First International Conference on
Bio-based Building Materials

June 22nd - 24th 2015
Clermont-Ferrand, France

STUDY OF THE MICROBIAL DEVELOPMENT IMPACT ON BIO-BASED MATERIALS

M. Gueguen*, S. Moscardelli, L. Van Schoors, I. Nour, S. Marceau
Université Paris-Est, IFSTTAR, MAST/CPDM, 77 447 Champs sur Marne, France
*Corresponding author; e-mail: marielle.gueguen@ifsttar.fr

Abstract

Materials from agroresources (granulates and fibres) are more and more developed in order to use renewable resources and to limit carbon footprint. These materials are used in many applications like building constructions. They are exposed to very high variations of temperature and humidity… In some cases, these conditions may be favorable for the development of microorganisms. Vegetal granulates or fibres are composed by a set of polysaccharides whose nature and function are different. Some microorganisms may use this sugar for growth. Microorganisms and more precisely mold growth may alter materials by consumption of constituent and induce health damage due to the liberation of spores in the atmosphere. In our study, we focus on the impact of microorganisms on agroresource materials. First, we only concentrate on mold growth on flax fibres. We have tested the evolution of chemical composition of lignocellulosic materials.

Keywords:
lignocellulosic materials, flax, micro-organisms, molds

1 INTRODUCTION

Many molds and bacteria are able to develop outside and inside buildings. They are able to colonize a large number of supports: painting, wallpaper, wood, cellulosic material and concrete [Nielsen 2002]… Fungal growth in building is mainly regulated by relative humidity of the substrate and of the air. The materials are gradually colonized by the successive appearance of different genera according to the water activity (Aw) in the materials, which corresponds to the available water. So *Penicillium* and *Aspergillus* colonize first in the substrate (Aw < 0.8) then we observe the development of *Closdrium* (0.8 < Aw < 0.9). Finally, when the substrate is very wet, *Ucladium* and *Stachybotrys* appear (Aw>0.9) [Pasanen 1992]. These various species play an important role in the air quality of the housing environment and in the human health: inflammations, allergies and dermatomycosis [Górny 2001].

These various genera are also known for their capacity to hydrolyze the lignocellulosic substrate. Currently, the use of agroresources is more and more developed in building sector. The resources arise from local plants: hemp, flax, wood, palm tree, sisal… Four main components constitute all the different plants: cellulose, hemicelluloses, lignin and pectin. Cellulose consists in semi-crystalline glucose chains bound in β1-4. Hemicelluloses are more complex molecule made of hexoses, pentoses and glucoronic acids. They link the cellulose molecules and the lignin, and can be classified in four groups: xyloglucanes, xylanes, mannames and β-1,3 ; 1-4-glucanes [Wertz, 2011]. Lignin is an insoluble and very heterogeneous polymer. It is an anti-pathogen thanks to its phenolic composition and can protect from herbivores. Pectins are glucoronic acid bound in β1-4. Fat and waxes are also present.

Although these four constituents are present in angiosperms, gymnosperms and herbs, the chemical composition greatly varies according to the species, genetic and environmental factors [Malherbe 2002]. Furthermore, variations are observed in accordance with cell localizations between aggregates and fibers [Thomsen 2005].

Many organisms are capable of degrading plants: insects (termites, beetles), mushrooms, molds and bacteria. These three last ones possess lingo-cellulosic enzymes able to degrade lignocellulosic materials. Cellulose is the most degradable molecule; it requires the use of three enzymes [Mussatto 2008] (fig 1):

(i) endoglucanases (EC 3.2.1.4.) which cleave randomly the β1-4 bounds;

(ii) exoglucanases (EC 3.2.1.91.) which remove monomers or dimers at the end of the chain ;

(iii) β-glucosidases (EC 3.2.1.21.) which hydrolyse dimmers.

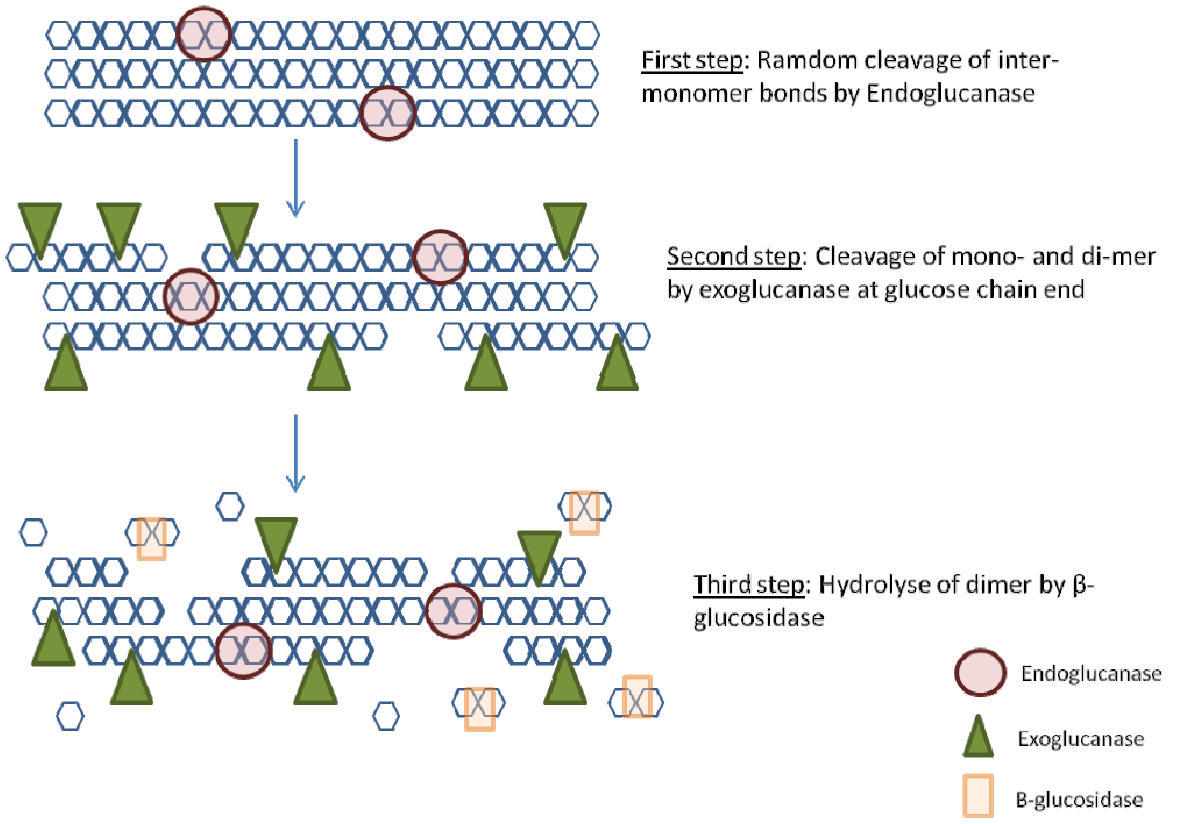

Fig. 1: Cellulose hydrolysis

The action of these three enzymes is essential for the complete hydrolysis of the cellulose. The rate-limiting step is the endoglucanase action which has to reach the amorphous regions between the crystalline zones and so creates new workable extremities for exoglicanases [Malherbe 2002]

The cellulose hydrolysis or saccharification is not a simple phenomenon. To be active, endoglucanases have to come into direct contact with the cellulose by absorption [Mussatto 2008]. However the cellulose is surrounded by the lignin and hemicellulose making the access of cellulose more difficult. The association of cellulose, hemicellulose and lignin prevents hydrolytic enzymes from accessing to their substrate [Malherbe 2002]. As a result, the presence of lignin and hemicellulose will reduce absorption of enzymes on cellulose, particularly as lignin inhibits cellulases. Furthermore, cellulose has amorphous and crystalline phases. Amorphous phases are more easily hydrolysed than crystalline phases. These latter phases require, in addition to the three enzymes required for the hydrolysis of cellulose, the synergistic action of a cellobiohydrolase enzym [Mansfield 1999]. Furthermore, a low degree of polymerization will be more readily degradable [Mansfield 1999; Chandra 2007]. Briefly, the cellulose hydrolysis depends on its physicochemical characteristics (degree of crystallinity and of polymerization...) and the lignin and hemicellulose distribution. During chemical and/ or biological treatment for separating the cellulose of lignin and hemicellulose, cellulose characteristics are modified, usually degrees of polymerization and crystallinity are reduced allowing for more rapid and more complete hydrolysis.

Degradation of hemicelluloses requires many other enzymes like endo-β-1,4-xylanase and β-xylosidase due to the complexity of the molecules [Malherbe Cloete 2002]. Lignin is even more difficult to degrade because of the non-hydrolysable bonds between the monomer units [Pellinen 1987].

Due to their chemical composition and to their very porous microstructure, the materials from agro-resources are characterized by a highly hygroscopic nature [Amziane 2013]. These materials will be attractive on many levels for mold They will serve as a nutrient and will have a high water content, thus promoting the growth of mold.

The global aim of our study is to investigate the impact of microorganisms and specifically mold on the durability of materials based on agroresources. Our first work is focused on the biodegradability of agricultural resources. So we are interested in the ability to degrade short flax fibres by mold.

2 MATERIAL AND METHODS

2.1 Fibres, micro-organisms and ageing

The length of flax fibres is about 2 mm.

Molds were isolated from hemp concrete aged 6 months at 30°C and 100% RH. Molds were cultivated on PDA (potatoes dextrose agar) medium. Three types of molds were selected. The three strains are cultivated in potatoes dextrose broth at 30°C during 72 hours. 4 mL of a strain mix was inoculated on 10g of flax fibres. The fibres were then incubated during 14 months at 30°C and 100 % RH.

2.2 Chemical analysis of flax

10g of native and ageing flax fibres was analyzed. Experiment was performed once. The chemical composition was obtained after a series of reactions and filtrations (fig. 2). Flax fibres composition was determined before and after mold ageing.

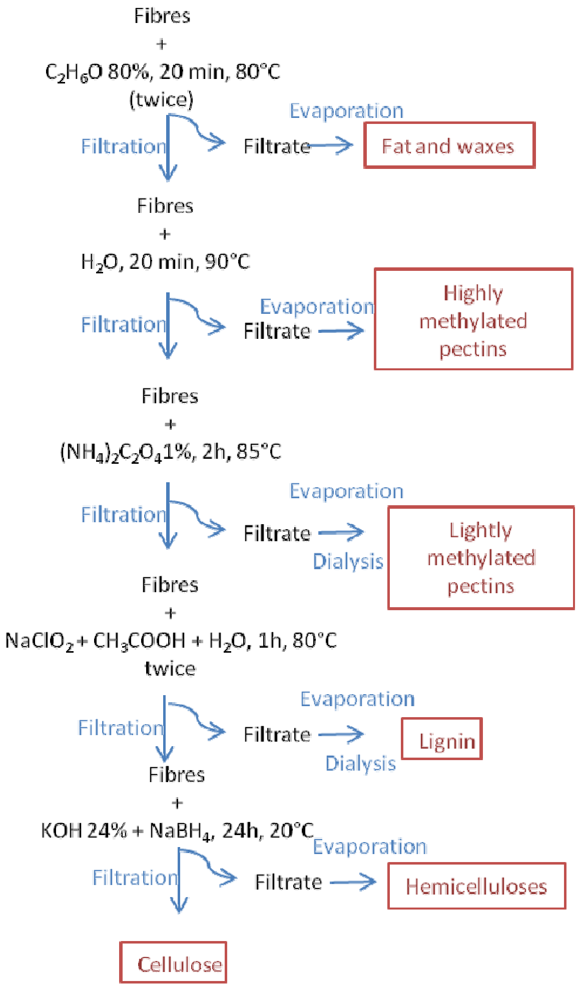

Fig. 2: Extraction method of the different natural fibre constituents [Sedan 2007; Garcia-Jaldon 1992].

3 RESULTS AND DISCUSSION

3.1 Ageing of flax fibres

The chemical composition of the flax fibres is reported on table 1 before and after ageing. Typical results observed in the literature are also included as references. We can notice that the chemical composition of the studied flax is very similar to what is described in literature. The quantity of cellulose is very important which is in accordance with the fact that fibres are richer in cellulose than granulates [Thomsen 2005]. Moreover, we noticed a lignin concentration more important in our fibres.

Table 1: Chemical composition of flax

Constituents	Literature	Native	Ageing
Cellulose	64-85 %	85.7%	76.5%
Hemicelluloses	4-18.5 %	4.3%	4.4%
Lignin	2-3.1 %	4.7%	3.2%*
Pectin	1.8-3.1 %	3.3%	3.7%
Waxes and fat	1.5-7 %	2.5%	6.9%
References	[Baley 2006; Magniont 2010]	Present study	Present study

* Some hemicelluloses were lost during the evaporation phase

If we focus on aged fibres, we can notice a serious decrease of fibre mass. Initially, the fibres are around 13 g and after 14 months, they represent only 9 g. This loss is surely due to consumption of lignocellulosic materials due to mold growth. Microorganisms don't consume all constituents in the same ratio. We have tried to make an estimate of the consumption of each constituent. For this we have considered that the initial 13 g of fibres have the same composition as the native fibres. Then we have compared the calculated native quantity of each constituent with the quantity obtained after 14 months. We can notice differences in the consumption of the constituents (Table 2). Concerning the three main components, lignin seems to be the more degradable molecule. This result needs to be confirmed because of the very little quantity of this product. Cellulose is clearly consumed by microorganisms with around 40 % degradation in 14 months. Some hemicelluloses were lost during the evaporation process, inducing a consumption rate lower than 29 %.

Cellulose seems to be more easily degraded by microorganisms, more than hemicelluloses, which is in line with literature [Malherbe Cloete 2002]. The lignin was present in little quantity, which may explain the good degradability of the cellulose.

It is very difficult to compare this result with the existing literature. Most of articles are focused on microorganism activities on the rumen or in biofermentor. These results are very different because one's usually pre-treats lignocellulosic materials.

In the table 1, we can notice an increase of the quantity of fat and waxes. This is probably due to the micro-organism presence. These results need to be confirmed by IR-ATR. Fat have indeed two characteristic peaks: 2850cm^{-1} (CH_2CH_3) and 1730cm^{-1} (C=O). Microorganisms may be identified by a peak at 1640 cm^{-1} corresponding to amid I [Mariey 2001].

Table 2: Estimation of consumption rate (in percentage) of each component during 14 months.

Constituents	% of consumption
Cellulose	38
Hemicelluloses	< 29*
Lignin	53
Pectin	23
Waxes and fat	Mix of waxes, fat and micro-organisms.

* Some hemicelluloses were lost during the evaporation phase

3.2 Biological ageing of flax fibres-reinforced composites

Previous results have shown that microorganisms are able to degrade flax fibres but are they able to consume them when fibres are embedded in a polyethylene matrix? To answer this question, we incubate the same microorganisms with composite PE/flax (50% w:w) during 3 months at 30°C and 100% RH.

Microorganisms are able to grow on this material without addition of nutrient (Fig.3). On this epifluorescent microscope view, the flax fibres have natural fluorescence (in blue on the picture). The microorganisms are visible in yellow, after 3 months. They have probably consumed some flax constituents for their growth.

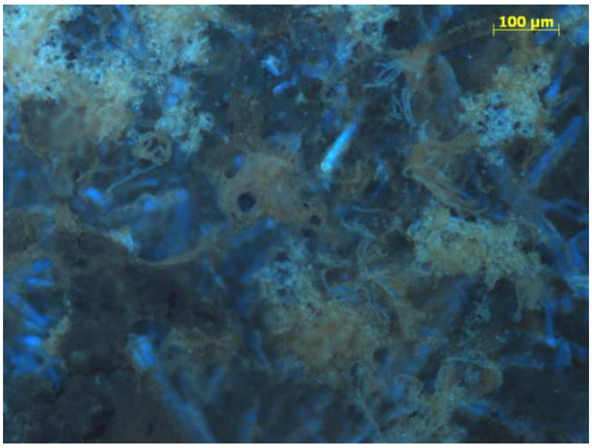

Fig. 3 : Photo obtained by epifluorescent microscope on PE/flax incubated during 3 months with molds

4 CONCLUSION

This preliminary study has shown that it is possible to underline the capacity of molds to consume cellulose in flax fibres. Moreover, molds are able to grow on PE/flax composites. Now, the next step of our study is to estimate the impact of microorganisms growth on the properties of materials (functional, structural and microstructural properties).

It is also necessary to study other natural fibres and granulates alone or used in different types of matrix to estimate the impact of biological ageing on material properties.

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