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Developing a technique to enhance durability of fibrous ion-exchange resin substrate for space greenhouses

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A B S T R A C T

One way to cut consumables for space plant growth facilities (PGF) with artificial soil in the form of fibrous ion-exchange resin substrate (FIERS) is on-board regeneration of the used medium. After crop harvest the procedure includes removal of the roots from the fibrous media with preservation of the exchanger properties and capillary structure. One type of FIERS, namely BIONA-V3, has been used in Russian prototypes of space conveyors. We describe a two-stage treatment of BIONA-V3 including primary microwave heating of the used FIERS until (90 ± 5) °C in alkali-peroxide solution during 3.5 hrs. The second stage of the treatment is decomposition of root vestiges inside pores of BIONA-V3 by using thermophilic and mesophilic anaerobic bacteria *Clostridium thermocellum, Clostridium cellulolyticum* and *Cellulosilyticum lentocellum* during 7–10 days at 55 °C. The two-stage procedure allows extraction of 90% dead roots from the FIERS' pores and the preservation of root zone hydro-physical properties. A posterior enrichment of the FIERS by minerals makes BIONA- V3 reusable.

1. Introduction

Space plant growth facilities are a promising part of bio-regenerative life support systems (LSS) because edible plants biomass may be generated in manned space vehicles during extended exploration missions. Several prototypes of conveyor space plant growth facilities (PGFs) with cylindrical planting surfaces have been developed earlier in Institute of Biomedical Problems (IBMP) of the Russian Academy of Sciences (Berkovich et al., 2004). Today, the PGF "Vita-cycle-T" project is under design in Russia. This vitamin PGF is intended for use into the Russian segment of the International space station. Constructional decisions of similar plant growth facilities imply using a specific root medium - fibrous ion-exchange resin substrate (FIERS) BIONA-V3 loaded with plant nutrients (Berkovich et al., 2003; Berkovich et al., 2004). FIERS capillary-porous structure simplifies water potential control and root zone gas exchange in microgravity, due to its stable cohesive matrix and does not pollute the cabin. Also, experiments showed that FIERS BIONA-V3 could buffer the substrate solution pH within the range from 6.0 to 6.6, favorable to most of growing crops. Therefore, plant cultivation in space flight becomes less labor-consuming and more convenient. Modern configurations for long term functioning PGF root feeding systems are composed of BIONA-V3 inside root modules and include mineral enrichment columns mounted into the PGF water supply system (Berkovich et al., 2016). Actually, the functioning time of the FIERS is limited due to accumulation of organics inside the root porous medium. During plant cultivation, roots are proliferating throughout the FIERS porous, changing hydro-physical performance of the medium and hampering of root zone moisturizing and aeration. Similar effects were described for granular root media (Steinberg et al., 2005). In addition decaying roots may help unwanted microflora to grow in space habitats (Tirranen, 2001; Van Houdt et al., 2012). Overall, decomposition of root residues is an essential condition of FIERS life cycle extension. Limited durability of FIERS is an urgent PGF problem for long-term space flights, since artificial soil is a central consumable for plants biomass production in weightlessness. Thus, prolonging the lifetime of artificial soil is an important task for space PGFs and greenhouses.

In the context of bio-technical life support systems, there are several known studies related to decomposition of plant waste products to produce or regenerate artificial soil for PGF with help of high temperature or/and electric current treatment (Strayer et al., 2002; Tikhomirov et al., 2010). However low melting point and small pore dimensions of BIONA-V3 restrains application of these methods to the FIERS. A basic challenge towards enhanced durability of the medium is an opportunity for the roots removal with preservation of exchanger properties and capillary structure of the substrate. This problem may be solved by the use of microorganisms and soft chemicals as cleaning agents. It stands to reason that required destructors should not pose a

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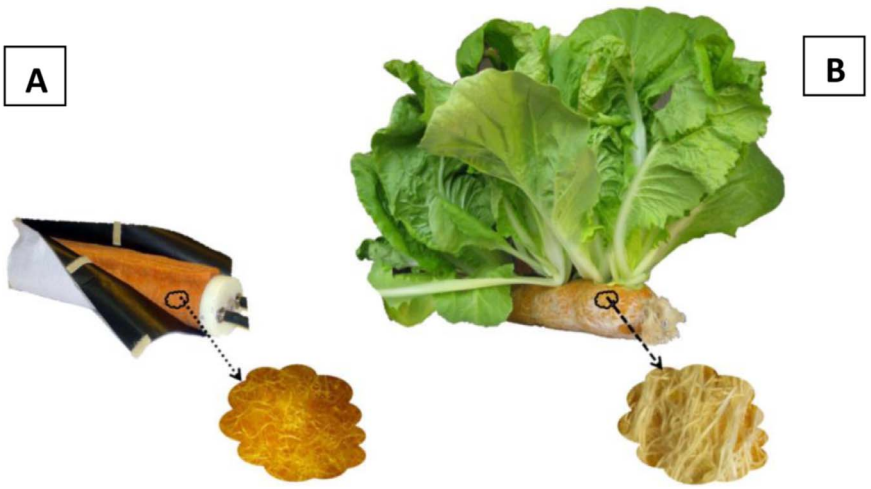

Fig. 1. Root module before (A) and after first salad growth cycle (B).

significant risk to human health and hardware material into low stability inside life support system (Novikova et al., 2006; Van Houdt et al., 2012). Adaptation features of microorganisms and the specifics of space conditions scale back the number of potential microbiological agents. In our work, we considered the possibility of FIERS cleanout using non-pathogenic extremophilic microorganisms. Their hard requirements to environmental conditions (anaerobiosis, a temperature beyond the 45 °C) reduce a risk of bacterial growth beyond the custom fermenter.

Under laboratory conditions, a rapid microbiological decomposition of the plant material into low-molecular products cannot occur without prior physical or chemical treatments targeted towards an extension of plants fibers surface and destruction of armor elements—lignin and microcrystalline cellulose (Lynd et al., 2002). The term “pretreatment” is widely used to refer to a conversation of native lignocelluloses into a form where enzymatic hydrolysis is effective. Alkaline pretreatment is one of the most effective methods for lignin degradation and swelling of cellulose fibers (Kumar et al., 2008). The alkali-based disruption of the intermolecular ester bonds of a cell wall is assumed to induce the formation of hydroperoxide anions ($HOO-$), which exhibit higher reactivity to electrophilic centers and lead to deprotonation of lignin (Curreli et al., 1997). In some studies it was noted, that supplement of peroxide promotes cellulose hydrolyzation during alkaline pretreatment of plants tissues (Lewis et al., 1988; Banerjee et al., 2011).

However, these chemical treatments are capable of plant polymers destruction only at high temperature or pressure. So, generally, pretreatment technologies include heating or autoclaving. Compared to conductive heating methods, microwave (MW) heating can reach the desired temperature in reaction zone more rapidly, consumes less energy and has a smaller hazardous emission potential (Park et al., 2004). The capabilities of MW applying for pretreatment processes has been observed before to the decomposition of agricultural lignocellulosic biomass and humans waste processing in bio-technical life support systems (Janker-Obermeier et al., 2012; Tikhomirov et al., 2012). It was assumed that MW treatment can induce “hot spots” in the plant biomass which disrupt the structure and improve the disintegration of the material (Hu, Wen, 2008). Authors show improved enzymatic saccharification of MW- pretreated straw compared to conventional heated samples. Other studies showed that MW-supported alkali pretreatment might act synergistically and increased energy-efficiency and cost-efficiency for organic wastes treatment (Janker-Obermeier et al., 2012). To increase root destruction we proposed pretreatment of root-filled FIERS in a low alkali-peroxide medium under microwave heating. Proposed pretreatment conception is potentially acceptable for space conditions, due to weak alkali and peroxide solution is authorized on ISS and MW heater is already on the American segment of ISS.

On the second stage of the treatment, we turn to describe microbial roots decomposition. We investigated microbial decomposition of *Brassica chinensis* L. roots after harvest and the pretreatment. Model microbial agents have been selected based on the literature data and previous experiments (Krivobok et al., 2012). Several reasons were taken into consideration. Filamentous fungi could lead to pores collapse inside FIERS because of accretion of fungi biomass. The use of microbial communities doesn't provide high biodegradation rate (Lynd et al., 2002) and could lead to the production of dangerous microbes in the community. Finally, for our investigations, we chose thermophilic bacteria *Clostridium thermocellum* and some physiologically similar species: *Clostridium stercorarium, Clostridium cellulolyticum, Cellulosilyticum lentocellum. Clostridium thermocellum* could coordinate substrate-specific regulation of cellulosomal subunit composition to suit the organism's needs for growth under different conditions.

2. Materials and methods

2.1. Plant material

Leafy cabbage *Brassica chinensis* L. was used as upload crop for root modules with FIERS. Description of the root modules (Fig. 1) and whole plant growing facility was described in detail earlier (Erokhin et al., 2006). Plants were growing on the cylindrical roll of BIONA-V3 reeled on a porous tube (diameter of 17 mm) with water inside. A specific volume of the FIERS was about 65 cm^3 per one plant. Water potential inside the tube was stabilized at (-1) kPa, that is equal to (-10) cmH_2O pressure. Crops were illuminated by high-pressure sodium lamps during 24 days continuously. Photon flux density (PPF) was approximately (450 ± 20) μmol m^{-2} s^{-1}. At 24 days plant shoot was clipped off and the crop roots were separated from FIERS mechanically, dried or kept at 4 °C. For chemical analysis roots material was dried at 60 °C for 72 h and crushed (< 1 mm) in a laboratory mill. Used Biona-V3 was utilized in the regeneration procedure after two successive 24 days growth cycles with root remains inside. Final root content inside BIONA-V3 was 0.25 ± 0.05 g/cm^3.

Properties of the root material and content of lignin, amorphous cellulose, hemicellulose, reducing sugars, proteins, pectin substances and ash content were investigated by two-step acid hydrolysis according to the previously published procedure (Yermakov et al., 1987). Microcrystalline cellulose was investigated with acetic-nitric agent (Updegraff, 1969). The total C and N contents were measured with a CHN auto-analyzer (Carlo Erba, model 1106, Italy). Measurements were done in triplicate and statistical data processing was performed at 5% level of significance.