NOVEL MYCO-COMPOSITE MATERIAL OBTAINED WITH *FUSARIUM OXYSPORUM*

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Modern world environmental challenges dictate the need for obtaining of alternative materials in various industrial sectors. Filamentous fungi strains are complex microorganisms that are able to produce rich enzymes batteries that are able of breaking down a wide variety of organic substrates. Present work explored the potential of *Fusarium oxysporum* strain for creating a novel biocomposite structure, based on an alternative substrate, composed of recycled shredded paper and worn coffee. Strain was successfully grown on an alternative nutritive substrate, which allowed formation of a 64.23µm biofilm on the surface of the substrate, and development of a homogenous hyphal matrix inside the aerial structure of the substrate, yielding a high rigidity material. Both optical and SEM analyses revealed even distribution of the hyphae and partial enzymatic hydrolysis of the recycled paper, which acted as a carbon source for cell development. Flammability analysis conducted on the biofilm surface of the material revealed very good fire retardant properties, withstanding close flame contact up to 80 seconds, after which smoldering fire occurred. Preliminary results are promising in exploiting the potential of an important plant pathogen in obtaining high added value materials, based on use of organic wastes which serve a double functionality, with nutritive role and mechanical substrate.

Keywords: biocomposite, fungi, *Fusarium oxysporum*

INTRODUCTION

Microorganisms derived biomaterials have lately enjoyed great attention, as self-growing natural composites, based on fungal mycelium, represent the latest trend in the biocomposites market. Creation of sustainable packaging materials is dictated by two major factors, regarding conventional materials: high consumption of nonrenewable resources and disposal of petrochemical based materials into the environment (Maachia *et al.*, 2005), which need a very long biodegradation process. The production of 1m³ of polystyrene consumes 4,667 Megajoules (MJ) of energy and release into the atmosphere a quantity of 462 kg of CO₂ while the production of 1m³ of fungal obtained material uses 652 MJ and releases 31kg of CO₂ (Holt *et al.*, 2012). Robust, low-cost, non-toxic manufacturing processes for the packaging industry represent major factors driving future markets and demands for bio-based materials in the European Union. Biomaterials obtained using fungal mycelium are safe and inert, lightweight, strong and durable, flame resistant and insulating, comparable with plastic packaging materials (polystyrene) and they can be used for large area of applications including decorative products, electronics packaging materials, building blocks, acoustic tiles etc. (Haneef *et al.*, 2017). No studies were conducted on *Fusarium oxysporum* (of Ascomycota Division) strain for obtaining of biocomposites, so far. This particular strain, which is a known plant pathogen agent, was selected because of the rich battery of enzymes it possesses (can produce cellulase, pectinase, protease, xylanase etc.) (Kashiwa *et al.*, 2016), fast growing rate and colonization capability of the substrate. Filamentous fungi strains are the most efficient and competitive microorganism used for the production of

mycelium derived materials due to their physiological, enzymological and biochemical properties.

MATERIALS AND METHODS

Microbial Strain and Substrate

The microbial strain used in obtaining of the myco-composite was *Fusarium oxysporum* obtained from Politehnica University of Bucharest strains collection. Fresh culture of the strain was obtained in Potato-Dextrose nutrient broth (Scharlau), in final volume of 100mL broth, previously sterilized at 121°C for 15'. Furthermore, the strain was incubated at 28°C for 10 days, until it reached metabolic maturity, and the appearance of the dark-purple aerial mycelium at the broth-air interface inside the flask.

Inoculation substrate was designed to act as both mechanical support substrate and nutritive substrate. Substrate was composed of shredded used paper and worn coffee, mixed in a ratio of 10:1 grams, and 0.5g of anhydrous D(+)-Glucose (Scharlau). All the substrate ingredients were thoroughly mixed, hydrated with sterile water, left at rest for 3 hours, at room temperature (28°C) for the water to drain, and then sterilized at 121°C for 15 minutes. After sterilization, the substrate was inoculated with the microbial strain by mixing the substrate volume with only the microbial biomass (approximately 4 grams of biomass) and thoroughly mixed in order to obtain a highly homogenized mix, and to allow the mechanical break of the biomass, for a better distribution in the substrate. The inoculated substrate was then incubated at 28°C, in a sterile Petri dish, in the dark, for 14 days. After the incubation period, the microbial growth was stopped by inactivating the biocomposite at 130°C for 3 hours, which also led to loss of constituent water.

SEM and Optical Microscopy Analysis

Microscopic analysis and morphological characterization on both obtained structure and constituent parts (paper and grounded coffee) was performed by scanning electron microscopy (SEM) using a Quanta 200, Fei (Netherlands) electron microscope. Morphological analyses were performed on the GSED detector, ESEM mode, spot beam size of 4.0, 10kV filament voltage, with image acquisition at 27.2 seconds.

Optical microscopy analysis was carried out on an Olympus SZX7 stereomicroscope, with 7:1 zoom ratio, built-in electrostatic discharge protection, and advanced Galilean optical system for highly resolved images. For assessment of surface biofilm morphological structure and specific filamentous growth on the back of the material, analyses were carried out at magnification levels of 0.67x and 2x.

Flammability Analysis

Flammability analysis on the developed composite was carried out on Flexiburn equipment, from James H. Heal & Co. Ltd. (England), according to SR EN ISO 6941:2004 international standard. Flame height was set to approximately 4.5cm, and the material (9x9cm dimension) was held above the peak of the flame in two time intervals, namely for 10 seconds and for 80 seconds.

RESULTS AND DISCUSSIONS

Following start of biomass development, microbial development on the surface of the substrate was possible starting with the 2nd day, where white mycelia net could be visible colonizing the surface. Growth during the incubation period allowed *Fusarium oxysporum* biofilm formation on the entire surface of the substrate, obtaining a highly homogenized surface, with dark-brown pigmentation and increased rigidity (Figure 1).

Figure 1. *Fusarium oxysporum* substrate surface biofilm development

However, due to possible high humidity content in the substrate formulation, combined with substrate thickness (~1.5cm), biofilm formation only occurred on the surface of the substrate, but microbial development can also be detected in the obtained biocomposite section (Figure 2), which presents both hyphal development, and hydrolyzed substrate.

Figure 2. Biocomposite section highlighting microbial development

The microbial developed matrix that occurred at the surface of the substrate allows for increased cells cohesion within the structure (Flemming *et al.*, 2010), consisting of various constitutional matrix components such as proteins and glycoproteins, neutral polysaccharides, carbohydrates (such as glucose, mannose), nucleic acids etc. This complex composition is in high dependency with a multitude of factors, starting from the strain used, substrate composition, ratio between the constituent components of the substrate, humidity, pH value, incubation temperature and period, affecting the balance between structural components, the most important being in protein composition variation (Zarnowski *et al.*, 2014).

Furthermore, for morphological characterization, substrate was visualized under a stereomicroscope, for both microbial biofilm specific morphology and in-section hyphal development (Figure 3).

Figure 3. Biocomposite stereomicroscopy analysis

Visual inspection shows clear separation between biofilm structure and hyphal development structure, with homogenous matrix development that occurred on the surface of the material. Section analysis shows short hyphal segments that developed within the aerial structure of the biocomposite. Even though the substrate was formulated having high thickness, combined with high humidity content, the strain development was able to penetrate even the inferior layers of the substrate, where hydrolysis occurred, and the whole process was driven as a self-sustaining one, with both substrate hydrolysis and cell growth occurring concomitantly.

In order to investigate the microscopic structure even more, SEM analysis was conducted on both biocomposite surfaces (biofilm surface and back of the biofilm) (Figure 4 & 5).

Figure 4. Surface of the biofilm structure SEM analysis

Figure 5. Back of the biofilm structure SEM analysis

Biofilm analysis revealed a smooth and homogenous structure, with no visible hyphal development on the surface, compared to the back of the biofilm, where hyphal structures can be visible, colonizing cellulose fibers. Furthermore, section analysis was carried out which revealed close interconnection of the biofilm development stage of the strain with the hyphal development, including measurement of the biofilm thickness, reported as 64.23 μ m (Figure 6).

Figure 6. Biocomposite section SEM analysis

Even though the highest microbial activity takes place at the surface of the substrate, hyphal development inside the substrate structure, due to its aerial structure, allowed production of exo-enzymes, which were responsible with substrate hydrolysis. Thus allowing for a double effect, on one side, this allowed breaking down of constituents which were used as nutrients, mainly carbon source, for synthesis of cell wall, and on the other side, mechanical cohesion of the substrate.

Flammability assays are very important in characterization of fire retardant properties of packaging materials. Polystyrene materials can release a complex mixture of toxic chemicals (including styrene gas), and even fire retardant compounds that are currently used in inducing fire retardant properties can cause significant environmental disruptions. Flammability analysis revealed fire retardant properties of the biocomposite, when flame was applied on the biofilm surface, both after 10 seconds and after extended to 80 seconds, the material did not maintain combustion (Figure 7). However, after maintaining the flame above 80 seconds threshold, the material was consumed by smoldering fire, during a time span of approximately 30 minutes.

Figure 7. Biocomposite flammability test

CONCLUSIONS

Fusarium oxysporum is a soil-borne plant pathogen in agricultural environment. Present work has successfully explored the ability of the strain to be used for obtaining a biocomposite structure, on recycled shredded paper and worn coffee. Flammability test showed promising results, with the material being able to withstand flame contact for as long as 80 seconds, without catching fire. In the near future, this kind of biotechnologies can completely replace obtaining of petrochemical-based packaging materials, with sound innovation in sustainable packaging design. This kind of materials propose novel structures, that are environmentally safe, fire resistant, VOC free, and 100% sustainable, with compostable mycelium materials that have a wide range of applicability: the packaging industry will be more competitive by replacing petroleum-derived polymers with sustainable and fully biodegradable compostable materials.

Acknowledgments

This work was carried out in Nucleu Programme TEX-PEL-2020, implemented with the support of MCI, project nr. PN 18 23 01 02, entitled “Exploitation of filamentous fungi for the production of bio-composite materials”, acronym Micopac.

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