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MICROBIAL PURIFICATION OF WASTE BIODEGRADATION LIQUID PRODUCTS

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Abstract. The problem of waste utilisation is very acute in view of a piloted spaceflight to Mars. A peculiarity of the life support on piloted spaceships is that there is no shower on board, therefore wipes and towels are the most common means for hygiene procedures and, therefore, they form the largest waste mass. The other potentially expected kinds of wastes are the non-edible residuals of greenhouse plants. Previous investigations considered these kinds of wastes to be well degradable. However, the liquid products of the biodegradation contain much soluble organics which are not suitable for straight admission to the water supply system. These liquid products of the biodegradation were purified with the aid of aerobic microbial associations. The purification was satisfactory, especially for plant wastes.

Keywords: cellulose waste, microorganisms, purification, biodegradation, life support system, spacecraft.

INTRODUCTION

Cellulose containing wastes, formed during spaceflight, are disposed personal hygiene means and non-edible residuals of plants. These wastes are large in mass and volume and contain products of human methabolism, which makes this kind of wastes dangerous from sanitary-hygienic point of view. On International space station these wastes are disposed in payload spaceship “Progress”. In prospective, coming out of the requirements of planetary quarantine one can no longer get read of wastes, having no previously disinfect them. These procedures require additional energy expenses. Besides, some kind of wastes, which could be expected in long-term interplanetary missions, could be degraded, and the products of this degradation should be included into different life support systems. At present time, these wastes are tested on their capability to be biodegraded using anaerobic and aerobic technologies. Previous investigations revealed effectiveness of transformation of these kinds of wastes into liquid state. This liquors contained high concentrations of organic solvents, therefore it looked problematic to forward this chemically aggressive liquor to traditional water supply system pathways (Ilyin et al. 2005).

The purpose of the work is to evaluate possibility of microbial degradation of cellulose containing substrates – personal hygienic mean, very familiar for the spaceflight body care (dry wipes) and vegetables wastes.

We investigated termophilic degradation of wipes samples with the aid of Clostridium thermocellum. Also we studied be-phased biodegradation of vegetable substrates, to be treated by associations of anaerobic and aerobic mesophiles.

The purpose of investigations:

- To study possibilities of active cultures growing on media, containing cotton wipes as the only source of carbon;
- To select strains for biodegradation of sanitary hygiene means in high concentration of the latter and without shredding;
- To investigate influence of pH on effectiveness of wipes degradation by Clostridium thermocellum F1;
- To study possibility of purification of liquid products of biodegradation by aerobic associations;
- To perform chromatomass spectrometry study of the biodegradation products before and after purification.

MATERIALS AND METHODS

The cotton consists mainly of cellulose fiber (70-100%) and synthetic polymers (30-0%). Clostridium is known to be the most active towards different structure of cellulose (Akimenko, 1988).

Their advantages are: high endoglukinase activity, which less depend on the nature of the substrate, and comparatively high grow velocity on cellulose-containing substrates.

There are 5 strains of Clostridium thermocellum in Collection of the G. K. Skryabin Institute for Biochemistry and Physiology of Microorganism. These strains possess different physiological and biochemical features. (Chuvilskaya et al., 1986).

Screening was performed while cultivation of strains on monocrystal cellulose MN-300 (10 g/l) using the following media: (g/l): NH_4Cl – 2.0, $MgCl_2$, $CaCl_2$ – 0.07, $FeSO_4$ – 0.0025, K_2HPO_4 – 3.0, KH_2PO_4, yeast extract – 6.0, rysasurin – 0.0002, cystein hydrochloride – 0.125, Na_2S – 0.125. Cultivation was made in Hangate tubes with total volume of 15 ml, working volume of media – 10 ml. Initial pH 6.8. Cultivation was made under +55^{0}Ñ till the culture gain stationary growth phase. The growth was controlled by measuring of optical density under λ = 600 nm on spectrophotometer Specol (Germany).

As an inoculum we used the strain Clostridium thermocellum F1. As an only carbon source we used standard cotton wipe “Dried wipe for hygienic purposes” in concentration 25 and 50 g/l. Cultivation was made on flasks of total volume 500 ml, working volume of the media – 200ml. Inoculum biomass was obtained on cellobiose. Cultivation was performed on cellobiose. Initial density of the media was 0.009. Cultivation was performed during 4 weeks under 55^{0}Ñ. Microbial growth was evaluated by optic density measuring.

There were 3 experiment groups:

1 – Control cultivation; 2 – cultivation with initial addition of chock (10 g/l); 3 – cultivation with addition of alkaline. The obtained products were analyzed on chromatomass spectrometer.

Also investigations of vegetable substrates were performed (non-edible residuals of cabbage, potato, carrot) by mesophylic associations of anaerobic bacteria. Cultivation was performed in flasks. As an inoculi the lyophilized associations, trophycally adapted to the substrates were used. Cultures were put in flasks, containing 50 g of the substrates, 4 g of peptone and 250 ml of water. Cultivation was performed under + 37 ^{0}Ñ during 1 - 4 weeks till complete vanishing of floating fractions and sediments. The products of biodegradation was analyzed on chromatomass-spectrometer.

For further purification of biodegradation products the microbial association was selected : *Pseudomonas esterophilus* ÂÊÌ-1736Ä, *Paracoccus denitrificans* ÂÊÌ Â-1324, *Achromobacter parvulus* ÂÊÌ-1541Ä è *Stenotrophomonas maltophila* ÂÊÌ Â-591.

Adapted monocultures were mixed in equal shares by flashing out from surface of agar media, and added to the flask with subtrate which afterwards was incubated on shuttle under 120 rev/min and 29 ^{0}Ñ for 7 days. Samples 10 ml each were taken for analysis on chromatomass – spectrometer. The purpose of chromatomass-spectrometric analysis of gases is to evaluate content and concentration of microadmixtures in gaseous content.

The samples of liquid products of biodegradation were placed in exicators of 9 l volume and stored for several days under room temperature and aerobic conditions. Analysis of air samples were performed under method recommended by ISO 16000 (“Air of confined habitat. Part 6 Determination of volatile substances in the air of confined habitats and testing chamber) by active sampling on sorbent Tenax ÒÀ with further chemical desorption and gas chromatography analysis”) Air sampling was performed by adsorption tubes “Gerstel” with sorbent layer, then chemical desorption was used under 290°Ñ for 4 minutes with simultaneous cryogenic fixation of volatile components under -30°Ñ and with further chromatography separation on capilliary column HP-5MS with programmed temperature from 30 to 280°Ñ and detecting electronic ion blow (ionizing energy 50 EV) in diapason m/z 2-500. Identification of substances was performed using libraries of mass-spectra of USA National Institute of Standards. Calibration of the unit à “Agilent GC 5973 MSD” with integrated systems of desorbtion “Gerstel” TDS3 è CIS4 was carried out using standard solutions from “Sigma-Aldrich” (Switzerland).

RESULTS AND DISCUSSION

The results of Clostridium thermocellum cultivation are presented on Table 1. Basing on obtained results the choice was delivered to the strain Clostridium thermocellum F1, since it has demonstrated highest velocity growth and largest biomass production on the ubstrate.

Table 1. Parameters of Clostridium thermocellum cultivation

Strain	Cultivation	OD_{600}	Growth velocity (h^{-1})	Biomass(g/l)
F1	Hangate tubes, 10 ml of media, 55°Ñ	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

Table 3. Consumption of substrate while Clostridium thermocellum F1 growth on cotton wipe

1 exp.	Initial quantity of substrate, mg	Average mass of cells,mg	Residual substrate, mg	Substrate consumption, %
1	5000	48	4466	10.7
2	10000	70	8544	14.6
3	5000	130	0	~100
4	10000	198	437	~95
5	5000	84	4107	17.9
6	10000	95	9900	1.0

The data on Clostridium thermocellum F1 growth on media containing medical cotton wipes are presented on Table 2. Biomass increasing and maximal decomposition of substrate was observed in cases when chock was added to the media for pH neutralization. Lag-phase was sufficiently shortened when using chock as an additive. The data on Clostridium thermocellum growth on the cotton wipes are presented on Table 3.

Increasing of substrate from 25 to 50 g/l led to prolongation of lag-phase to 5 and more days and to fast acidification of media, when chock was not added. Addition of alkaline did not influenced on uptake of substrate. When chock was used, the lag-phase period decreased, biomass acceleration and decomposition of substrate (25 g/l) within 14 days and within 28 days in concentration of 50 g/l) under 55°Ñ. Possibly, stimulating activity of endoglukinases of F1 strain was performed by calcium ions, which obtain while neutralization.

Addition of small portions of substrate could evidently optimize the process. Chromatomass-spectral analysis of liquid products of cotton biodegradation showed prevalence of quantitative and qualitative content of substances in gaseous media in control samples, without addition of chock and alkaline (Table 4). In samples, where chock was added, there were much less volatile components and large evaporation of methane. (Table 5). The explanation of this may be that while neutralization of pH the volatile products of C. thermocellum metabolism is transferred to non-volatile salts, e.g. acetates.

Further purification of the liquid substance was made by fungi and yeasts. First, several cultures were tested on their capability to change pH of the substrate liquor, i.e.: Candida sp Saccharomyces cerevisiae, Rhodotorula sp., Hypomyces sp., Cladosporium cladosporioides, Chaetomium globosum and Trihoderma viridae (Table 6). Basing on obtained results the prevalence was given to Hypomyces sp., Trihoderma viridae, Chaetomium globosum and Rhodotorula glutiuius. These microbes were very active in purification of liquid products of wipe degradation (Table 7).

Table 5. Content of equilibrated vapor of liquid products of biodegraded cotton wipes (with addition of chock)

Substance	Sample 1 * , mg/m^3	Sample 2 **, mg/m^3
methane	6,224	6,226
Isopropanol	0,009	0,086
acetic acid	0,232	0,188
Limonene	0,032	0,076
Toluene	0,078	0,101
ethylbenzene	0,056	0,042
Xylene	0,144	0,086
threemethylbenzenes	0,098	0,022
hydroxypropanone	0,112	0,118
methylstyrene	0,020	nd
9-methylacridine	0,054	nd
1-methyl-3-ethyladamantane	nd	0,822
Phtalates	nd	0,344
1,2-cyclo-hexandimethanol	0,062	nd
methoxyphenyloxime	0,388	nd
benzaldehyde	0,112	0,068
1-bromo-11-iodoundecane	0,212	nd
anthracenamine	nd	0,082
Total mass	1,609	2,033
Total substances	17	13

*) – initial substrate concentration - 50 g/l

**) – initial substrate concentration - 25 g/l

Anaerobic degradation of vegetables led to formation of non-transparent liquor containing viscous substance and sediment. After aerobic purification liquid phase looked like transparent liquor of less density. Chromatomass-spectral analysis (Table 8) shown that microbial consortium actively consumed products, which present in homogenates of vegetables. Thus, as a result, the proposed bacterial association was capable for transportation of liquid products of first, anaerobic phase of biodegradation of vegetable (cabbage, potato, carrot). This association consists of well-studied non-pathogenic bacteria Pseudomonas esterophilus ÂÊ Ì-1736D, Paracoccus denitrificans ÂÊ Ì Â-1324, Achromobacter parvulus ÂÊ Ì-1541 D and Stenotrophomonas maltophila ÂÊ Ì Â-591.

Thus the obtained results revealed possibility of purification of liquid products of biodegradation. It means, that after pretreatment this “grey water” can admit to water supply system of piloted spaceship, if this technology will be applied in piloted missions. It can be used further, for example, as washing water or can be treated in electrolysis for oxygen production. Our further investigations will be directed to study biodegradation and purification processes in experiments on Russian segment of International Space Station and on automatic spaceship “Bion M#1”. Also practical testing are planned in groundbase experiment “Mars-500” simulating long-term autonomous exploration of confined habitat.

Table 2. Growth of Clostridium thermocellum F1 on the media containing cotton wipe mass

[1] exp.	Concentration of wipes (g/l)	Time (days)																Substrate consumption, %
		0		2		5			7			14			17			20			29	
		OD	pH	OD	pH	OD	pH	NaOH[2]	OD	pH	NaOH	OD	pH	NaOH	OD	pH	NaOH	OD	pH	NaOH	pH	
1	25	0.09	6.8	nd [1]		0.09	6.2	2.0	0.13	7.1		nd			nd			0.22	5.5		5.6	10.7
2	50	0.09	6.8	nd		0.15	5.2	5.0	0.22	7.1		nd			nd			0.32	5.2		5.2	6.2
3	25	0.09	6.8	nd		0.34	6.4		nd	6.8		0.37	6.5		nd			0.59	5.9		6.0	100
4	50	0.09	6.8	0.13	6.8	0.34	5.9		nd	6.7		nd			nd			0.90	5.8		5.9	95.6
5	25	0.09	6.8	0.14	6.8	0.16	5.8	2.5	nd	5.2	4.0	0.37	6.0	2.0	0.3	5.9	2.0	0.38	6.4	1.0	5.7	17.9
6	50	0.09	6.8	nd		0.13	6.6	1.0	nd	6.8		0.17	6.7	1.0	0.35	5.2	4.0	0.43	5.5	3.0	5.6	1.0

[1] – non determined; [2] – 2N alkaline, ml, bold: experiment with addition of chock

Table 4. Content of equilibrated vapor of liquid products of biodegraded cotton wipes

Substance	25 g/l	25 g/l	50 g/l	50 g/l
	3rd day of exposition	4th day of exposition	3rd day of exposition	4th day of exposition
isopropanol		-	0,032	-
butanol-1	0,022	-	0,033	-
3,6-dimethoxyfluorenol -9	0,031	0,021	0,033	-
fluorobenzothiazol	0,059	-	Nd	-
limonene	0,012	0,012	-	-
butyl formate	0,011	0,011	-	-
ethylalanine	2,200	2,200	0,333	0,333
3-ethylborane	-	-	0,031	0,041
acethylacetone	0,002	0,002	0,041	0,041
oxyran	Nd	Nd	0,031	0,031
1 Í -indole	Nd	Nd	0,013	0,023
3,5-dihydroxybenzamide	nd	nd	0,079	0,064
2-phenylbenzothiazol	-	-	0,012	-
anthracenamine	-	-	0,042	-
4-methylpentannitrile	0,013	0,013	0,045	0,045
styrene	Nd	Nd	0,038	0,038
3-methyloil acid	0,078	-	0,164	-
phenole	Nd	-	0,056	-
furfural	0,023	0,023	0,124	0,124
Toluene	0,011	0,011	0,026	0,026
dihydroxybenzamide	0,010	0,010	0,034	0,034
acetic acid	0,239	0,139	0,272	0,312
2-ethylhexanol	0,008	0,008	1,239	0,039
propionic acid	Nd	Nd	2,221	2,221
benzaldehyde	0,068	0,011	0,168	0,168
acetfenchone	0,025	0,025	0,348	0,348
dymethylpyridine	0,015	0,015	0,074	0,074
methoxyphenyloxime	0,512	Nd	0,210	0,810
naphthalincarbazol	0,096	0,096	0,044	0,044
didodecylphtalate	Nd	Nd	0,112 î	0,120 î
dibutylphtalat	-	-	0,099	0,099
bis(2ethylhexyl)phtalat	6,444	2,444	0,260	0,260
2,6,10,14,18-pentamethyldecatriene	0,231	1,211	0,109	-
,2,3,5,8- tetramethyldecatriene	1,223	1,223	0,612	-
Total substances	28	28	22	19
Total mass	6,935	6,1023	11,333	7,528

*) – initial substrate concentration - 50 g/l; **) – initial substrate concentration - 25 g/l; Nd – not determined; - absent in the samples

Table 6. pH modifications of wipes degradation liquid product while treatment by different fungi and yeasts

Test-culture	Initial pH	pH after 5 days of treatment
Candida sp.	5.5	6.96
Saccharomyces cerevisiae	5.5	6.75
Rhodotorula sp.	5.5	7.32
Hypomyces sp.	5.5	7.44
Cladosporium cladosporioides	5.5	6.12
Chaetomium globosum	5.5	7.28
Trihoderme viride	5.5	7.21

Table 7. Dynamics of chemical content in wipes degradation liquid product while treatment by different fungi and yeasts

Chemical	Rhodotorula glutiuius	Trihoderme viride	Chaetomium globosum
propanamine	0,012	0,022	0,001
lemonen	0,026	-	-
Pinen	0,066	-	-
Toluol	0,003	0,005	0,005
Nonan	0,013	-	-
Hexanal	0,011	-	-
Nonanal	0,014	-	-
Acetone	0,028	0,034	0,032
3-caren	0,026	-	-
Butanol	-	0,006	-
metylstyrol	-	0,027	-

- absent in samples

Table 8. Content of equilibrated vapor of liquid products of biodegraded carrot (mg/m3)

Substance	Initial sample	After 48 hours	After 6 days	After 8 days
methane	-	2,200	0,008	0,002
α-pinen	0,110	-	-	-
Oil acid methylate	0,610	0,002	-	-
Oil acid ethylate	1,318	-	-	-
Cyclobutanole	1,023	-	-	-
2,4-dimethylheptane	1,034	-	-	-
Buthylformiate	-	0,013	-	-
Butanal	0,438	-	-	-
Ethylacetate	0,434	-	-	-
Ethanol	0,422	0,007	-	-
Buthylacetate	0,228	-	-	-
2-methylpropanol-1	0,137	-	-	-
Butanol	7,364	-	-	-
Xylene	0,124	-	-	-
Toluene	0,216	-	-	-
Hydroxyacetic acid	0,141	0,010	-	-
Acetic acid	0,612	0,039	0,023	0,011
D-limonene	0,100	0,008	-	-
Oil acid	0,621	0,018	-	-
Benzaldehyde	0,012	0,011	-	-
Acetophenone	0,328	0,025	-	-
I-ole	0,442	0,015	0,005	-
Phenole	0,110	-	-	-
Naphtalyncarbonitryle	-	0,096	-	-
methylstirole	-	-	-	0,023
Total mass	16,724	2,444	0,036	0,036
Total substances	21	12	3	4

- absent in samples

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Ðåçþìå: Âúâ âðúçêà ñ áúäåùèòå ïèëîòèðàíè êîñìè÷åñêè ïîëåòè äî Ìàðñ, ïðîáëåìúò ñ îïîçíîâàíåòî íà òîïàäúöèòå å îñîáåíî îñòúð. Ïîðàäè ñïåöèôèêàòà íà ñèñòåìèòå çà æèçíåîáåçïå÷àâàíå íà êîñìîíàâòèòå íà áîðäà íà êîñìè÷åñêèÿ êîðàá ëèïñâà äóø è çà õèãèåííè íóæäè ñå èçïîëçâàò îñíîâíî ñàëîêîè, êîèòî ôîðìèðàò íàé-ãîëÿìàòà ÷àñò îò îïàäúöèòå. Äðóãà ïîòåíöèàëíà ÷àñò îò îïàäúöèòå ïðåäñòàâëÿâàò íåïîãîäÿùèòå çà õðàíà îñòàòúöè îò ðàñòåíèÿòà, îòãëåæäàíè íà áîðäà, êîèòî îáà÷å ñà ëåñíî ðàçãðàæäàùè ñå. Òå÷íèòå ïðîáêè îò áèîäåãðàäàöèÿòà ñúáúðàò ãîëÿìî êîëè÷åñòâî ðàçòâîðåíà îðãàíè÷íà ìàòåðèÿ è çà òîâà íå ñà ïîäõîäÿùè çà äèðåêòíî ïîäàâàíå â ñèñòåìàòà çà âîäîñíàáäÿâàíå. Òåçè ïðîáëåìè ñå ïðå÷èñòâàò ñ ïîìîùòà íà àåðîáíè ìèêðîáíè àññîöèè, êàòî ïðå÷èñòâàùèòå å çàâîäîëèòåëíî, îñîáåíî çà ðàñòèòåëíèòå îòïàäúöè.