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IMPACTS OF ALKALINITY AND ENVIRONMENTAL AGEING ON FUNGAL GROWTH IN HEMP CONCRETES

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Abstract

Hemp concrete is a building insulation material based on vegetal aggregates and a mineral binder. Due to the lignocellulosic constitution of this material and to the amount of fungi naturally present in hemp, concerns exist about risks of fungal growth, which induce deterioration of the material but also indoor air pollution.

In this paper, we focus on the fungal origin on hemp concrete and the fungal growth during ageing. Firstly, the comparison between the fungi population in aggregates and in fresh hemp concrete is done to investigate the capacity of the pH to kill spores present in the material. Secondly, fungal population in concrete is followed for 18 months under a static (50%RH–20°C) and dynamic (variation between 98 and 40% RH – 30°C) ageing protocols.

For fresh hemp concrete, the fungal population remains important, despite the alkalinity of the binder.

After ageing, no fungal growth is observed, which is explained by the low RH% of the static ageing and of the drying step coupled with the mineralization of vegetal aggregates in the dynamic ageing.

Based on these results, no fungal growth should appear in real buildings if the material is well manufactured and without exterior damage.

1. INTRODUCTION

Air pollution is an issue studied for decades. The indoor air quality is also more and more monitored and analyzed. Indeed, the way of life, air ventilation, materials used to build or to equip a building, or even cleaning products are the source of indoor pollution, partly due to VOC or to proliferation of microorganisms [1]. To compare and assess the resistance of material to the development of microorganisms, various protocols are used, like visual assessment of mould growth or quantification of viable cells [2,3]. However the results

obtained with these protocols are not comparable and do not represent the conditions of use. The proliferation of microorganisms depends of a wide variety of parameters: the chemical composition of the carrier material, the presence of nutrients, the relative humidity in the environment, the water absorption capacity of the material, the amount of spores initially present in the material, the pH, the rugosity or the surface conditions [4].

Biobased materials are increasingly used in buildings, as wood for structures, vegetal wools or biobased concretes for insulation. The higher presence of vegetal biodegradable materials raises questions about the indoor air quality and their durability. Indeed, cellulosic materials, which are a source of nutrients for microorganisms, can have a strong water absorption capacity and also contain a significant amount of spores [5]. These viable cells can give rise to a colony in appropriate conditions, and consequently lead to fungal growth, associated with spores release in air and material degradation.

Hemp concretes are an example of biobased materials used in buildings. These efficient insulation materials which ensure comfort in habitat are based on vegetal aggregates called hemp shiv, corresponding to the crushed woody part of the stem, covered and bonded by a coating of mineral binder. Therefore, hemp concretes contain vegetal aggregates, rather sensible to fungal growth as mentioned before, and a mineral part, less vulnerable to microorganisms thanks to its high pH value [6]. Fungal growth has been already observed in hemp concrete walls, often after incorrect implementation such as too important water content in formulation, insufficient drying conditions and time or wrong choices of render [7,8]. In laboratory, fungal growth has been noticed under environmental conditions considered as extreme: relative humidity higher than 90%, after inoculation of fungi and storage at high humidity and after climatic simulations in presence of vapour and liquid water [9-10]. In other cases, no fungal growth was observed despite wetting and drying cycles with a maximum at 95% RH or successive inoculations of fungi and storage at 80% RH for months [11, 12]. Besides water activity, the pH is also a significant parameter to consider. Marceau *et al.* showed that no fungal growth is observed after inoculation of fungi on fresh hemp concrete while a fungal development appears after inoculation on aged specimens [11]. Carbonation of binders, particularly of portlandite, induces a decrease of pH from values higher than 12 to pH values lower than 10. Conditions become then more favorable to a fungal growth. The same hypothesis is proposed by Walker *et al.* [12] to explain the absence of fungal growth despite successive inoculations of fungi.

In buildings, the stress of the environmental conditions is less important than that applied in laboratory experiments quoted above. However, it is important to study the risk of fungal growth on well-manufactured hemp concretes under environmental conditions closer to the real conditions, in order to effectively address the durability issue of hemp concretes. It is the aim of the present study, divided in three parts. Firstly, the quantification and differentiation of viable fungal cells from hemp shiv are performed. Secondly, for fresh hemp concretes, the impact of the alkalinity of the binder on the total amount of fungal cells is investigated. Finally, the fungal growth and the pH are monitored and followed in core and in surface of hemp concretes for 18 months under different ageing conditions.

2. MATERIALS AND METHODS

2.1 Materials

One type of commercial hemp shiv (HS) for insulation and one binder commonly used for hemp concretes are selected. The binder is a commercially formulated binder based on natural lime (70%) and hydraulic and pozzolanic binders (30%), referred as FL in the text.

2.2 Hemp concretes

Manufacturing and curing of hemp concretes

Hemp concretes for ‘wall’ application were manufactured as detailed in Delannoy *et al.* [13]. Hemp concrete is called HC. After manufacturing, 90 days of curing at 65% RH and 20°C ensure the hardening of the hemp concretes by both hydration and carbonation reactions of binders. The manufacturing and curing protocols are detailed in Delannoy *et al.* [13]. Specimens studied after curing time and before ageing are defined as the initial time A_0 (ageing 0 month).

Protocols of ageing

After the manufacturing and the curing time, specimens of hemp concretes have been divided in two groups to be aged under different conditions for 18 months: a static ageing and a dynamic ageing.

The first set is stored in a conditioned room at 50% RH and 20°C. This set serves as reference and this ageing condition is called “Ref”.

As the material is sensitive to humidity, an accelerated ageing protocol in climatic chamber has been chosen to study the durability of hemp concretes. The temperature was set up at 30°C, a good temperature for fungal growth. Humidification and drying cycles of 1 week are applied. During the 5 first days, a high relative humidity is applied (98% RH) follow-up by 2 days at 40% RH as a drying step. Transitions between the two RH occur in one hour. These humidification and drying cycles ageing protocol is referred as HD.

At different stages of ageing, specimens are characterized.

2.3 Quantification of viable fungal cells

The fungal cells present in 10 g of shiv and in 10 g of fresh hemp concrete (2 hours and 48 hours after the end of the manufacturing) are extracted with a sodium dodecyl sulfate (SDS) water solution (1% weight/volume) for 1 min with the stomacher 400 circulator (320 rpm). Successive 1/10 dilutions are then applied with the SDS solution and 100 μ L of the liquid is pipetted onto PDA (Potatoes Dextrose Agar) growth medium in Petri dishes. Dishes are incubated for 2 days at 30°C, after which the colonies are counted, and the numbers of viable fungal cells are presented as colony forming unit per gram of hemp (cfu/g_{hemp}). Two replicated are done. All the procedure takes place in a biological safety cabinet with sterile instruments.

2.4 Analysis of fungal growth

To monitor a potential fungal growth on hemp concrete depending on time and on the ageing protocol, one 25 cm² square of adhesive tape is applied for 1 min on the surface or inside the specimens after having been split in two parts. In order to compare with hemp shiv, adhesive tape is also applied on hemp shiv in bulk. Then the adhesive tape is placed and vortexed in 1 mL of the SDS water solution. The same dilution and inoculation protocols

presented in section 2.3 are used, with in this case 5 days of incubation. The results are presented in cfu/cm² of material.

2.5 pH analysis

Phenolphthalein test is performed to follow the evolution of the pH. This indicator is used to determine if a mineral binder is carbonated or not. The colour indicator has its transition for a pH value around 10: below 10 the material is uncoloured and carbonated, higher than 10 a pink colour appears.

2.6 Microstructure observations and identification of fungi

The microstructure of hemp concrete and of fungi developed on Petri dishes are observed on FEI Quanta 400 Scanning Electron Microscope (SEM) with low vacuum and in secondary electron mode (SE).

3. RESULTS AND DISCUSSION

3.1 Fungi present in hemp shiv and at fresh state in hemp concrete

The total amount of viable cells determined with the experiment described in section 2.3 for hemp shiv is 37.8×10^6 cfu/g_{hemp} (Table 1). 6 kinds of colonies are visually differentiable on growth medium. An example of Petri dish is presented Figure 1-a with SEM observations. *Penicillium sp.* (Figure 1-1) or *Aspergillus sp.* (Figure 1-4) seems to be present.

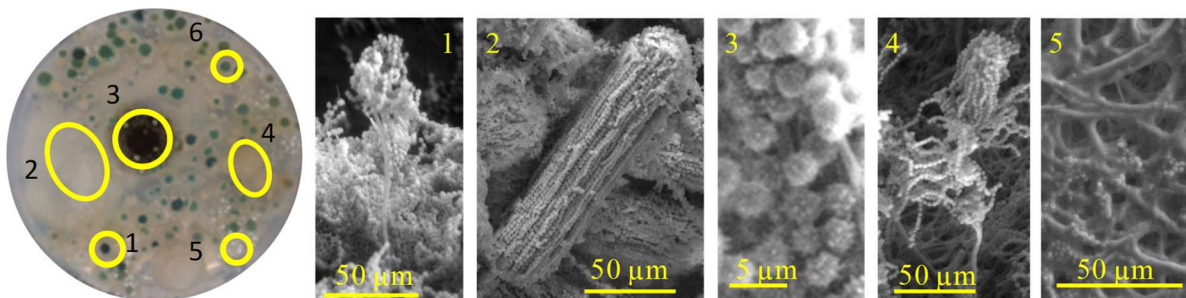


Figure 1. Picture of a hemp shiv Petri dish and SEM pictures of fungi

A high amount of viable cells is found in hemp shiv as described in the last paragraph. In alkali-conditions during the manufacturing of hemp concretes, these fungi can either: grow if they are alkali compatible, wait for more favourable conditions by producing spores or be killed [6]. The study of the impact of alkalinity of binders on the total amount of viable cells helps to determine the origin of fungi observed in buildings or in laboratory studies under high humidity: these fungi can be initially present in shiv and/or come from an external contamination if the initial fungi from the shiv are killed during the manufacturing.

Table 1. Viable fungal cells in hemp shiv and in sample of hemp concrete taken 2h and 48h after manufacturing

	Hemp shiv	HC 2h after manufacturing	HC 48h after manufacturing
cfu/g _{hemp}	$37.6 \times 10^6 \pm 2.3 \times 10^6$	$4.3 \times 10^6 \pm 1.4 \times 10^6$	$1.5 \times 10^6 \pm 0.7 \times 10^6$

The total amounts of viable cells for hemp concrete are presented Table 1 for samples taken 2 hours and 48 hours after the end of the manufacturing. A decrease of the $\text{cfu/g}_{\text{hemp}}$ about 1 log is observed for HC compared to hemp shiv. Even if the values for hemp concrete are getting closer to the limit between potential risk and important risk, fixed at 1×10^6 cfu/g [8], the amount of viable fungal cells remains important. The conclusion is that the pH of the binder does not mainly kill fungi during the fresh state of the material. Then if a fungal growth appears later on hemp concrete with the appropriate environmental conditions, it could be coming from fungi initially present in hemp shiv as from external contamination.

3.2 Monitoring of the fungi development in and on hemp concretes during ageing

As we saw that the alkalinity of binder does not kill fungi cells present in hemp shiv, fungal growth is possible if appropriate conditions are met, such as a good range of temperature, a sufficient water activity or a lower pH on the surface of the material. The amounts of viable fungal cells per cm^2 in core and in surface of specimens of hemp concrete with log scale are presented Figure 2 as function of time and of the type of ageing. First, whatever the type of ageing, at least a 3 log decrease of the amount of fungal cells is observed compared to the amount of cells for hemp shiv in bulk. In the food industry [14], the amount of viable cells is considered bad for the hemp shiv, and most of the time fair, good or even excellent for hemp concrete. No significant differences can be concluded for all measurements in hemp concrete whatever the time and the type of ageing. Moreover, the fungal cells in hemp concrete do not seem active. Indeed, 5 days of incubation are necessary to allow the counting of colonies on Petri dishes for hemp concretes against 2 days for hemp shiv. Spores are sleeping and need more time to develop on the growth medium. Finally, only 2 different kinds of colonies are visually differentiable compared to the 6 for hemp shiv (Figure 1). The reasons of these results are different according to the ageing protocol applied.

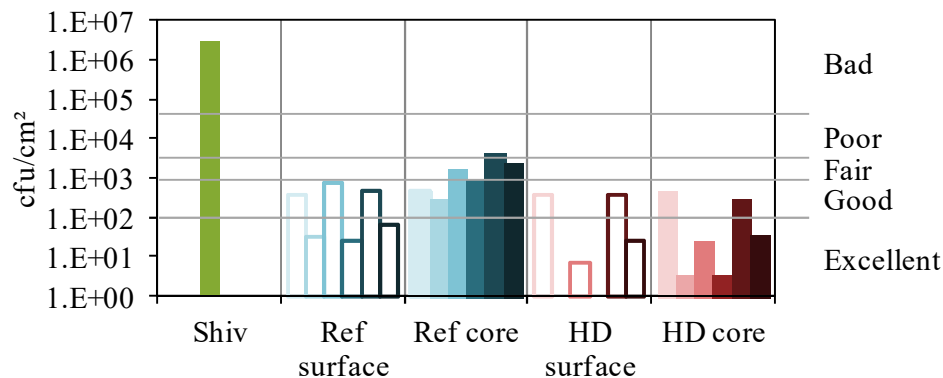


Figure 2. Amount of viable cells on surface and in core of HC with time (at A₀ and at 1, 3, 6, 12 and 18 months of ageing) under different ageing conditions (Reference in blue and HD cycles in red) compared to viable cells counted on hemp shiv in bulk (green)

Concerning the static ageing, the explanation to the lowest fungal content compared to hemp shiv is first that the relative humidity applied (50% RH) is lower to the 70% RH necessary to a fungal growth [4], despite a pH lower than 10 in surface observed with the 1

cm uncolored layer on cross-sections of hemp concrete for A₀ and after 18 months in Ref ageing (Figure 3-a).

More favourable conditions for fungal growth are brought by the HD ageing, where a high value of relative humidity is fixed during 70% of the time (mean RH per week = 81%). In a first step, this higher relative humidity applied than in Ref ageing impacts the carbonation of FL binder. Indeed, Figure 3.a for HC tested with phenolphthalein after 18 months in HD shows a carbonated depth higher than 2 cm. Even if more volume fraction of hemp concrete has a pH lower than 10, no fungal growth are observed, despite the high value of relative humidity applied. Then, it is the use of alternating periods of high and low relative humidity which could limit the fungal growth [15, 16]. In fact, the two days at 40% RH seem to be enough to neutralize the development of hyphae which might start during the 5 days at 98% RH. Moreover when the RH is higher than 95%, a part of the mineral binder is dissolved in the condensate liquid water in hemp concrete, which can migrate into the shiv porosity, and precipitate again during the drying step of HD [13]. This mineralization inside the shiv, observed by SEM on cross-sections of aggregate of HC sample in Figure 3-b (binder in white, cell wall in light grey and pores in dark grey), hinders the accessibility of the lignocellulosic part of hemp, and interferes with the potential fungal growth.

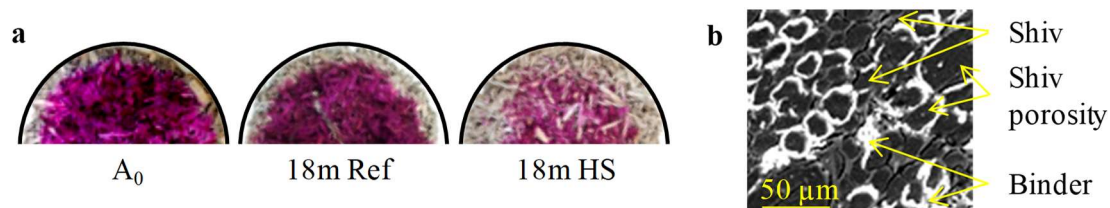


Figure 3. (a) Pictures of HC tested with phenolphthalein at A₀, 18 months after Ref and HD ageing - (b) SEM picture of hemp concrete cross-section

4. CONCLUSIONS

Hemp concrete is an insulation material mainly made of vegetal aggregates, and consequently considered to be sensitive to fungal growth. In addition to being a source of nutrient thanks to the presence of lignocellulosic molecules, a high amount of viable fungal cells is measured on hemp shiv from the living period of the plant and the retting process.

One formulation of hemp concrete containing a binder based on lime is manufactured for this study. During the manufacturing, the alkalinity of binders does not significantly impact the number of viable fungal cells in the concretes.

After manufacturing, curing time and ageing in static or dynamic conditions, no fungal growth is observed on hemp concrete. Fungal cells remain inactive, despite more favourable conditions brought by the carbonation reaction, leading to a decrease of the pH lower than 10 in the surface. That is explained by a low relative humidity for the static ageing and by the drying step of the dynamic ageing coupled with a mineralization of the porosity of shiv.

In buildings with both outdoor and indoor environments, hemp concrete is submitted to important variations of relative humidity and temperature. Projecting the results obtained in this study to the case of hemp concrete implemented with a render in buildings, with values of relative humidity measured in real wall of hemp concrete (< 70% RH), no fungal growth should be observed without wrong manufacturing or accidental damages.

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