

## Review

# Capacity of fungi for biodegradation of cellulose wastes generated at manned space flight

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## Abstract

The amount of waste generated on manned long-duration space missions away from Earth orbit creates the daunting challenge of how to manage the waste through reuse, rejection, or recycling. Microbial degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of cellulose containing organic waste in the space stations. This approach offers several advantages: low energy, mild operation conditions, control on biological hazard of the wastes, etc. Fungi are the main cellulose-degrading microorganisms in the nature and their cellulolytic features are object of intensive studies. This review describes the ability of anaerobic, aerobic, and microaerophilic fungi to degrade organic wastes generated during manned space flight.

**Keywords:** fungi, cellulose-containing waste, biodegradation, manned space-flight

## Резюме

По време на пилотируемите космически мисии, далеч от земната орбита в станциите се натрупват големи количества органични целулоза съдържащи отпадъци. Тяхното управление чрез повторна употреба, изхвърляне или рециклиране е изключително предизвикателство за науката. Микробното разграждане на място, в космическите станции, се превръща във все по-популярна алтернатива по икономически и екологични причини. Този метод предлага няколко предимства: ниска енергия, меки условия на работа и контрол върху биологичната опасност на отпадъците. Гъбите са основните микроорганизми, които разграждат целулозата в природата и техните целулозолитични свойства са обект на интензивни проучвания. Този обзор разглежда способността на анаеробните, аеробните и микроаерофилните гъби да разграждат органичните отпадъци, натрупвани по време на пилотируемите космически полети.

## Introduction

Popular depictions of space exploration as well as government life support research programs have long assumed that future planetary bases would rely on small scale, closed ecological systems with crop plants producing food, water, and oxygen and with bioreactors recycling waste.

Among the wastes that are formed in the conditions of manned space flight, a significant proportion are cellulose-containing materials such as personal hygiene products, uneaten parts of plants from greenhouse, etc. (Ilyin *et al.*, 2004; 2018). Organic waste collection and containment present

difficulties and challenges for the each manned space mission. These materials must be processed onboard the spacecraft, which requires technologies based on low energy consumption, high processing speed, on the area, volume and mass of the processing system. Microbial degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of organic waste in the space stations. It offers several advantages: low energy, mild operation conditions and control on biological hazard of the wastes.

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For the most part the organic wastes are disposable personal hygiene items used in large quantities containing human body products, which are very dangerous from the sanitary-epidemiological standpoint. Thus, it is very important to develop a process of 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.

### Structure of cellulose materials

Cellulose-containing wastes include agriculture residue, water plants, grasses, and other plant, food and hygiene materials and substances. In general, agricultural waste biomass is composed of cellulose ( $C_6H_{10}O_5$ )<sub>n</sub>, hemicellulose ( $C_5H_8O_4$ )<sub>m</sub>, lignin [ $(C_9H_{10}O_3(OCH_3)_{0.9-1.7})_x$ ], pectin, extractives, glycosylated proteins and several inorganic materials (Akhtar *et al.*, 2015). The main occurrence of cellulose is the accessible lignocellulosic substance in forests, with wood as the most essential source (Sundarraj and Ranganathan, 2018). Cellulose occurs in almost pure form in cotton fiber (Kalia *et al.*, 2011). However, in wood, plant leaves and stalks, it is found in combination with other materials, such as lignin and hemicelluloses. In addition to cellulose and hemicellulose, wood also contains lignin.

Cellulose is the most abundant form of organic waste, including these of space stations. It is a fibrous, tough, and water-insoluble polymer and plays an essential role in maintaining the structure of plant cell walls. It has been shown to be a long-chain polymer with repeating units of D-glucose linked by  $\beta$ -1,4-glycosidic bonds to form linear polymeric chains of over 10 000 glucose residues. These residues are highly organized into micro and microfibrils.

Cellulose contains both highly crystalline regions where individual chains are linked to each other and less-ordered amorphous regions; material in amorphous regions may not be able to participate in reactions if blocked by a preceding crystalline region. The stabilities of crystalline forms are similar and may depend on the temperature. A variety of different crystalline forms of cellulose are known. Cellulose Ia, Ib are meta-stable and after recrystallisation or treatment with strong base yield cellulose II. In each crystalline state the patterns of hydrogen bonding differ; the orientation of chains differs between phase I, where the chains are parallel, and phase II where the chains are anti-parallel. The different phases have different chemical and physical properties although it is not known to what extent these differences influence the degradation

rates of celluloses.

Native cellulose (exemplified by cotton wool) has a greater degree of polymerization and a smaller fraction of reducing end groups than treated cellulose (e.g. tissue) and therefore the rate and extent of hydrolysis of cotton wool is less than that of tissue (Bassil *et al.*, 2015).

Cellulose and hemicellulose have very different physical and chemical characteristics which have direct relevance to their degradation in repositories e.g. cellulose is insoluble in alkali and hemicellulose is soluble. Hemicellulose is branched polymer which contain a range of different monosaccharides. It has considerably smaller degree of polymerisation when compared to cellulose. Hemicelluloses from different plant species have different compositions but the major constituents are xylans, galactoglucomannans, glucomannans and arabinogalactans.

Lignin, the third largest available biopolymer in nature, consists of a phenyl propane (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) unit linked with ester bonds that forms a complex with hemicellulose to encapsulate cellulose, making it resistant towards chemical and enzymatic hydrolysis. Lignin-enriched biomass is more resistant for the depolymerisation of holocellulose (cellulose and hemicellulose) to produce fermentable sugars. In addition, during the degradation process, it may form furan (furfural and hydroxymethyl-furfural) compounds that could inhibit fermentation (Akhtar *et al.* 2015). In generally, the saccharification of lignocellulosic biomass is still technically problematic because of digestibility of cellulose, which is hindered by structural and compositional factors (Akhtar *et al.*, 2015).

### Cellulose-containing wastes generated during the manned space flights

Recovery of various organic wastes accumulated in the manned space flight is an actual problem of modern astronautics and future interplanetary missions. Optimal and rapid biodegradation of lignin and other cellulosic materials by fungi is paramount in the use of these organisms to achieve effective biomass recycling in ALS. It is thus imperative for a spacecraft's environmental control and life support system (ECLSS) to be able to handle all types of waste, be they solid, liquid, or gaseous. Such waste accumulation also presents considerable vehicle storage and crewmember handling challenges. Each space mission needs logistics that includes, but are not limited to, water, oxygen, nitrogen, clothing, waste collection, hygiene, health-

care and consumables (Lopez *et al.*, 2015; Wang *et al.*, 2018). A necessary element of the closed LSS of manned spacecraft is considered greenhouses for the reproduction of the vegetative part of the diet of cosmonauts. Among the crops suggested for growth in space are wheat, rice, carrots and mushrooms (Nyochembeng *et al.*, 2019). Uneaten parts of plants are waste from greenhouses. Cellulosic wastes include feces, food refuse, paper, cotton, clothing, wipes, grey tapes etc. In addition, wastes may be in mixed form such as “semi-solid” (Pul-lammanappallil and Dhoble, 2010).

The advantages of the biodegradation waste management in general are the following: it allows to diminish the volume of organic wastes, the biological hazard of the wastes is controlled, and this system may be compatible with the other systems Life Support Systems for long-term space flights.

Among the wastes that are formed in the conditions of manned space flight, a significant proportion is spent on personal hygiene (Ilyin *et al.*, 2018). Astronauts use for hygiene needs chiefly napkins and towels on cellulose basis, forming the greater part of the wastes (Simeonov *et al.*, 2010). These waste contains products of human body secretion, which are dangerous in the sanitary-epidemiological sense. According to Caraccio *et al.* (2013) hygiene items generated by four persons during one year mission could be more than 280 kg.

### Mechanisms of cellulose-containing waste degradation by fungi

Most of the cellulolytic microorganisms belong to eubacteria and fungi, even though some anaerobic protozoa and slime molds able to degrade cellulose have also been described (Fig. 1).

They produce a battery of enzymes with dif-

ferent specificities, working together. Cellulases are enzymes that hydrolyze the  $\beta$ -1,4-glycosidic linkages of cellulose, such as endo-1,4- $\beta$ -glucanases (Egs, EC 3.2.1.4), cellobiohydrolases (or exo-1,4- $\beta$ -glucanases) (CBHs, EC 3.2.1.91) and  $\beta$ -glucosidases (EC 3.2.1.21) (Pérez *et al.*, 2002). EGs breakdown cellulose by attacking the amorphous regions to produce more accessible new free chain ends for the action of CBHs. An effective hydrolysis of cellulose also requires  $\beta$ -glucosidases, which break down cellobiose releasing two glucose molecules.

Theoretically, this enzyme assemblage is sufficient to degrade cellulose completely into glucose, and commercial cellulase mixtures typically include these enzymes in varying proportions. However, the crystalline portion of cellulose is only partially attacked by hydrolytic enzymes, and in practice, its degradation proceeds slowly and is incomplete. For cellulose degradation additional components must be present to achieve complete hydrolysis, a cocktail of  $\beta$ -glucosidases, endoglucanases, and exoglucanases are required. Hydrolysis of hemicellulose requires enzymes with additional functionality, including xylanases and mannanases. To access these sugar polymers from crude biomass, it is often necessary to solubilize lignin, which is crosslinked within cellulosic and hemicellulosic fibers. For this process, accessory enzymes such as polysaccharide deacetylases, peroxidases, and esterases are required (Fig. 2) (Gilmore *et al.*, 2015).

The anaerobic microorganisms utilize complexed cellulase systems for cellulose degradation, called cellulosomes. Bacterial cellulosomes typically contain enzymes required only for cellulose degradation while fungal enzyme complexes contain a richer diversity of enzymes to enable degradation of crude plant material.

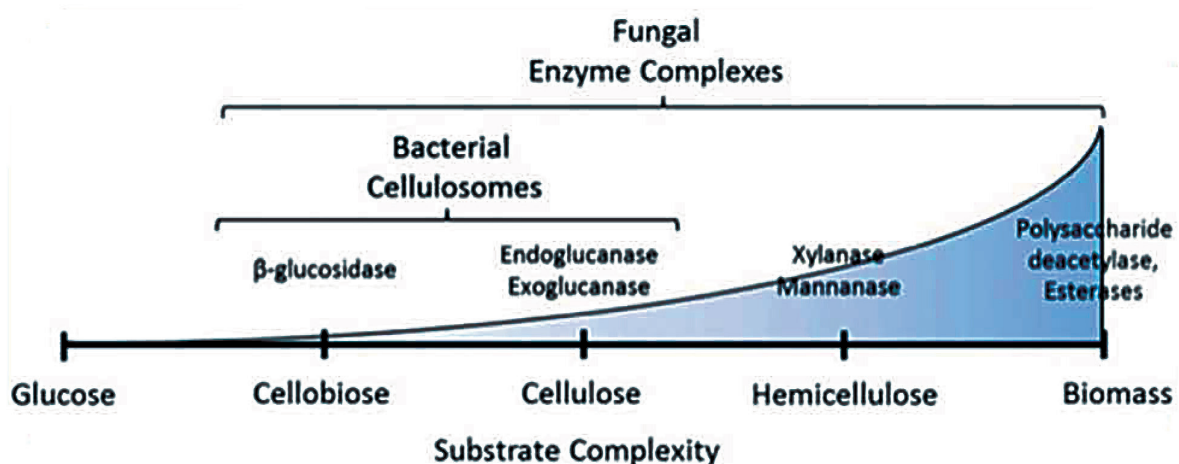
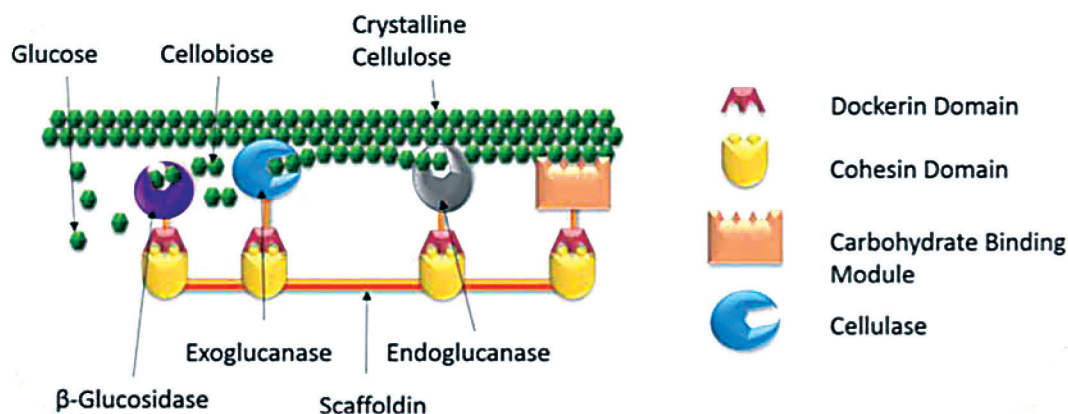


Fig. 1. Enzyme degradation of cellulose-containing biomass by bacteria and fungi (Gilmore *et al.*, 2015).





**Fig. 2.** Synergistic action of cellulases within a cellulosome (Gilmore *et al.*, 2015)

Cellulases assemble in close proximity on a noncatalytic protein called a scaffoldin. The endoglucanase reduces the degree of crystallinity of the cellulose substrate and liberates both cellulose chain ends. The exoglucanase processes along a free chain, freeing cellobiose with each cleavage. This cellobiose is then transferred to a nearby  $\beta$ -glucosidase, which hydrolyzes it into two glucose monomers.

Fungal genera *Trichoderma*, *Fusarium* and *Aspergillus* are thought to be cellulase producers, and crude enzymes produced by these microorganisms are commercially available for use. Fungi of the genus *Trichoderma* produce relatively large quantities of endo- $\beta$ -glucanase and exo- $\beta$ -glucanase, but only low levels of  $\beta$ -glucosidase, while those of the genus *Aspergillus* produce relatively large quantities of endo- $\beta$ -glucanase and  $\beta$ -glucosidase with low levels of exo- $\beta$ -glucanase production.

Fungi are the main cellulase producing microorganisms, though a few bacteria and actinomycetes have also been reported to yield cellulase activity (Kadarmoidheen *et al.*, 2012). They are capable of degrading and utilizing cellulose and hemicellulose as carbon and energy sources. Most emphasis has been placed on the use of fungi because of their capability to produce copious amounts of cellulases and hemicellulases which are secreted into the medium for easy extraction and purification.

One of the most important features of cellulose as a substrate for microorganisms is its insolubility. Bacterial and fungal degradation of cellulose and other insoluble polymers occurs exocellularly, either in association with the outer cell envelope layer or extracellularly. This suggests that the assembly of enzyme systems, which may be extremely complex, also occurs exocellularly. To function, these enzyme systems must be stable in the exo-

cellular environment; for example, they must be reasonably resistant to proteolytic attack. Also, the products of cellulose hydrolysis may be available as carbon and energy sources for other microbes that inhabit environments in which cellulose is biodegraded, thereby forming the basis of many interactions between microorganisms in these environments (Leschine, 1995).

#### *Degradation by anaerobic fungi*

The mechanism of cellulose degradation in anaerobic environments is very complex; it involves numerous, varied interactions of metabolically diverse microorganisms whose activities are influenced by a wide range of environmental factors. The mechanism by which cellulases from anaerobic fungi catalyze the depolymerisation of crystalline cellulose is clearly fundamentally different from that of the cellulase systems of most aerobic fungi. Anaerobic fungi gain energy from the fermentation of carbohydrates. Of the common plant monosaccharides, fructose, glucose, xylose, cellobiose and gentiobiose were used by all isolates in several different studies, whereas galactose and mannose utilization varied and L-arabinose was not used (Gordon and Phillips, 1998). Utilization of oligosaccharides and polysaccharides is more varied though the plant polysaccharides cellulose and xylan were used by all isolates. Kameshwar and Qin (2018) investigated the genes, enzymes and the mechanisms involved in structure and functioning of the cellulosomes of the anaerobic fungi. They revealed the genomic machinery underlying the extrinsic plant cell wall degrading abilities. According to Couger *et al.* (2015), cellulose-degrading anaerobic fungi achieve fast and effective results by the simultaneous employment of a wide array of constitutively-transcribed cellulosome-bound and free enzymes with considerable functional

overlap. The authors consider that the utilization of this indiscriminate strategy could be justified by the evolutionary history of anaerobic fungi, as well as their functional role within their natural habitat. Anaerobic fungi are the only fungi which possess cellulosomes.

A significant amount of research on anaerobic cellulose degradation has focussed on landfill sites since domestic waste contains cellulosic materials such as paper and wood. It is well established that anaerobic fungi reside in the guts of herbivorous mammals where cellulose is abundant and oxygen is absent. Moreover, other anaerobic environments may also support these fungi. For example, molecular ecological surveys have unequivocally demonstrated that anoxic zones contained within landfills harbor *Neocallimastigomycota*, suggesting that anaerobic fungi are not exclusively gut inhabitants (Haitjema *et al.*, 2014). Transitional stages where fungi migrate between anoxic environments may be made possible by a third life cycle stage characterized by an aero-tolerant cyst or resistant spore-like structure. Such structures have been observed in cultures of *Anaeromyces*, and it was suggested that their presence enabled cultures to stay viable over extended time periods of greater than 272 days (see Haitjema *et al.*, 2014). Anaerobic fungi have been found also in freshwater lakes, landfill and deep-sea sediments. Moreover, they have been shown to be part of the microbial community in biogas reactors (Ivarsson *et al.*, 2016).

The anaerobic fungi are important members of the rumen microbial community. Because they are found in large numbers attached plant fragments removed from the rumen, and they ferment the major plant polysaccharides, they are believed to take part in decomposing cellulose in the rumen. The following genera of fungi have been described for effective degradation: *Neocallimastix*, *Caecomyces*, *Piromyces*, *Orpinomyces*, and *Ruminomyces* (Leschine, 1995; Thareja *et al.*, 2006). These fungi reproduce through the asexual production of flagellated zoospores from sporangia, with no sexual reproductive life stage identified to date (Gruninger *et al.*, 2014). Three rumen anaerobic fungal strains belonging to these genera, namely *N. frontalis* MCH3, *P. communis* FL, and *Caecomyces communis* FG10, were cultured on cellulose filter paper alone or in association with one of two rumen cellulolytic bacteria, *Ruminococcus flavefaciens* 007 and *Fibrobacter succinogenes* (Bernalier *et al.*, 1992). Similar results have been reported for *Neocallimastix* and

*Piromyces* species on paper cellulose degradation (Teunissen *et al.*, 1991). The fungal monocultures were evaluated as markedly more effective than the co-cultures and bacterial monocultures. The rumen anaerobic fungus *O. jayonii* A4 has been used for waste paper degradation (Kovář *et al.*, 2000). The strain *N. frontalis* C5-1 isolated from the rumen of a Korean native goat has also proved very suitable for the degradation of cellulose-containing materials (Ha *et al.*, 2001). Co-cultivation of *Neocallimastix* sp. L2 and metanogenic bacteria (*Methanobacterium arboriphilus*, *Methanobacterium bryantii*, or *Methanobrevibacter smithii*) increase the rate of filter paper degradation (Marvin-Sikkema *et al.*, 1990). This cultivation strategy activated the process by a shift in the fermentation products to more acetate and less lactate, succinate, and ethanol. On the other hand, cellulolytic power was inhibited when fungi were exposed to sugars they did not metabolize, suggesting a general mode of catabolite repression (Henske *et al.*, 2018).

Many ruminal fungi require specific nutrients from ruminal fluid for digestion of cellulose, but they can be cultured in medium lacking ruminal fluid when it is amended with the appropriate growth factor(s). Gordon and Phillips (1989) reported that ruminal fungal strains *Neocallimastix* sp. LM-1, *Piromonas* sp. SM-1 and *Sphaeromonas* sp. NM-1 growing in a semidefined medium can digest cellulose and plant cell walls when grown in media lacking ruminal fluid (Table 1).

**Table 1.** Breakdown of some components of wheat straw by ruminal fungi grown for 10 days in a semidefined medium or in a ruminal-fluid medium (Gordon and Phillips, 1989)

Fungal strain	Ruminal-fluid in medium	% of component solubilized	
		ADF*	Cellulose
<i>Neocallimastix</i> sp. strain LM-1	No	47.0	54.1
	Yes	46.4	55.2
<i>Piromonas</i> sp. strain SM-1	No	45.6	53.8
	Yes	45.0	53.4
<i>Sphaeromonas</i> sp. strain NM-1	No	35.0	38.7
	Yes	32.8	38.4

\*Acid detergent fiber (ADF)

Anaerobic fungi possess a potential to decompose agriculture and kitchen wastes. Wilken *et al.* (2018) propose *in silico* identification of micro-

bial partners to form consortia with anaerobic fungi aiming to degrade corn stover. Rumen microbial community containing anaerobic fungi and bacteria has been used for fresh grass waste degradation in a 4 day solids retention time (Nair *et al.*, 2005). Anaerobic fungi have been included effectively in efficiently hydrolyze of cellulose-containing domestic and agricultural wastes (Garg, 2017).

Three anaerobic communities isolated from lime-kiln exhibited a great power for cotton fibers degradation (Bassil *et al.*, 2015). Dominated microorganisms are bacterium *Geobacter sulfurreducens*, archaea *Methanohalophilus halophilus*, and fungus *Handkea fumosa* (Fig. 3.).



**Fig. 3.** The triplicate of the tissue microcosms, incubated for 30 months at 25°C and in the presence of Ca(OH)<sub>2</sub> at saturation. Samples 1, 2 and 3 differ in the amount of sediment.

#### *Degradation by microaerotolerant/microaerophilic fungi*

A microaerotolerant (facultative) anaerobe is an organism that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation if oxygen is absent. They show growth in an anaerobic system; 5% CO<sub>2</sub>, 10% H<sub>2</sub>, 85% N<sub>2</sub>; a candle extinction jar, and in air. The data on the species diversity of facultative anaerobic fungi, their abundance, and occurrence in various habitats are extremely scarce. Several species of facultatively anaerobic fungi were detected in various type of soil. *Mucor hiemalis*, *M. circinelloides*, *Rhizopus oryzae*, *Fusarium solani*, *F. oxysporum*, *Trichoderma atroviride*, *T. polysporum*, *T. harzianum*, *T. aureoviride*, *T. viride*, and *T. koningii* were the fungi most often found in soil (Kurakov *et al.*, 2008). Such microaerotolerant fungi have been isolated from oceanic crust, deep soft sediments in the Bosphorus outlet area of the Black Sea, groundwater-sediment of varying depths, wood probes etc. (Sergeeva and Kopytina, 2014; Ivarsson *et al.*, 2016; Hoque and Fritscher, 2017).

Microaerotolerant fungi are capable to decompose cellulose effectively. The genus *Trichocladium* is a saprotrophic soil fungus commonly found in soil and aquatic environments. It is known for its cellulolytic activity; when grown under microaerophilic conditions, certain *Trichocladium*

species can convert ca. 90 to 96% of available cellulose to ethanol (Eichorst and Kuske, 2012). The fungal strains *T. canadense*, *Geotrichum* sp., and *Fusarium* sp. cultivated under microaerophilic and combined (aerobic, followed by microaerophilic) conditions were able to grow and effectively degrade lignocellulosic materials, such as paper filter, Avicel, and agriculture residues at a final concentration 0.5% (dry wt/v) (Pavarina and Durrant, 2001; 2002). The results suggested that they prefer lower oxygen concentration for growth and enzyme production. Durrant *et al.* (1995) isolated two strains of morphologically and physiologically distinct cellulose-fermenting fungi from soil samples under anaerobic conditions. Based on morphology, one isolate was identified as a species of *Trichocladium* and the other as a basidiomycete species. Both strains grew and utilized cellulose more rapidly when incubated under microaerophilic conditions. They used pentoses, hexoses, cellobiose and xylan as microaerophilic growth substrates. A Japan research group reported a novel filamentous fungus strain characterized as a facultative anaerobic member of the class *Lecanoromycetes* of the phylum *Ascomycota*, which grows in both aerobic and strict anaerobic conditions (Tonouchi, 2009).

#### *Degradation by aerobic fungi*

The aerobic fungal isolates bring about most of the cellulose degradation occurring in various environments. The diverse subdivisions *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* contain large numbers of cellulolytic species. Among the most studied aerobic fungal genera are *Chaetomium*, *Coriolus*, *Phanerochaete*, *Poria*, *Schizophyllum*, *Serpula*, *Aspergillus*, *Fusarium*, *Geotrichum*, *Paezilomyces*, *Penicillium*, and *Trichoderma* (Viikari *et al.*, 2009). Clustering of the fungi based on their predicted enzymes confirmed that *Ascomycota* and *Basidiomycota* use the same enzymatic activities to degrade plant cell walls (Busk *et al.*, 2014).

*T. reesei* is the most studied cellulolytic fungus (Gunda, 2011). This species has been characterized for over 60 years and is considered an industrial workhorse for cellulase enzymes. Along with *T. reesei*, *Phanerochaete chrysosporium* and members of genus *Fusarium* are also the thoroughly studied (Soliman *et al.*, 2013). The strains *T. viride*, *A. niger* and *F. oxysporum* have been used in studies of cellulosic waste biodegradation (Kadarmoidheen *et al.*, 2012). Among the three fungal isolates studied *T. viride* was found to be the most efficient in degrading the cellulosic wastes viz., paddy straw, sugarcane



bagasse and banana stalks decreasing the cellulose content by 53.70, 51.59 and 55.28 % respectively. *A. candidus* has been established as a good agent for degradation of rice husk, millet straw, guinea corn stalk and sawdust (Milala *et al.*, 2009).

Marine fungi have been found to produce a wide range of lignocellulose-active enzymes that could explain their great potential to biotransformation of plant wastes (Balabanova *et al.*, 2018). The consortium of ligninolytic and cellulolytic marine-derived fungi prove to have the potential for application in the effective utilization of agricultural refuse (Ramarajan and Manohar, 2017). Similar enzyme profile were identified in fungal strains belonging to species *T. harzianum*, *A. niger*, *A. flavus* and *Penicillium expansum*, exhibiting kitchen waste decomposition at temperature of 25°C (Ashraf *et al.*, 2017). Moreover, degradation of cellulosic materials by fungi like *Cladosporium cladosporioides*, *C. sphaerospermum*, *P. chrysogenum*, *Scopulariopsis brevicaulis*, *Stachybotrys chartarum*, *Verticillium cyclosporum*, and *Chaetomium hamadae* is well documented. *Pleu-*

*rotus ostreatus*, *P. florida*, and *T. viride*, isolated from natural habitats, have been used as immobilized cells in continuous systems for bioconversion of cellulose wastes (Petre *et al.*, 1999). Effective cellulose degrading enzyme systems demonstrated aerobic fungi from genera *Helminthosporium*, *Cladosporium*, *Trichoderma*, and *Aspergillus* (Sivaramanan, 2014). They have been obtained from sawdust, straw dust and sprinkled soil, decaying wood particles and decaying leaf and could be used for treatment of filter paper or agro-wastes such as rice straw. Isolates from soil and decaying wood caused aerobic degradation of cellulose waste materials, e.g. paddy straw, sugarcane bagasse and banana stalks (Kadarmoidheen *et al.*, 2012). The best were obtained with the use of *T. viride*, *A. niger* and *F. oxysporum*.

Parihar *et al.* (2012) found that, among 212 species wood decaying fungi 33 of them were able to decompose cellulose at varying degree (Table 2). The authors concluded that cellulose decomposition pattern was vary not only among the genera but also between the species of same genus.

**Table 2.** Cellulose decomposition by wood decaying fungi (Parihar *et al.*, 2012)

Name of test fungus	% weight loss	Growth Rate/24h, mm	CAI*
<i>Trametes cubensis</i>	8.792	2.3	3.823
<i>Poria fulviseda</i>	9.596	8.3	1.156
<i>Trametes lactinea</i>	10.426	10	1.043
<i>Polyporus gramocephalus</i>	11.125	8.3	1.34
<i>Trametes pocas</i>	12.083	6.5	1.859
<i>Lenzites aspera</i>	15.51	7.5	2.068
<i>Hapalopilus nidulans</i>	18.501	2	9.251
<i>Earliella scabrosa</i>	19.429	3.8	5.113
<i>Pycnoporus sanguineus</i>	19.718	6.6	2.988
<i>Ganoderma lucidum</i>	21.848	3.8	5.749
<i>Trametes feei</i>	24.907	9.3	2.678
<i>Lenzites platyfylla</i>	25.703	9	2.856
<i>Lenzites palsoti</i>	26.179	8.3	3.154
<i>Schizophyllum commune</i>	27.466	8	3.433
<i>Trametes cingulata</i>	34.25	6.2	5.524
<i>Trametes leonina</i>	36.683	7.8	4.703
<i>Lenzites steroids</i>	41.134	8.3	4.956
<i>Pycnoporus cocineus</i>	43.269	5	8.654
<i>Polyporus brumalis</i>	44.767	9	4.974
<i>Innonotus tabacinus</i>	45.034	6.6	6.823
<i>Daedalea sulcata</i>	45.076	7.7	5.854
<i>Navisporus floccosus</i>	63.241	5	12.648

\*CAI=Percentage wt. loss/Growth rate of test fungus in 24 h (*Cellulolysis Adequacy Index (CAI)* for each isolate was calculated from the weight of cellulose respired by the fungus growing on filter paper (percent weight loss) and its linear growth rate in mm per 24 hrs growing on potato dextrose agar medium at 28°C ± 2°.)

The high cellulose content (about 94%) and hygroscopic nature of the cotton fibers facilitate its decomposition by fungi. Very suitable for such a process could be noted *Trichoderma* sp., *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., and *Helminthosporium* sp. (Sivaramanan, 2013).

the agriculture wastes. The authors noted the positive treatment by *Serpula lacrymans*, *Coniophora puteana*, *Meruliporia incrassata*, *Laetoporeus sulphureus* and *Gleophyllum trabeum*, *Xylariaceae ascomycetes*, *Daldinia concentric*. Tuomela *et al.* (2000) reviewed the possibility of edible fungi to degrade compost (Table 3).

**Table 3.** Fungi from different subdivision which effectively degraded cellulose (see Tuomela *et al.*, 2000)

Fungus	Subdivision	Lignocellulose degradation
<i>Aspergillus fumigatus</i>	<i>Deuteromycotina</i>	Wood degradation, cellulose degradation
<i>Chaetomium thermophilum</i>	<i>Ascomycotina</i>	Very active cellulose degradation
<i>Malbranchea cinnamomea</i>	<i>Deuteromycotina</i>	Cellulose degradation
<i>Melanocarpus albomyces</i>	<i>Ascomycotina</i>	Cellulose and hemicellulose degradation
<i>Myceliophthora thermophila</i>	<i>Ascomycotina</i>	Very active cellulose degradation, wood degradation
<i>Paecilomyces spp.</i>	<i>Deuteromycotina</i>	Cellulose and some lignin degradation
<i>Phanerochaete chrysosporium</i>	<i>Basidiomycotina</i>	Effective lignin degradation, newspaper degradation
<i>Scytalidium thermophilum</i>	<i>Deuteromycotina</i>	Cellulose degradation
<i>Stibella thermophila</i>	<i>Deuteromycotina</i>	Cellulose degradation
<i>Talaromyces emersonii</i>	<i>Ascomycotina</i>	Wood degradation
<i>Thermomyces lanuginosus</i>	<i>Deuteromycotina</i>	Cellulose degradation

The experiments of the Ilyin research group concerning 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 arouse a great interest (Ilyin *et al.*, 2018). They test the ability of *T. 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. The results 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. Effective degradation has been established after fermentation of vegetable waste products.

#### Edible Fungi

Cellulose-containing wastes such as raw cotton, rice straw, paper, etc. provide an ideal substrate for the growth of some edible mushrooms notably *Volvariella volvacea*, *Pleurotus* spp, etc. Optimal and rapid biodegradation of cellulosic material of the crop residues (wheat, rice, carrots, and mushrooms) by edible white rot fungi has been achieved (Nyochembeng *et al.*, 2019). Akhtar *et al.* (2015) reviewed the efficacy of brown-rot and soft-rot fungi for hydrolysis of cellulose and hemicellulose in

Nyochembeng *et al.* (2019) carried out experiment on degradation of wheat, rice, carrots and mushrooms, wastes from long-term crewed space flights. Optimal and rapid biodegradation of lignin and other cellulosic material of the crop residues by candidate edible white rot fungi is paramount in the use of these organisms to achieve effective biomass recycling in ALS. They investigated mycelial growth and fruiting of two strains of *P. ostreatus*. Growth and fruiting of the two strains on rice straw mixed with solid thermophilic aerobic reactor (STAR) effluent for degradation and recycling were also studied. Both wheat and rice straw used were dried and milled to a size of 2 mm prior to incorporating different concentrations of urea solution in the wheat straw, or varying concentrations of STAR sludge in the rice residue. High concentration of STAR residue enhanced mycelial growth, however, a relatively lower concentration (20%) was required for abundant fruiting.

#### Application of technologies for waste degradation in space-flight conditions

According to Paavola (2009), the unique constraints of developing a technology for use in space may be beneficial for small-scale biomass conversion facilities. It is necessary to minimize the kind of consumable chemicals that are used in the process, to minimize the hazardous chemicals used in the process, and also to minimize ener-



gy required for the process and the weight of the equipment.

Experiments on cellulolytic waste degradation under conditions of space-flight have been not founded. There are publications about the effect of microgravity on fungal development. The stressful environment of space causes changes to all forms of life, from bacteria and fungi, to animals and people. Investigations about the effect of microgravity on growth, gene expression, physical responses, and metabolism of a fungus *A. nidulans* provide data for increase virulence and resistance to UV (Singh *et al.*, 2016).

Microscopic characteristics of spores from dried spores samples have been investigated, as well as the morphology of the colonies obtained from spores that survived during mission (Gomoiu *et al.*, 2016). For the experiment, authors selected the fungal species *A. niger*, *C. herbarum*, *Ulocladium chartarum*, and *Basipetospora halophila* based on their involvement in the biodeterioration of different substrate in the International Space Station (ISS) as well as their presence as possible contaminants of the ISS. The results revealed that spores used in the long term experiment lost the outer layer of their coat without affecting the viability since they were still protected by the middle and the inner layer of the coating. This research highlights a new protocol to perform spaceflight experiments inside the ISS with fungal spores in microgravity conditions, under the additional effect of possible cosmic radiation. According to this protocol the results are expressed in terms of viability, microscopic and morphological changes.

Large-scale farming in presence of an Earth-like atmosphere in space faces two main challenges: plant yield in microgravity and plant nutrition in extraterrestrial soils, which are likely low in nutrients compared to terrestrial farm lands. Liu *et al.* (2018) propose a plant-fungal symbiosis (i.e. mycorrhiza) as an efficient tool to increase plant biomass production in extraterrestrial environments. The experiments concerned mycorrhization of *Solanaceae* on the model plant *Petunia hybrida* using the arbuscular mycorrhizal fungus *Rhizophagus irregularis* under simulated microgravity (s0-g) conditions obtained through a 3-D random positioning machine. Based on the results, the authors propose that in nutrient limited conditions root exudation can challenge the negative microgravity effects on mycorrhization and therefore might play an important role in increasing the efficiency of future space farming.

Phenotypic and genotypic changes in *A. niger* and *P. chrysogenum*, spore forming filamentous fungi, with respect to central chitin metabolism were studied under low shear modeled microgravity, normalgravity and static conditions (Sathishkumar *et al.*, 2014). The results collectively indicate that the low shear modeled microgravity (LSMMG) has shown no significant stress on spore germination, mycelial growth, cell wall integrity of potentially pathogenic fungi.

The only information available in the literature about experiments in space with fungi are these carried out by the research group of Ilyin. The authors discussed a possibility of applying anaerobic digestion for reduction and stabilization of the organic fraction of solid wastes generated during piloted spacecraft flights (Ilyin *et al.*, 2018). The culture of *T. viridae*, isolated from the internal environment of the ISS, has been tested for anaerobic decomposition of gauze tissue under space flight conditions. The results showed a reduction in the total concentration of volatile organic impurities remaining after the biodegradation of gauze from 34 mg/m<sup>3</sup> to 5 mg/m<sup>3</sup>. 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.

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