, much less volatile organic substances were detected than in liquid products of primary

biotransformation, and the concentration found compounds decreased to trace values.

The most suitable technology for the use of this method in LSS of space vehicles is the cultivation of microorganisms in specialized fermenters in flow-cascade or batch mode, since such a technological scheme ensures the continuity and closure of successive biodegradation processes.

The first link should be represented by the sterilizer block (Fig. 14), from which the disinfected substrate enters the bioreactor with the *Clostridium thermocellum* culture. As gauze fermentation, liquid biodegradation products, biogas and a dry residue consisting mainly of synthetic polymers will be formed. Liquid products of biodegradation gauze enter the next bioreactor, where fermentation is carried out through fungal cultures. The substrates cleared of most organic impurities, after filtration, are potentially suitable as technical water in the LSS: so they can be incorporated into the SRW-C system. The water thus obtained can be used for various purposes: recycle as a component of the fermentation medium, obtain oxygen and hydrogen from it by electrolysis (Sinyak Yu.V., Skuratov VM, 2001) or use as a source of mineral elements (in this case, the stage of distillation in SRW-C is not needed) for nutrient solutions of hydroponic greenhouses. The post-cleaned substrates are saturated with mineral compounds - products of aerobic mineralization and residual salts - components of synthetic media for microorganisms. Plants will absorb mineral compounds, and return water to SRW-C in the form of transpiration moisture.

Microbial degradation of plant waste can also be carried out in two stages (Fig. 15). Preliminary sterilization of the substrate in this case is not necessary, since the applied consortium of microorganisms is resistant to contamination by extraneous microflora. The efficiency of the process is affected by the grinding of the substrate - the rate and degree of biodegradation increases. The culture liquid does not need to add any additional compounds, water and vegetable food waste are sufficient components of the medium. Fermentation occurs in the mesophilic regime and is accompanied by a significant biogas release, which can be used as fuel for spacecraft engines (Maksimenko KV, Nazarov VP, 2011) or as fuel in a fuel cell. Compared with biodegradation gauze, the mass fraction of dry residue is higher (4-30%, in gauze 0-10% with pH correction), but it has a purely biogenic nature.

Fig. 14. Block diagram of disposal of used personal care products

Fig. 15. Block diagram of waste management

The liquid waste formed after fermentation undergoes sterilization (the presence of autochthonous microflora is unfavorable for monocultures producing post-treatment) and then, depending on the pH of the medium, they are sent either to the aerobic or anaerobic bioreactor. In the first case, liquid biotransformation products having neutral pH are purified by association of aerobic bacteria. A liquid with an acidic pH falls into an anaerobic fermenter with a mushroom culture, where along with the absorption of organic substances, the pH level is corrected to neutral and weakly alkaline.

To implement the above technologies for the utilization of cellulose-containing substrates, a system consisting of several bioreactors connected to each other in successive chains is suitable. The very process of decomposition of waste should proceed in several stages. On the first gauze and plant residues are subjected to shredding by means of shredders, and then they enter fermenters, where biotransformation of solid substrates into the liquid takes place with the formation of biogas and a dry residue. In the case of gauze wipes, the components of the synthetic medium necessary for *Clostridium thermocellum* are added to the bioreactor. Sterilization of substrates and media is then carried out. For this purpose, the fermenters intended for primary biotransformation should function in 2 modes: a bioreactor and an autoclave. In the second stage, suspensions of cultures initiating biodegradation of cellulose-containing waste are added to the bioreactors. Further, during the fermentation, biogas is withdrawn, the pH level is corrected and the culture liquid is stirred. Anaerobic biodegradation proceeds until the medium is saturated with the products of the exchange of bacterial cultures, after which the bioreactor is again transferred to the autoclave regime, and the liquid decomposition products are sterilized. As the dry residue accumulates in the cultivation blocks, it should be removed.

In the third stage, the obtained liquids are transferred to the fermentors for aerobic and anaerobic post-treatment, after which the substrates are filtered.

As the fourth stage, one can consider the exposure of the obtained water in hydroponic greenhouses with the enrichment of its mineral compounds, if necessary.

CONCLUSIONS

1. With the aid of an obligate anaerobic culture of *Clostridium thermocellum*, it is possible to transform gauze with an initial concentration of 50 g/l into solutions of organic compounds and biogas at a

specific rate of 1.8 g/l * day and a dry matter degradation ratio of 95-100%.

2. Sixteen species of microorganisms present in the association transforming vegetable waste with an initial concentration of 200 g/l into solutions of organic compounds and biogas under microaerophilic conditions with a specific rate of 29 g/l day and a proportion of dry matter degradation of 70 to 75 were isolated and identified %.

3. Aerobic mesophilic bacteria and cellulolytic fungi mineralize the solutions remaining after the fermentation of gauze and vegetable waste products, significantly reducing the total concentration, total molar mass and the pollution factor of volatile organic compounds.

4. *Trihoderma viridae* culture demonstrated the ability to produce aerobic after-treatment of the substrate obtained after anaerobic biodegradation of gauze tissue in the bioreactor BTS.084.011.000, Head # 03 "Fragmenter" under conditions of orbital flight.

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БИОТРАНСФОРМАЦИЯ НА ИЗПОЛЗВАНИТЕ СРЕДСТВА ЗА ЛИЧНА ХИГИЕНА НА КОСМОНАВТИ И НА РАСТИТЕЛНИ ОТПАДЪЦИ, ПРИЛОЖИМА В СИСТЕМИТЕ ЗА ЖИЗНЕОБЕЗПЕЧАВАНЕ НА КОСМИЧЕСКИТЕ ЕКИПАЖИ

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Резюме: В тази статия се обсъжда възможността за прилагане на анаеробна биодеградация с цел намаляване и стабилизиране на органичната фракция от твърди отпадъци, генерирани по време на полети с пилотирувани космически кораби. Изследвана е култура от *Trihoderma viridae*, способна да използва субстрати, съдържащи целулоза, при мезофилни условия. Щамът е изолиран от вътрешността на Международна космическа станция.

Основната цел на работата е да се изследва способността на култура от *Trihoderma viridae* да консумира продуктите от анаеробната биодеградация на тъкани от марля в условията на космически полети, т.е. като субстрат за култивиране на гъбичките са течни среди, образувани след разграждането на марлените кърпички от термофилните клостридии. При условията на космически полет културата на *Trihoderma viridae* намалява общата концентрация на летливите органични примеси, останали след биоразграждането на марлята, от 34 mg/m³ до 5 mg/m³. Броят на определяните летливи съединения намалява почти два пъти. Извършените проучвания показват, че последващото очистване на течни продукти от хидролизата на тъкани от марля с помощта на целулозни гъбички може да бъде ефективен компонент за утилизация на лични хигиенни материали с помощта на микробни съобщества в условията на космически полети. Също така, при наземни условия, течните продукти от биоразграждането на растителни отпадъци бяха допълнително пречистени с помощта на съобщества от аеробни бактерии. Хроматографският и мас спектрометричен анализ показаха, че консорциумът съставен от аеробни мезофилни бактерии активно консумира продукти, присъстващи в растителни хомогенати.

Ключови думи: биоразграждане, анаеробна биодеградация, отпадъци от целулоза, *Trihoderma viridae*, пилотирувани полети на космически кораби, условия на микрогравитация

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