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Evaluating mold growth in tannin-resin and flax fiber biocomposites

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ABSTRACT

Flax fiber is an industrially available, renewable product with attractive technical properties for the composite industry such as density, mechanical strength and sound/heat insulation. Applications are developing quickly for biocomposites based on these fibers. However, unlike plastics, there is no standard used to assess its degree of contamination by microorganisms. Thus, the aims of this work were to detect and quantify molds in samples of nonwoven fibrous materials from industry and high resin content natural matrix-natural fiber biocomposites. In a first attempt, we tried to evaluate the fungal development on the nonwoven materials as defined in the ISO 846 standard. Despite the materials having been inoculated with molds, no fungal mycelium could be detected by this way. To validate this observation, we developed a qualitative approach based on the specific aniline blue method to stain the mycelium present in the material. This allowed us to detect the mold occurrence in the material and to evaluate the colonization extent on the nonwoven fibrous materials incubated under different conditions. The biocide efficiency of a boric acid pretreatment of flax fibers used in the manufacturing process of nonwoven fibrous materials and high resin content natural matrix-natural fiber biocomposites was determined by grading the mold development using the microscope-based method that we developed. The results showed clearly that in optimal mold growth conditions, (Aw > 0.6, 26 °C) the colonization of the material was hampered by the boric acid pretreatment of the flax fibers. To get semi-quantitative data of the colonization extent, we used a molecular method. DNA was extracted from the different materials and the ribosomal region of the fungal DNA was amplified by PCR allowing the detection and the quantification of fungal colonization in the biosourced material. Results confirmed the microscopy-based method. Work is currently in progress to determine risk classes of mold colonization in wood-derived and fibrous-based composite materials.

1. Introduction

Currently, bio-based materials from natural fibers have been the focus of academic and industrial research interest from the viewpoint of reducing impact on the natural environment (Faruk et al., 2012). The use of flax and hemp is seen frequently on technical markets such as the automotive industry and the building market.

Flax is mainly cultivated in France (75% of European land) but is often transformed to yarn in Asia. Hemp is a more rustic plant that is cultivated in France in 5 main production areas (8000 ac are cultivated in France (2008), approximately 17600 t fiber/year). Currently, these fibers are used essentially for isolation in building and paper industry; the technical industry is starting to develop (CELC 2014).

In recent years, many studies have been carried out with the goal of developing relatively low-cost composite materials with superior or comparable performance to traditionally used materials.

Vegetable tannins are a wide family of polyphenolic compounds produced by the majority of plants. They are divided in two major classes: hydrolysable tannins and condensed tannins. Tannin-based resins were initially developed as wood adhesives (Pizzi, 2003, 2006; Lei et al., 2008), using the polymerization properties of condensed tannins, which have a reactivity close to resorcinol at the appropriate pH (Stephanou and Pizzi, 1994). Tannin can be used alone-paraformaldehyde is then the common hardener or in combination with phenolic or aminoplastic resins (Dunky, 2003). They are only a niche market in Western Europe but remain still in use in the south hemisphere (Dunky, 2003). The three main species of tannin industrially used are mimosa tannins, quebra-

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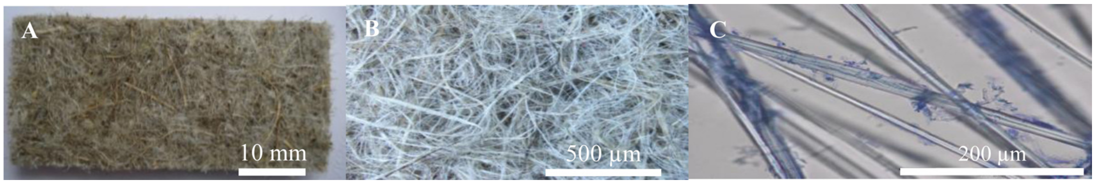

Fig. 1. Evaluation of the presence of mold in a sample of nonwoven material. A sample was inoculated with 2.5×10^5 conidia of *A. niger* and placed on a sterile wet filter paper in a petri dish. Sample was incubated for 10 days in the dark at 26 °C and 70% RH. Thus, the presence of mold was investigated either by macroscopic observation (A), stereomicroscope screening as recommended in the ISO 846 standard and (B) by observation with the optical microscope after aniline blue staining (C). Scale bars represent 10 mm in (A), 500 µm in (B) and 200 µm in (C).

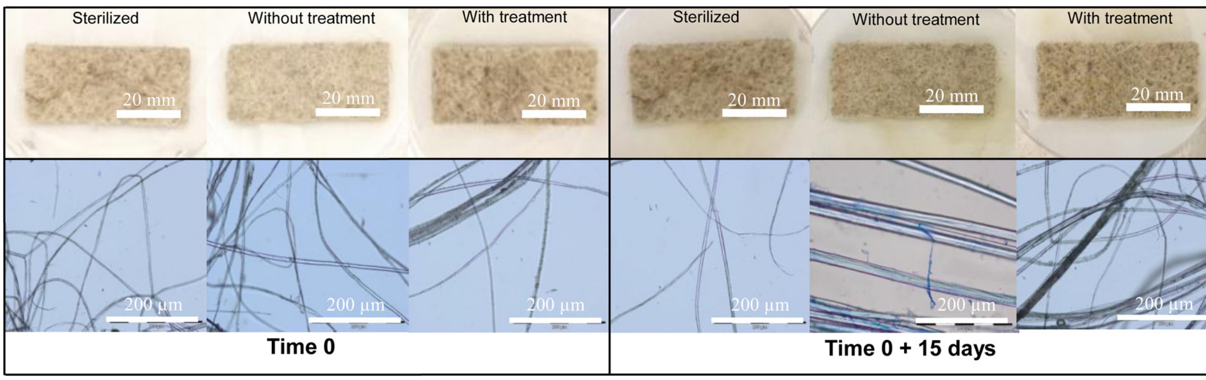

Fig. 2. Visual evaluation of the mold growth on nonwoven material. Non inoculated samples of nonwoven materials were or not pre-treated with boric acid incubated for 15 days at 26 °C and 70% RH. Autoclaved samples were used as negative controls. Mold occurrence was monitored at the beginning of the experiment (Time 0) and after 15 days of incubation (Time 0 + 15 days) by stereomicroscope screening as recommended in the ISO 846 standard (upper panels) or by optical microscopy after aniline blue staining (bottom panels).

cho tannin and pine tannin. Tannin-based adhesives can also be used with a reinforcement of long fibers like flax, hemp, or kenaf to create composite materials with good environmental properties such as being formaldehyde-free materials (Pichelin et al., 2006; Kamoun and Pizzi, 2000a,b). Research on this kind of composites is very recent but under constant growth due to the future scarcity of fossil resources and to environmental concerns. The initial development of tannin-based composites started in 2009 with student research projects in the University of Lorraine (Carpentier and Krebs, 2009; Masseteau and Loiseau, 2009). Some materials of good performances have then been prepared in this way and described in following studies (Pizzi et al., 2009; Kueny et al., 2012; Nicollin et al., 2012). The manufacturing process of tannin-hexamine matrix and flax fibers biocomposites is now well known and yield stable products with good mechanical performances (Sauget et al., 2013; Zhu et al., 2013).

It was demonstrated that some mold species are found in association with materials in buildings (Andersen et al., 2011). Toxic compounds such as organic volatiles or mycotoxins are released by mold during their development (Bush et al., 2006). Therefore, to ensure the proper design and use of a new bio-based material, a main purpose is the detection and the quantification of the fungal biomass that occurs in the material. Molds are ubiquists and when the appropriated water availability (Aw) and temperature conditions are encountered, germination of conidia occurs. The optimization of the test condition and in particular the humidity of the material is crucial since it influences the fungal growth (Johansson et al., 2012). However, beside the occurrence of the ISO 846 standard that provides guidelines for the visual quantification of mold growth on plastic substrates, no standard are currently defined for fibrous materials. Ergosterol is a lipid found in the plasma membrane of the true fungi (Weete, 2012) that is commonly used to quantify fungal biomass in different environments (Mille-Lindblom et al., 2004). However, molecular methods based on DNA amplification by PCR were found to be more efficient in fungal biomass quantification either in soil (Filion et al., 2003) or in wood material (Eikenes et al., 2005; Pilgård et al., 2011).

To evaluate the mold growth in tannin-resin and flax fiber composites, we have taken advantage of the fungal DNA quantification sensitivity and we developed a semi-quantitative PCR method. This technique allows the detection at early developmental stage of molds or other cellulolytic fungi at the surface but also in the volume of fiber-based material. The main challenge was to set up a reliable and sensitive method allowing to ensure the health status of flax- based biocomposites over time. Effect of a boric acid pre-treatment of the material on fungal contamination is discussed.

2. Methods

2.1. Nonwoven

Flax which in generally classified in bast fibers are delivered by AGYLIN Company. After harvesting by cutting or pulling and before the decortification in order to separate the fibers, the bast fibers are retted by a traditional method during 2–3 weeks on the field. The micro-organism attacked the gum and pectinous substances. These flax fibers were characterized by the CETELOR. The chemical composition is as follows: 78.6% holocellulose, 14.8% lignin, 2.7% off wax, and 2.7% of others components. A needle punched nonwoven reinforcement was prepared on the pilot line of CETELOR. The process is similar to geo-textiles; it's widely used in civil applications at lower cost than woven reinforcement. The first step is to open the fibers from the bale with a one-cylinder ditches equipped opener. After, the bast fibers are carded in order to form a web of approximately 16 g/m^2. A cross-lapper is used to laminate the layers of webs in order to obtain a final weight of 600 g/m^2. Finally a needle punched machine with one plate of needles is used to give cohesion and tensile properties to material with a density of 80 punch of needle/cm^2. A density of 600 g/cm^3 and average thickness of 3 mm are obtained.