Institute of Biomedical Problems of the Russian Academy of Sciences

Regeneration of ion-exchange fibrous artificial soil for space greenhouses

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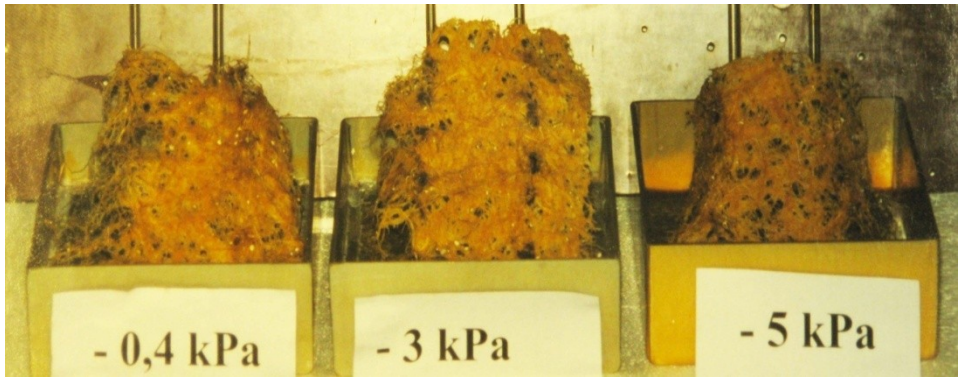
BIONA-V3 artificial soil produced by **INSTITUTE OF PHYSICAL ORGANIC CHEMISTRY OF THE NATIONAL ACADEMY OF SCIENCES OF BELARUS**

Composition	Weight producing capacity of substrate, mg/g	Extensional producing capacity of substrate mg/cm^3	Volume density (kg/dm^3)	Available moisture content (g per 1 g of substrate)
Artificial fibrousion-exchange resins ”FIBAN K-1”, “AK 22-1”	72	10	0,4-0,6	3-5

Exhaustion of nutrients (mainly potassium and nitrogen ions) and accumulation of roots residues reduce productive time of of ion-exchange artificial soils

Prototype of space greennhouse «Vitacycle T»



Plants root systems, grown under various watter pressure conditions.

Vegetation module

Productivity of BIONA-V3 may amount up to 180 g of salade dry biomass from 1 kg of artificial soil, and roots biomass accounts for 30% of the total biomass.

Development of substrates regeneration pathways for vegetation modules of greenhouses

- Biodegradation of of root inside the artificial soil
- Correction of substrates ion composition over the range of macro- and microelements acceptable combination

Research tasks:

- Screening of basic grupes cellulose-decomposing microorganisms for biodegradatione of Brassica pekinensis roots.
- Selection of effective microorganisms association and re-engineering of biodegradation process.
- Development of preliminary technique of BIONA-B3 regeneration with help of salts solutions.

Chemical constitution of dry
Brassica pekinensis **root biomass**
(percent of dry weight)**:**

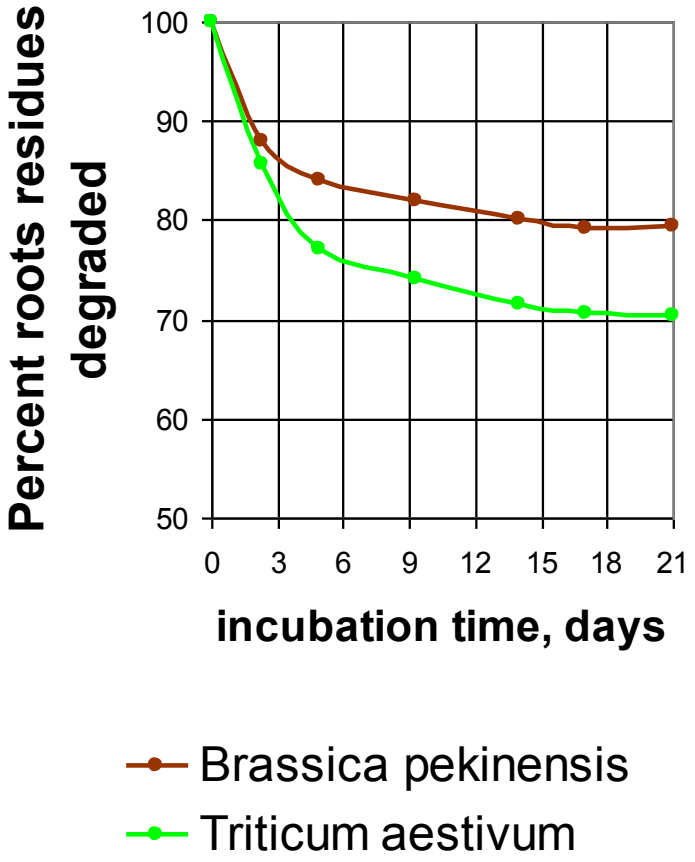
• cellulose	30 %
• noncrystalline	2 %
• crystalline	28 %
• hemicellulose	11 %
• lignin	12 %
• protein	14 %
• water extractable carbohydrates	5 %
• mineral constituents	7 %
• pectin substances	1 %

Application of aerobic cellulolytic bacterial association to biodegradation of root inside the artificial soil in liquid phase

Species composition:

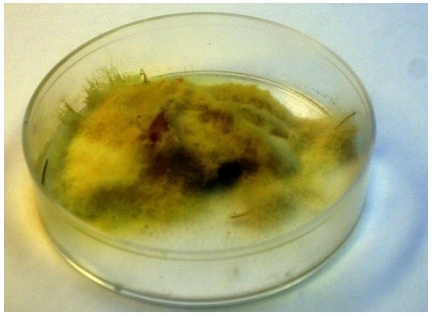
predominant

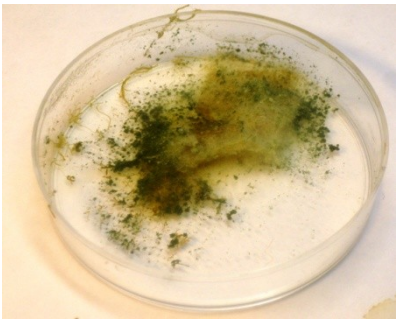
- *Clostridium cellobioparum*
- *Clostridium pasterianum*
- *Bacillus subtilis*
- *Bacillus polymyxa*
- *Citrobacter freundii*
- Azomonas agilis
- *Azotobacter macrocytogenes*
- *Flavobacterium breve*
- *Pseudomonas fluorescense*
- *Cytophaga diffluens*
- *Bacillus cereus*
- *Cellvibrio gilvus*
- *Cellulomonas cellulans*



The decrease of root residues mass don't exceede 30 % for 20 days in case of applicatione aerobic cellulolytic bacterial association as a main agent of biodegradation.

Aerobic solid state fermentation of roots with help of Trichoderma reesii and Trichoderma harizanum





Comparative degradation of *Brassica pekinensis* roots residues by Trichoderma.

Strain	Percent of degraded Roots, % d/m	Amount of cellulose degraded, % d/m
control	0	0
J1C*	17,22±3,6	8
M99/5**	33,06±6,3	0,5
МГ6*	38,5±5,9	13
Th7**	19,36±5,6	2,8

Comparative degradation of *Triticum aestivum* roots residues by Trichoderma

Strain	Percent of degraded Roots, % d/m	Percent of degraded cellulose, % d/m
control	0	0
J1C*	30,78±4,9	40,1
M99/5**	27,22±8,5	39,7
МГ6*	15,48±6,8	36,5
Th7**	46,5±13,7	37,7

*Trichoderma reesii ** Trichoderma harizanum

Roots biodegradation with help of Trichoderma in liquid phase

Incubation time - 10days

Brassica pekinensis roots degradation by Trichoderma.

Strain	Minimal increase of dry biomass (g/ g roots)	Endoglucanase activiti, mg/ml	Percent of degraded cellulose, % d/m
control	0	0	-
J1C*	0,25	1,6	47
M99/5**	0,31	1,5	39
МГ6*	0,29	1,3	36
Th7**	0,24	1,3	33

Triticum aestivum roots degradation by Trichoderma

Strain	Minimal increase of dry biomass (g/ g roots)	Endoglucanase activiti, mg/ml	Percent of degraded cellulose, % d/m
control	0	0	-
J1C*	0,21	1,3	31
M99/5**	0,33	1,3	29
МГ6*	0,29	1,4	37
Th7**	0,4	1,6	44

*Trichoderma reesii ** Trichoderma harizanum

Biodegradation of *Brassica pekinensis* root with help of anaerobic thermophilic bacteria *Clostridium thermocellum*

Strain	Bacterial optical density (14 days) 600 nm	Reducing sugars of cell culture fluid in the stationary growth phase (14 days), Δ C, M	Endoglucanase activiti of cell culture fluid in the stationary growth phase (14 days), Δ C, M	Percent of degraded roots, % d/m	Percent of degraded cellulose, % d/m
5	0,35	0,0008	0,0026	53	43
F15	0,25	0,0038	0	48	20
F19	0,27	0,0027	0,0005	42	6
F4	0,14	0,0024	0,0014	50	37
F9	0,18	0,0028	0,0002	44	30
LQRL	0,17	0,0034	0	44	13
NCYB	0,14	0,0029	0,0005	46	20

Fermentation of pre-treated *Brassica pekinensis* root materials by Clostridim thermocellum

Strain	Bacterialoptical density, 600 nm		Reducing sugars of cell culture fluid in the stationary growth phase (14 days), Δ C, M	Endoglucanase activiti of cell culture fluid in the stationary growth phase(14 days),Δ C, M	Total endoglucanase activiti (7 days), M	H2 conc., % d/m	Percent of degraded roots, % d/m	Percent of degraded cellulose, %d/m
	7 days	14 days						
5	0,22	0,13	0,0005	0,0023	0,001	15,2	62,1	54
F15	0,65	0,13	0,0022	0,0016	0,018	18,3	75,9	74
F19	0,75	0,2	0,0017	0,0019	0,005	19,9	76,9	82
F4	0,45	0,15	0,0025	0,0017	0,092	14,7	73,1	74
F9	0,6	0,26	0,0018	0,002	0,003	19,7	73	75
LQRL	0,6	0,62	0,0012	0,0028	0,108	21,8	70,5	67
NCYB	0,53	0,15	0,002	0,0016	0,003	20,7	67	65

Pre-treatment conditions : alcaline hydrolysis (1% NaOH), 1 atm, 30 min.

Alkaline pre-treatment of roots provide 2 folds decrease of roots mass and promote the availability of roots tissues to the microorganisms.

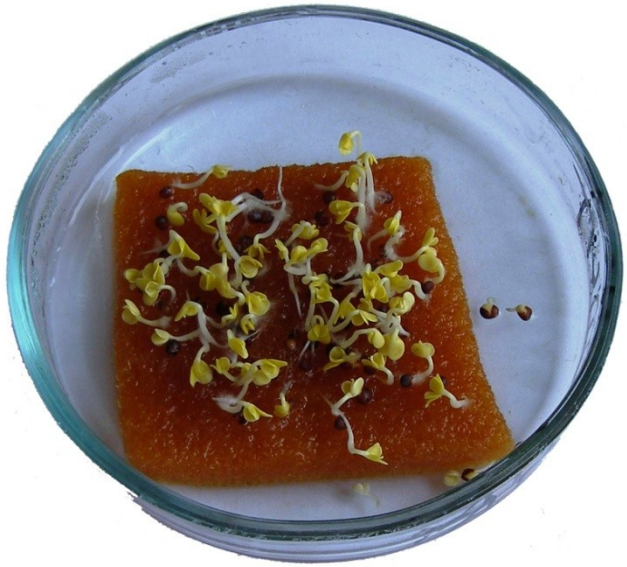
It has been shown, that ion exchange resin substrate is stable against of examined strains enzymes, during both solid and liquid fermentation phases

Preliminary technique of BIONA-B3 regeneration

1. Pre-treatment of exhausted substrates with 1% NaOH
2. Biodegradation with help of Clostridim thermocellum
3. Alkali washing of substrate with 1% KOH solution
4. Treatment by 0,2% H_2O_2 solution
5. Submersion in 1% KNO_3 solution

Regeneration of ion-exchangeable properties of fibrous artificial soils did for nothing in germinative ability, germinative energy and seedlings viability.

	Germinative ability of Brassica pekinensis seeds %	Germinative energy of Brassica pekinensis seeds %
Control	94	88
Regenerated artificial soil	92	89



Conclusions:

- Decrease of cellulose content of 45 % in the case of *Brassica pekinensis* roots and 40 % in case of Triticum aestivum roots has been achieved by using Trichoderma micromyces as a main agent of BIONA-B3 biodegradation .
- In the case of *Brassica pekinensis* roots preliminary treated by 1% NaOH decrease of residue mass about 70 % and decrease of cellulose content about 70 % has been achieved by using an anaerobic thermophilic bacteria Clostridium thermocellum as an agent of biodegradation.
- It has been shown that ion exchange resin substrate BIONA-B3 is stable against of examined strains enzymes, during both solid and liquid fermentation phases.