FT-IR ANALYSIS OF *FUSARIUM OXYSPORUM* GROWN MYCO-COMPOSITE

OVIDIU IORDACHE[1], IULIANA DUMITRESCU[1], CIPRIAN CHELARU[1], ELENA PERDUM[1], CORNELIA MITRAN[1,2], ANDREEA CHIVU[1], IRINA-MARIANA SĂNDULACHE[1]

[1]*The National Research Development Institute for Textiles and Leather, 16 Lucretiu Patrascanu, 030508, Bucharest, Romania*

[2]*Politehnica University of Bucharest, 1-7 Polizu Street, Bucharest, Romania*

Present study was focused on obtaining a novel biocomposite obtained from a plant pathogenic strain, *Fusarium oxysporum,* on wastes from renewable sources, and assessment of microbial development on both the substrate surface and inside the aerial structure, by FT-IR analysis. Fourier Transform Infrared (FTIR) spectroscopy was successfully used for recording of infrared spectra specific to various components of microbial biomass of *Fusarium oxysporum*, grown on an alternative substrate composed of recycled shredded paper and worn coffee. FT-IR analysis allowed highlighting of various functional groups, specific of constituents of microbial biofilm and hyphae: C–H asymmetric and symmetric stretching of CH, $–CH_2$, and $–CH_3$ highlighted at 2923 and 2857cm^{-1} which were indicators of the microbial cell fatty acids component, C=O stretching at 1631cm^{-1} of amide I of protein β-turns, N–H and C–N stretching at 1542cm^{-1} of amide II from α-helix of proteins, polysaccharide components, as a major constituent of the fungal biofilm, was confirmed by FT-IR bands at 1380cm^{-1}, 1195cm^{-1} and 1029.8 cm^{-1}. The verso side of the composite showed similar absorption values as the paper, but with higher intensity peaks, which indicated substrate degradation. Therefore, FT-IR analysis made possible the assessment of existence in the developed biocomposite of various biomolecules such as proteins, lipids, polysaccharides.

Keywords: FTIR, biofilm, 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. Innovative biotechnological processing for transforming and improving exploitation of waste and by-products from household waste, agricultural waste, fruits and vegetables processing industries, plastic and textile industries, will reduce the environmental impact of wastes, will enhance the sustainable management of organic as it can be used as feedstock for value-added bio-based materials. The production of packaging materials with the use of fungal strains and organic waste uses 12% of the energy normally required in the manufacture of traditional plastic packaging and can reduce carbon emissions by up to 90%. Various patents have explored the use of filamentous fungi strains for obtaining mycelium-derived biocomposites, with the aid of *Pleurotus djamor, Pleurotus eryngii, Pleurotus ostreatus, Pleurotus ostreatus* var. *columbines, Grifola frondosa, Ganoderma lucidum, Ganoderma oregonense, Lentinula edodes, Agrocybe aegerita*, or *Coprinus Comatus* (U.S. 2011/0306107 A1 and U.S. 8283153 B2). Patent U.S. 2011/8001719 B2 provides the method of growing Basidiomycetes and Ascomycetes fungal fruiting bodies into a low density, chitinous material that can replace balsa, bass, other woods, and also many foamed plastics. Patent U.S. 5854056 discloses a process for the production of "phylum fungal pulp" but the method is optimized for *Neurospora crassa* as the filamentous fungus that can be used in the production of paper products and textiles. Patent U.S. 2015/0342138 provides a method for producing dehydrated mycelium which can be re-

https://doi.org/10.24264/icams-2018.VIII.8

hydrated and rapidly re-formed into many different shapes, such as bricks, blocks, pellets and the like elements wherein the adhesion of the elements is achieved through "re-animation" of a fungal organism which grows the elements together.

The main challenge in this novel class of biomaterials remains the high heterogeneity of the mixture components with different structures which makes difficult the uniform growth of the mycelium mass (Zeller *et al*., 2012).

MATERIALS AND METHODS

Biotechnological Process

Plant pathogen *Fusarium oxysporum* (obtained from Politehnica University of Bucharest strains collection) was used in the present experiment. Fresh culture was firstly grown on Potato-Dextrose-Agar media (Scharlau) (Fig. 1) and incubated at 28°C for 14 days. After the incubation period, small biomass samples were further passed in Potato Dextrose nutritive broth (100mL volume) and incubated for 10 days at 28°C.

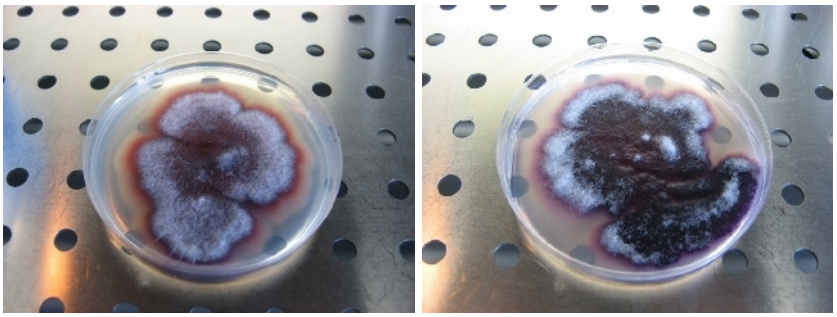

Figure 1. *Fusarium oxysporum* growth on solid media

Furthermore, flask obtained biomass was used for inoculation of an alternative substrate, composed of shredded recycled 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 3hours, 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 (Figure 2). The biocomposites was then incubated for 14 days, in the dark, at 28°C. Following incubation period, the composite was inactivated by sterilization at 130°C for 3 hours.

https://doi.org/10.24264/icams-2018.VIII.8

Figure 2. Mix of microbial strain, shredded paper and grown coffee

Optical Microscopy Analysis

Specific surface biofilm morphology assessment 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 analysis was carried out on at a magnification level of 0.67x from two different sites of the surface.

FT-IR Analysis

The FT-IR spectroscopy analysis was carried out on a Jasco FT/IR-4700 spectrometer, with max resolution of 0.5cm^{-1} and S/N ratio of 30,000:1, and Specac Golden Gate ATR accessory, with single reflection monolithic diamond ATR sampling accessory, featuring a Type IIIA diamond ATR element metal-bonded into a tungsten carbide mount. Spectrometer parameters were set to: measuring range: 4000-600cm^{-1}; readings/specter: Auto; resolution: 16cm^{-1}; type of measurement: Abs. For FT-IR analysis, the biocomposite material was dried at 105°C for 3 hours, for components moisture reduction. Composite was analyzed on both surfaces, by depositing a small quantity on the sensor, for ATR analysis.

RESULTS AND DISCUSSIONS

Fusarium oxysporum strain is an abundant and active soil saprophyte, with some specific pathogenic forms of the plant, the strain being part of the Eukaryotes (Smith *et al*., 1988). The saprophytic capacity allows the strain to survive in the soil between harvest cycles of infected plant remains. The fungus can survive either as a mycelium or as any of the three different types of spores it can produce.

The strain presented a fast growing rate on the alternative substrate used, allowing development of a microbial biofilm on the surface of the substrate (Figure 3a). Further analysis of the microbial biofilm was conducted by stereomicroscopy analysis, which highlighted the homogenous growth of the biofilm (Figure 3b-c)

https://doi.org/10.24264/icams-2018.VIII.8

a *b* *c*

Figure 3. Biofilm structure formed at the surface of the alternative substrate

Significant microbial growth could be best highlighted on the surface of the substrate, which may be due to too high humidity in the initial substrate, which did not allowed sufficient penetration by microbial hyphae, thus colonizing the most accessible portion as a nutrient substrate, respectively the surface of the alternative substrate. Final biocomposite material had a thickness of approximately 1.5cm, with increased rigidity and microbial biofilm developed at the interface substrate-air (Figure 4).

Biofilm detail (front) *Back detail (verso)*

Section detail

Figure 4. Final biocomposite version

FT-IR analysis was conducted on three component parts of the composite: biofilm surface (composite face), back of the biofilm that was in close contact with the recycled paper and worn coffee (composite verso) and the recycled shredded paper. Obtained spectra for all components were then overlapped for a better visualization of bands distribution in the 4000-600cm^{-1} range (Figure 5).

https://doi.org/10.24264/icams-2018.VIII.8

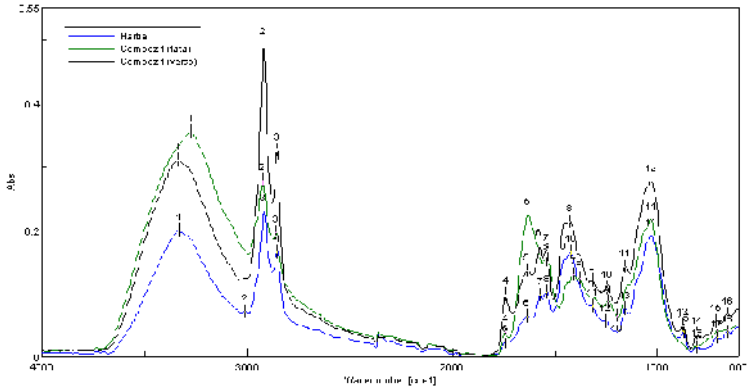

Figure 5. Biocomposite components FT-IR spectra overlapping

The FT-IR spectrum of the **composite front (biofilm)** shows modified positions and intensities of the peaks compared with feeding substrate and verso side of the composite. The broad band between 3600- 3000cm^{-1} with a maximum situated at 3274 cm^{-1} is attributed to the stretching vibrations of hydrogen bonded O-H coming from H_2O or bonded N-H (Gupta *et al*., 2015). The sharp and intensive peak at 2923cm^{-1} accompanied by the less intensive peak at 2857cm^{-1} are specific for C–H asymmetric and symmetric stretching of CH, –CH_2, and –CH_3 from the fatty acids produced in fungal cells (Diehl *et al*., 2003). All these bands and the small peak at 1743cm^{-1} attributed to the ester C=O stretching vibration are similar with those found by other authors for lipids (Muhammad *et al*., 2017) of the biofilm grown on cellulose substrates. Phospholipids could be characterized by the peak situated at 1238cm^{-1} (Wang *et al*., 1997), and the cell lipids by the band at 1407cm^{-1} (C=O symmetric stretching of COO groups) (Gupta *et al*., 2015).

Except few bands (3012, 1454, 1029cm^{-1}), **the verso side of the composite** absorbs at almost the same wavenumbers as the paper but the intensities of the peaks are highly increased indicating the substrate degradation. More than that, the band at 3332cm^{-1}, becomes broader demonstrating that the -OH groups of cellulose are largely affected by the fungal growth. Furthermore, the bands situated at 2919 and 2854cm^{-1}, assigned to CH_2 asymmetric and symmetric stretching vibrations are sharper and show a significant increment probably due to the mycelia fibers formed on the feeding substrate. The hypothesis is confirmed by the increase of the peaks intensities absorption in the 1538-600cm^{-1} domain and the absence of specific cellulose bands situated at 1454 and 1029cm^{-1}.

The FT-IR results of **paper used as substrate** for fungal growth show absorbance at the following wavenumbers: 3332cm^{-1} (OH stretching), 3012cm^{-1}, 2919cm^{-1} (C-H asymmetric stretching, CH_2 group), 2854cm^{-1} (C–H symmetrical stretching in methylene groups), 1739cm^{-1} (C=O stretching vibration), 1635cm^{-1} (OH bending of absorbed water), 1569cm^{-1} (C-H bending), 1538 cm^{-1} (C=C), 1454cm^{-1} (C=C), 1423 cm^{-1} (HCH and OCH in plane bending), 1319 cm^{-1} (CH2 rocking vibration at C6), 1253 cm^{-1} (G ring stretching), 1157 cm^{-1} (C-O-C asymmetrical stretching), 1029 cm^{-1} (C-C, C-OH, C-H ring and side group vibrations), 871 cm^{-1} (COC,CCO and CCH deformation and stretching), 806 cm^{-1} (glucosidic bond), 709 cm^{-1} and 659 cm^{-1} (C-OH out-of-plane bending) (Mizi *et al*., 2012).

https://doi.org/10.24264/icams-2018.VIII.8

The bands situated at 1631cm^{-1} (C=O stretching, amide I of protein β-turns) (Hu *et al*., 2006) and 1542cm^{-1} (N–H and C–N stretching, amide II from α-helix of proteins) are characteristic for proteins (amide I at 1700–1600cm^{-1}, amide II and III at 1575–1300 cm^{-1}). The presence of polysaccharides is indicated by the bands situated at 1380cm^{-1}, 1195cm^{-1} and 1029.8 cm^{-1}.

As the results demonstrate, **the composite front** is composed entirely from biomolecules characteristic for fungal mycelia (lipids, proteins, nucleic acids and polysaccharides).

CONCLUSIONS

Despite the massive work already done by the scientific community, biotechnologies based on fungi are not mature for full scale application in packaging systems. Present work has successfully obtained a novel biocomposite from *Fusarium oxysporum* plant pathogen fungal strain grown on alternative nutritive substrate composed of recycled shredded paper and worn coffee, as source of cell grow nutrients and carbon source. Fourier Transform Infrared analysis stands out as a highly versatile analysis tool for monitoring in situ components of various biomolecules, constituents of both microbial biomass and hydrolyzed substrate, and allowed identification of functional groups stretching and vibrations specific to microbial hyphae developed within the aerial structure of the substrate and biofilm formed at the interface air-substrate surface, such as polysaccharides, proteins, fatty acids etc.

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.

REFERENCES

Diehl, S.V. *et al*. (2003), “Use of fatty acid profiles to identify white-rot wood decay fungi”, in B. Goodell, D. D. Nicholas, and T. P. Schultz, Eds., *Wood Deterioration and Preservation—Advances in Our Changing World*, p. 313, American Chemical Society, Washington, DC, USA.

Gupta, B.S. *et al*. (2011), “Characterization of wood mould fungi by FTIR—a valuable step for prediction of initiation of decay”, *Proceedings of the 12th DBMC International Conference on Durability of Building Materials and Components*, Porto, Portugal, April 2011.

Gupta, B.S. *et al*. (2015), “Application of ATR-FTIR Spectroscopy to Compare the Cell Materials of Wood Decay Fungi with Wood Mould Fungi”, International Journal of Spectroscopy, Volume 2015, Article ID 521938, 7 pages, https://doi.org/10.1155/2015/521938.

Hu, X. *et al*. (2006), “Determining beta-sheet crystallinity in fibrous proteins by thermal analysis and infrared spectroscopy”, *Macromolecules*, 39, 6161–6170, https://doi.org/10.1021/ma0610109.

Mizi, F. *et al*. (2012), “Fourier Transform Infrared Spectroscopy for Natural Fibres”, in Dr Salih Salih (Ed.), *Fourier Transform - Materials Analysis*, IntechOpen, Open access peer-reviewed chapter.

Muhammad, H. *et al*. (2017), “Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties”, *Scientific Reports*, 7, 41292, https://doi.org/10.1038/srep41292.

Smith, I.M. *et al*. (1988), *European handbook of plant diseases*, Blackwell Scientific Publications: Oxford, 583pp.

Wang, H.P. *et al*. (1997), “Microscopic FTIR studies of lung cancer cells in pleural fluid”, *Science of the Total Environment*, 204(3), 283–287, https://doi.org/10.1016/S0048-9697(97)00180-0.

Zeller, P. and Zocher, D. (2012), “Ecovative’s Breakthrough Biomaterials”, *Fungi Magazine*, 5, 51–56.

https://doi.org/10.24264/icams-2018.VIII.8