Development of bio-based earth products for healthy and sustainable buildings: characterization of microbiological, mechanical and hygrothermal properties

Alexis Simons, Aurélie Laborel-Préneron, Alexandra Bertron, Jean-Emmanuel Aubert, Camille Magniont, Clément Roux, Christine Roques

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Développement de produits de construction biosourcés à base de terre crue pour des bâtiments sains et durables : caractérisation des propriétés microbiologiques, mécaniques et hygrothermiques

A. Simons[1], A. Laborel-Préneron[2], A. Bertron[2], J.E. Aubert[2], C. Magniont[2], C. Roux[3], C. Roques[1]

[1] Université de Toulouse ; UMR 5503 CNRS – UPS - INPT ; LGC (Laboratoire de Génie Chimique), 35 chemin des maraîchers, 31062 Toulouse cedex 9, France

[2] Université de Toulouse; UPS, INSA; LMDC (Laboratoire Matériaux et Durabilité des Constructions), 135 avenue de Rangueil, F-31 077 Toulouse cedex 4, France

[3] Université de Toulouse; UMR 5546 CNRS – UPS ; LRSV (Laboratoire de Recherche en Sciences Végétales), Pôle de Biotechnologie Végétales, 24 chemin de Borde Rouge, B.P. 42617 Auzeville, 31326 Castanet-Tolosan, France

Abstract

The impacts of buildings on the environment and on the health of the inhabitants are priority issues nowadays. For many environmental, social and economic reasons, the demand for building products made of materials such as earth and bio-based materials is increasing. Under certain conditions, mold growth can be observed on the surface of such materials, which raises many questions about their use in buildings. In the framework of the "BIOTERRA" ANR project, the aim of the study was to develop and characterize an earth based material incorporating plant fibers from both abiotic and biotic points of view. Compressive strength, thermal conductivity and water vapor permeability of this material were determined. Microorganism sampling methods intended for raw materials and cylindrical specimens were optimized, and the microflora profile of these materials was then obtained. The results showed that the straw addition led to a decrease of compressive strength and an increase of thermal insulation. However, it did not influence water vapor permeability coefficient. Raw materials and manufactured specimens contained mainly *Bacillus* sp., *Aspergillus* sp. and *Penicillium* sp. Other compositions of this bio-based material will be characterized. Sampling methods developing here can also be used to identify the microflora of existing earthen buildings.

Keywords: bio-based building material, Sick Building Syndrome, mechanical properties, hygrothermal properties, mold.

Résumé

Les impacts de la construction sur l'environnement et la santé des occupants sont devenus aujourd'hui des enjeux prioritaires. Pour de nombreuses raisons environnementales, sociales et économiques, les produits de construction à base de matériaux tels que la terre crue et des matériaux biosourcés connaissent un essor important. Sous certaines conditions, le développement de moisissures peut être observé, soulevant de nombreuses questions quant à leur utilisation. Incluse dans le projet ANR « BIOTERRA », cette étude a eu pour objectif de développer et caractériser, à la fois sur le plan abiotique que biotique, un matériau à base de terre crue avec ajout de fibres végétales. Sa résistance à la compression, sa conductivité thermique ainsi que sa perméabilité à la vapeur ont été déterminées. Des méthodes de prélèvements des microorganismes dans les matières premières ou les produits fabriqués ont été optimisées. Un profil de la microflore de ces matériaux a ainsi été obtenu. Les résultats abiotiques ont montré que l'ajout de paille faisait diminuer la résistance en compression du matériau composite mais permettait une diminution de la conductivité thermique. Cet ajout n'a toutefois pas influencé le facteur de résistance à la perméabilité à la vapeur d'eau. Les essais biotiques quant à eux ont révélé que les différents matériaux contenaient principalement des microorganismes appartenant aux genres *Bacillus* sp., *Aspergillus* sp. et *Penicillium* sp. D'autres compositions de ce matériau biosourcé seront caractérisées. Les méthodes de prélèvement développées ici pourront également être utilisées pour l'identification de microflores de bâtiments en terre crue existants.

Mots-clefs: matériaux de construction biosourcés ; Syndrome du Bâtiment Malsain ; propriétés mécaniques ; propriétés hygrothermiques ; moisissures.

1. Introduction

Recent years have seen renewed interest in low-environmental impact housing in industrialized countries, and the impacts of building materials on the health of their inhabitants and on the environment have become priority issues. Some old building materials, such as earth, are being examined from this point of view. Scientific research on earth construction has been expanding significantly for about thirty years. Nevertheless, there are very few publications focusing specifically on unfired earth, although this material is widely used around the world. Nowadays, more than two-thirds of the world's population still live in unfired earth houses [1]. This building technique was used in France for centuries and a large heritage of unfired earth building methods (mud-bricks, cob, etc.) exists in different regions. Earth building has several advantages, such as improving comfort in the house, providing good thermal inertia [2] and offering natural regulation of the humidity of indoor air [3]. Earth can also be transformed into bio-based materials with the addition of aggregates or fibers of plant matter (straw, flax, hemp, etc.), in order to enhance the thermal insulation and lighten the material. In addition, these materials are low cost and have very low environmental impact (local and renewable raw materials with low embodied energy that contribute to carbon storage).

However, microbial proliferation can sometimes be observed on these materials [4], as in other common building materials. Under certain conditions, such as high and uncontrolled humidity (minimal water activity between 60% and 90%) and a temperature between 10°C and 35°C [5], molds may grow and form visible mycelia on building walls [6]–[10]. When molds are visible to the naked eye, development of the mycelium is already very advanced, which can imply health risks. Molds and bacteria may then cause poor indoor air quality, which is one of the most important issues in building. The pollution of indoor air is linked to Sick Building Syndrome (SBS), and may cause health problems for inhabitants [11]. The main microorganisms involved in SBS are molds. Fungal development can cause production of allergens, mycotoxins or volatile organic compounds (VOC), and also fungal infections and diseases [12]–[17]. Genera involved in health problems are mainly *Aspergillus*, *Cladosporium*, *Penicillium*, *Stachybotrys*, *Ulocladium* and *Chaetomium* [10], [18], [19]. Bacterial involvement in these problems is less common or less well known and there are few studies discussing the problem. The main bacteria identified on wet area inside buildings are Gram positive bacteria [20], such as *Streptomyces*, and also mycobacteria [21]–[23]. Adverse effects observed are similar to those of fungi, and include mycobacteria parietal compound in the ambient air, or the production of toxins by *Streptomyces*, which may cause inflammatory reactions [24], [25]. Molds on building materials may be initially present in raw materials, or brought in during the fabrication process or by the outdoor air. Potential origins of microbial contaminations are many and varied, and a large diversity of microorganisms may be encountered.

The ANR collaborative project "BIOTERRA" aims to identify, characterize and provide solutions to microbial growth on earthen bio-based products (bricks and plasters) used in the construction and renovation of healthy, sustainable buildings. The final ambition is notably to identify how the properties of earthen bio-based products - especially the hygroscopic properties, condition the possible growth of microorganisms on these materials, in relation to the environmental conditions. This project will also aim to develop and validate methodologies for the sampling and identification of microbial strains and the study of their growth on building products. With this in mind, a preliminary study was carried out in order to develop and characterize an earth based material incorporating plant aggregates from both the abiotic and biotic point of view.

In the present paper, mechanical and physical characteristics of manufactured specimens were measured, such as compressive strength, thermal conductivity and water vapor permeability. Microorganism sampling methods intended for

raw materials and building products were set up and optimized. Finally, microbial isolates were characterized, and a first microbial profile of these materials was obtained.

2. Material and methods

2.1. Material

Quarry Fines from Washing Aggregate Sludge (FWAS) were used for this investigation. These fines were composed of calcite (63%), dolomite (3%), kaolinite (11%), illite (9%), quartz (10%) and goethite (3%). FWAS had a pH of 7.8, which is an optimal value for the development of many microorganisms. FWAS were extremely fine: 99% of the particles were below 80µm and the average particle size (D50) determined using the pipette analysis was equal to 6.5 µm. Before being used, they were stored in plastic bags at room temperature.

Barley straw, in pieces 10 to 30 mm long, was also tested in different proportions in the earth matrix. The straw was also stored in plastic bags at room temperature.

2.2. Manufacturing

Three different mixtures were prepared for the various tests: (i) specimens made with FWAS only and specimens containing (ii) 3% and (iii) 6% of straw by weight content, marked S3 and S6 respectively. No binder (cement or lime) was added to the mixtures. The water content of the mixtures, determined by the Proctor test, was around 14% for FWAS, 19% for S3 and 21% for S6. To manufacture the specimens, earth and straw fractions were poured into a blender and mixed by hand. Then, water was added and the materials were mixed mechanically in the blender until a homogeneous mix was obtained (3 min). The raw materials were mixed the day before molding.

Cylindrical specimens 5cm in diameter and 5cm high (Φ5H5) (Figure 1), intended for compressive strength tests, vapor permeability measurements and biotic tests, were manufactured by double static compression at the Proctor density. Specimens (150x150x50 mm^3, Figure 1) for thermal conductivity measurements were rectangular prisms, manufactured in the same way.

The specimens were first dried at 40°C for 24 hours, then the temperature was increased by 0.1°C/min to 100°C and kept at 100°C until the weight became constant (weight variation less than 0.1% between two weightings 24 hours apart). This rise in temperature, inspired from the industrial process, was kept slow to keep shrinking homogeneous and to avoid mechanical stresses. The specimens were then stored in a room regulated at 20°C and 50% relative humidity (RH) and were tested as soon as they were in equilibrium with the environment (about one week later). It is important to note that the specimens used for the two parts of this paper (mechanical and hygrothermal properties and microbiological study) were prepared using the same procedures. The dry densities of the FWAS, S3 and S6 specimens were 1.99 $g.cm^{-3}$, 1.52 $g.cm^{-3}$ and 1.20 $g.cm^{-3}$ respectively.

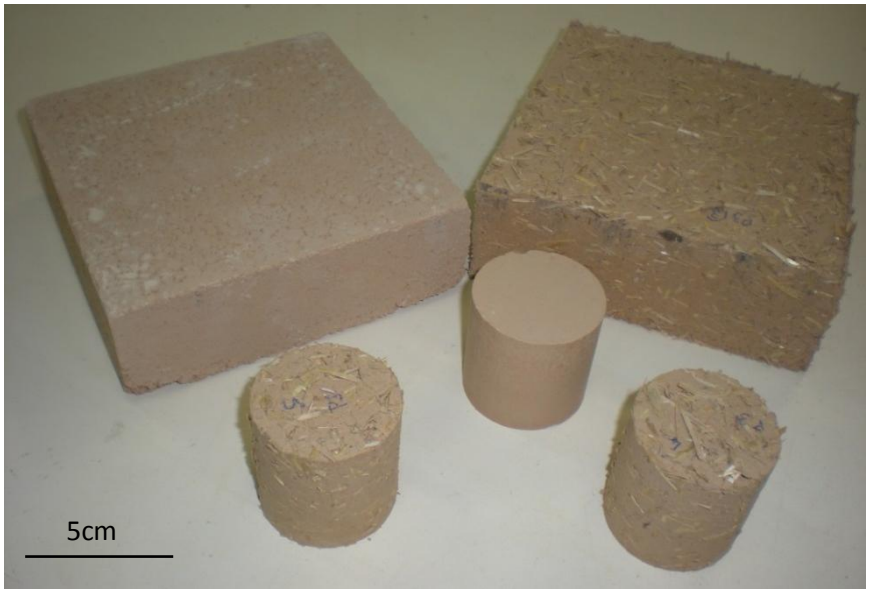

Figure 1. Cylindrical and parallelepiped specimens

2.3. Mechanical and hygrothermal characterization methods

2.3.1. Compressive strength

The compressive strength tests on the Φ5H5 specimens were performed using a 100 kN capacity hydraulic press. The values of compressive load and vertical displacement were registered during each test. The vertical displacement was measured with a transducer that was in contact to the lower steel plate. The load was applied at a constant deflection rate of 3 mm/min. This speed was chosen as an intermediate value between the 1.2 mm/min specified in the French standard NF XP 13-901 [26] (intended for compressed earth blocks) and the 5 mm/min used in [27] (intended for hempcrete). Three specimens of each mixture were tested in two different tests: one test with the specimen in direct contact with the steel plates (generating friction) and the other including a system avoiding friction. In the latter case, a 2-mm-thick piece of Teflon and a thin neoprene piece - with a drop of oil between the layers - were put between the earth sample and the steel (neoprene in contact with the specimen, and Teflon in contact with the steel). Teflon was used because of its low friction coefficient and neoprene because of its high mechanical resistance. Specimens were tested as soon as they were in equilibrium at 20°C and 50% relative humidity. The Young's modulus of each specimen was then calculated from the linear part of the stress-strain curve (elastic deformation).

2.3.2. Thermal conductivity

Thermal conductivity properties were assessed on three 150x150x50 mm^3 rectangular prisms for each composition. The measurements were carried out with the EP500 guarded hot plate apparatus for earth alone and for earth with 6% of barley straw. Before testing, the specimens were dried at 100°C and placed in a desiccator to cool. They were wrapped in a thin plastic film to avoid any humidity uptake during the measurement, which was performed at 25°C with a difference of temperature of 10 K between the two plates. Steady state was assumed to be reached when the change in conductivity was lower than 1% in 60 minutes.

2.3.3. Water vapor permeability

The experiment used Φ5H5 specimens, which were kept at 20°C and 50% relative humidity. This test was performed with a wet cup, as presented in the French Standard NF EN ISO 12572 [28]. The cup contained a saline solution of potassium chloride used to regulate the relative humidity at 86% (Figure 2). This cup, with the specimen on its top, was placed in a chamber regulated at 23°C and 50% relative humidity. The difference of humidity created an outgoing flow

of water vapor, measured by regular weighing. The specimens were surrounded by an adhesive waterproof aluminum tape on the lateral face. They were then placed on a plastic support so as not to be in contact with the saline solution, as can be seen in Figure 2. The whole setup was finally sealed by a mix of 60% beeswax and 40% paraffin. The water vapor diffusion resistance factor (μ) was determined for the three compositions.

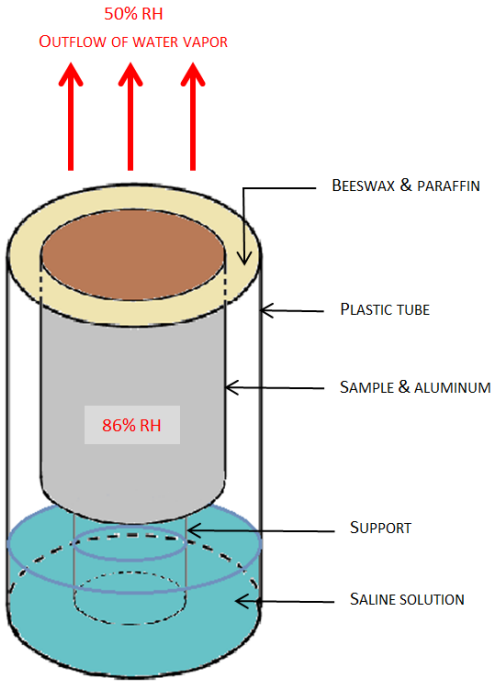

Figure 2. Schematic diagram of the wet cup

2.4. Microbial sampling and characterization methods

In order to sample the microorganisms contained in raw materials, the materials were suspended in aqueous sterile saline solutions and the influence of some key parameters (shaking time, addition of detergent, etc.) was evaluated. Sampling was more difficult on manufactured specimens because microorganisms were included in the matrix. A specific method, using adhesive sterile tape, had to be set up and optimized. All microbial assays were conducted under controlled conditions. Each assay was performed in triplicate in 2 independent tests.

2.4.1.Sampling and quantification on raw materials

Several techniques were used on the raw materials in order to optimize microorganism sampling methods. Each material (FWAS: 1g; straw: 0.25g) was mixed with 10mL of sterile Phosphate Buffer Saline (PBS) at room temperature. Sterile detergent (Tween80) was added to make the sampling of conidia easier. Different final detergent concentrations (1%, 5% and 10%) were tested. Suspensions were shacken at 300rpm for 10 minutes. Shaking time was also extended to 30 minutes with a final detergent concentration of 5%. Three masses of FWAS in different volumes of buffer (1g/10mL, 5g/20mL and 25g/100mL) were also tested with 30 minutes' shaking time. After homogenization by vortex, a range of dilutions of suspension were prepared in sterile distilled water**.** The suspensions and dilutions were deposited on various nutrient media, which were incubated at different temperatures: Tryptone Soy Agar (TSA) medium was incubated for 2 days at 32.5°C to enumerate aerobic and aero-anaerobic bacteria; Potato Dextrose Agar (PDA) with 0.05mg/mL of chloramphenicol (Cm) was incubated for 5 days at 22°C to enumerate fungi. After incubation, the colonies formed were counted (CFU: Colony Forming units).

Another method to sample microorganisms on straw was tested, using a Smasher™ blender (AES Laboratories). Straw (2.5g) was placed in a sterile bag with a membrane inside to separate solid particles from liquid after blending. Then, 100 mL of PBS with detergent was added at room temperature. Two final concentrations of detergent (1% and 5%) were tested. The bag contents were blended for 2 minutes. Blending time was extended to 5 minutes with a 5% final concentration of detergent. A range of dilutions was used and CFU were enumerated as described above.

2.4.2.Evaluation of release of microorganisms from adhesive dressing

An adhesive dressing was artificially contamined in order to validate the release of microorganisms from it. Adhesive sterile dressings (Hydrofilm®) were cut into pieces (about 4 cm x 4 cm). One milliliter of a suspension of *A. brasiliensis* (*niger*) (ATCC 16404 / CBS 733.88) conidia (10^7 CFU/mL) was deposited on each piece of adhesive dressing. Then, the pieces were put face with deposited conidia up under a laminar flow in a Biosafety Cabinet (BSC). Water containing fungal conidia evaporated by the air of the laminar flow. When all the water had evaporated, the pieces were removed from the BSC. Adhesive dressings were put into a tube and 10mL of PBS with a 5% final concentration of detergent was added. They were agitated by a vortex for 5 or 10 minutes. The suspension and a range of dilutions were deposited on nutrient medium (PDA with 0.05 mg/mL of Cm) and were incubated at 22°C for 5 days. After incubation, the colonies formed were counted.

2.4.3. Sampling and quantification on manufactured specimens

Sampling with pieces of adhesive dressing was carried out on Φ5H5 specimens made of only FWAS and S3 specimens at 2 different times of the manufacturing process: before the drying stage, directly after the compression step, and after the drying stage, when specimens were taken out the thermal chambers. Adhesive dressings were pressed on to manufactured specimens for 5 minutes. Then, the same protocol as described in section 2.4.2 (with 5 minutes' agitation and without evaporation step) was used to put the sampled microorganisms in suspension. A range of dilutions and a numeration were done as described at section 2.4.1.

2.4.4.Characterization of microorganisms

Some aspects (color, size, relief) of the colonies and mycelia were first observed on the isolation medium. Bacterial isolates were Gram stained and mold isolates were stained with cotton blue. Then, the aspect of cells and hyphae was observed by optical microscopy (X400 to X1000) so that bacilli/cocci Gram+/Gram- could be distinguished for the bacteria, and molds could be identified at the genus level.

2.4.5.Statistic tools

Averages and standard deviations were calculated for each condition. A Student test was performed to compare means. The tests were carried out on R software. A p-value below the threshold for statistical significance (0.05) is shown by an asterisk above the means concerned in Figure 5 to Figure 8.

3. Mechanical and hygrothermal characteristics of the products

3.1. Compressive strength

Figure 3 presents typical stress-strain plots for the different compositions and protocols (with friction at the interface between the specimen and the press and with reduced friction). For each composition, the compressive strength measured in the tests with friction was greater than that in tests with reduced friction because of the confinement. The compressive strength of the specimen composed of earth alone was higher than that of S6 and S3, which is in accordance with density values of the various specimens. The average strengths for the test with friction were 4.1 MPa for the FWAS, 3.2 MPa for S3 and 3.8 MPa for S6. All the results were above the minimal value of 2 MPa imposed by the New Mexico standards [29] for adobe construction and by the German Standards DIN 18945 [30]. The ultimate compressive strength

of S6 specimens was higher than that of S3 specimens. This can be explained by a consolidation phenomenon due to the compressibility of the straw. Specimens not reinforced with straw showed an abrupt rupture after the maximum load whereas ductility improved with the increase of barley straw content. However, it is important to note that the ultimate strain was high for S3 and S6 specimens: between 5% and 8% for S3 and between 15% and 20% for S6, whereas it was only between 1% and 1.3% for FWAS. The addition of straw increased the ductility of the composite. In calculating building structures, such deformations of the material cannot be tolerated. Thus the maximal stress does not constitute a relevant indicator for compressive mechanical performance.

In order to compare the materials, it was chosen to limit the strain to 1.5% and to keep the corresponding compressive strength value, as described by [27] for hemp concrete. The maximal compressive strength was kept in cases when the failure occurred before 1.5% strain (for the specimens of FWAS alone). These values are presented in Figure 4 with the Young's modulus values for a test with friction. It may be noted that for a given deformation, compressive strength decreases with the density, which is in accordance with the decrease of elastic modulus with the addition of straw.

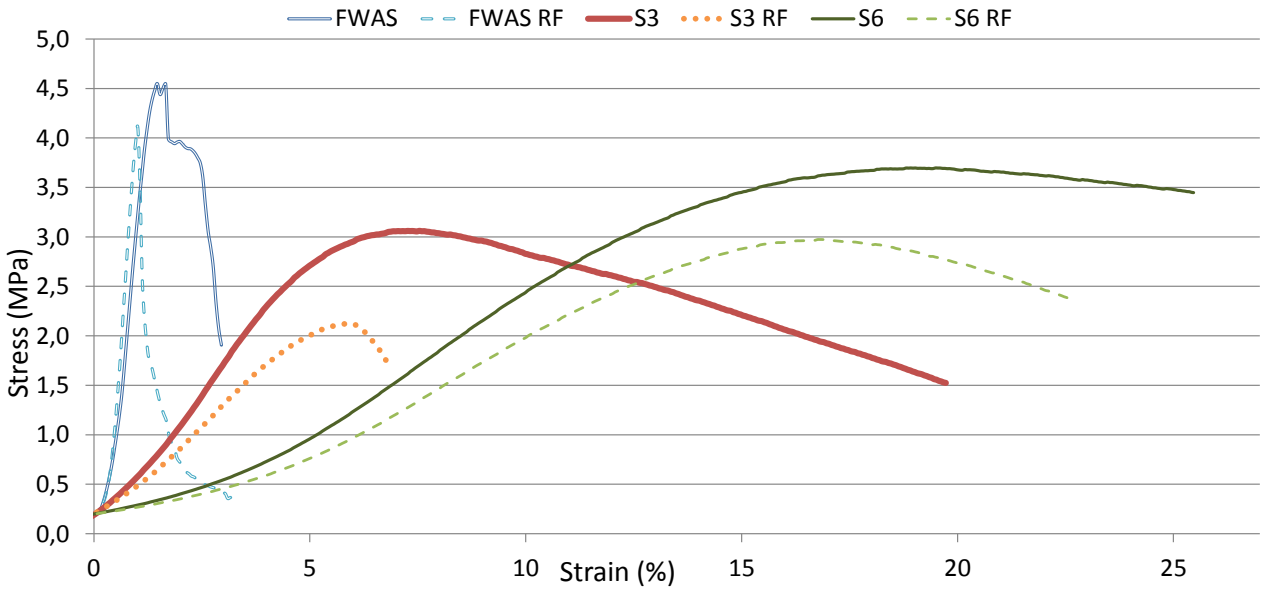

Figure 3. Typical stress-strain curves of the different specimens with friction at the interface with the plates and reduced friction (RF)

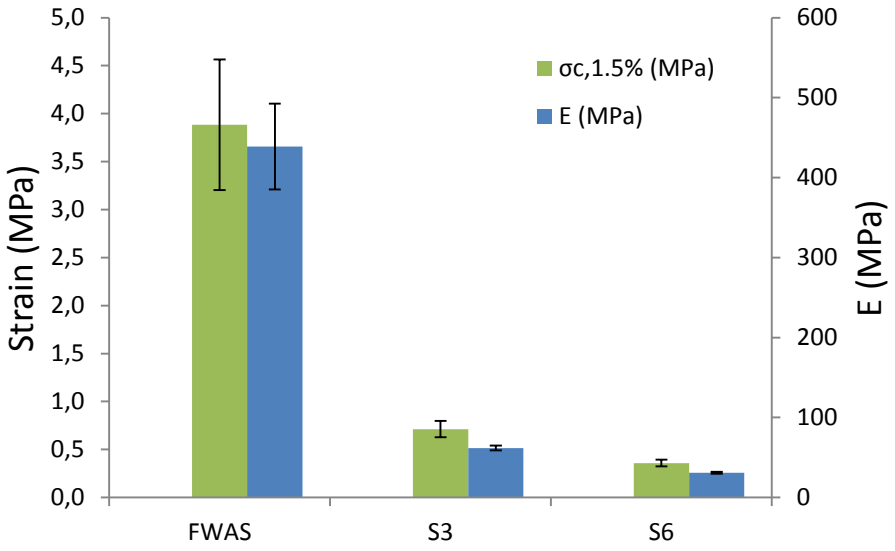

Figure 4. Compressive strength at 1.5% strain ($\sigma_{c,1.5\%}$) and Young's modulus with friction at the plate- specimen interface

3.2. Thermal conductivity

Tests were performed on three specimens for the two compositions. The average values of thermal conductivity obtained were 0.57±0.04 $W.m^{-1}.K^{-1}$ for FWAS specimens and 0.14±0.01 $W.m^{-1}.K^{-1}$ for S6 specimens. The results show that the thermal conductivity decreased by about 75% with the addition of straw in comparison with the FWAS specimen. This decrease of thermal conductivity with an addition of plant aggregates has been widely reported in the literature [31], [32]. It is linked with the decrease in density of the composite material. The improvement of thermal insulation demonstrates the interest of studying fibered earth bricks.

3.3. Vapor permeability

The assemblies were weighed daily over ten days. Using the measurements and the calculation presented in the NF EN ISO 12572 standard [28], the water vapor diffusion resistance factor (μ) was deduced for each composite specimen. The average values calculated from three specimens are presented in Table 1.

	FAC			P3			P6		
μ	4.2	4.8	4.8	5.0	4.5	4.6	6.1	6.3	6.4
μ_{moy}	4.6 ± 0.3			4.7 ± 0.3			6.3 ± 0.2		

Table 1. Water vapor diffusion resistance factor (μ)

All the average factors were very close (5-6), but these results do not bring out the influence of the straw. These values were very low, which means that earth is a very permeable material. The results could be compared with reference values of other materials according to the 2012 French Thermal Regulations [33]. For instance, the concrete factor is around 80 and the calcareous stone factor is around 150. The permeability behavior of earth bricks is comparable to that of porous construction materials such as wood concrete or gypsum (lower than 10).

4. Sampling and characterization of microbial flora of raw materials and products

Preliminary tests showed that the microflora of the raw materials was mainly composed of bacterial spores and fungal conidia. Therefore only these two types of microorganisms were considered in the following tests.

4.1. Sampling on raw materials

Various parameters were tested, in order to enhance microorganisms sampling on FWAS (Figure 5) or straw (Figure 6). For FWAS, an increase of the shaking time (Figure 5.A) did not improve the recovery of bacterial and fungal spores. FWAS was easily suspended in the buffer by a simple vortex and FWAS particles in suspension could be directly deposited on a medium or diluted. Similarly, the detergent concentration (Figure 5.B) did not have any effect on the recovery of bacterial spores. However, the use of detergent at 5% final concentration doubled the recovery of fungal conidia. Finally, an increase in the FWAS / volume of buffer had no effect on the recovery of the bacterial and fungal spores (data not shown). Therefore, the conditions chosen were a shaking time of 30 minutes, a final detergent concentration of 5% and a solid/liquid ratio of 1 g/10mL.

Regarding the straw, a 30-minute shaking time (Figure 6.A) significantly improved (by about 1 log10) the recovery of both bacterial spores and mold conidia. Straw was rougher than FWAS and microorganisms could be blocked on it, so were not easily suspended. As observed previously, the use of detergent had no significant effect on the sampling of bacterial spores (Figure 6.B). However in the case of molds, an addition of detergent significantly enhanced the recovery of conidia by about 1 log10, even with a final concentration of detergent of 1%. The use of a surfactant enabled a better suspension of conidia, thanks to lipophilic interaction with conidia membrane and hydrophilic interaction with PBS [34].

Figure 6.C presents the results of the sampling when a Smasher™ was used. The detergent still did not have any significant effect on the recovery of bacterial spores but it increased the recovery of fungal conidia as observed with the previous method. In addition, a 5-minute blending time (instead of 2 minutes) did not have any effect on the recovery of microorganisms. Compared to the shaking method, the blending method enhanced the recovery of bacterial spores by a factor 4 but no difference occurred for fungal sample. The use of the Smasher™ for straw was therefore advantageous in comparison to a shaking step, with a better recovery of bacterial spores and a shorter processing time.

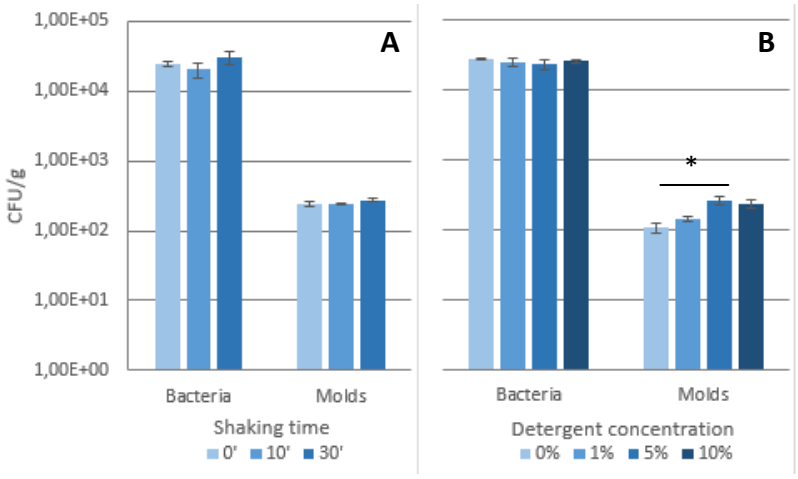

Figure 5. Colony-forming units (average ± standard deviation; 2 independent assays in triplicate) sampled per gram of FWAS according to shaking time (A) or detergent concentration (B).

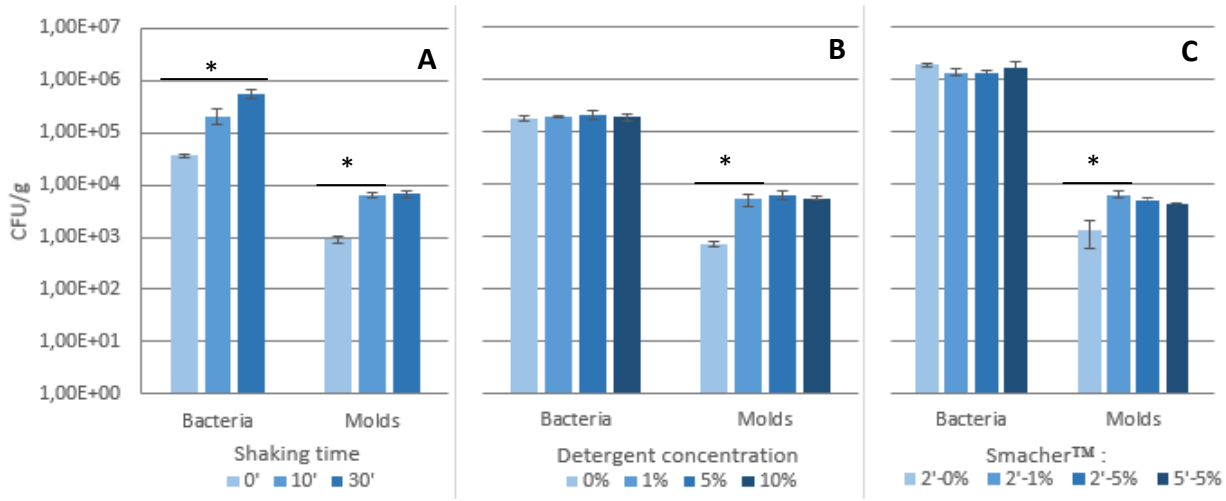

Figure 6. Colony-forming units (average ± standard deviation; 2 independent assays in triplicate) sampled per gram of straw according to shaking time (A), detergent concentration (B) and Smasher™ blending (C).

4.2. Evaluation of the release of microorganisms from the adhesive dressing

To determine the influence of the vortexing time on the release of microorganisms from the adhesive dressing, fixed conidia of *A. brasiliensis* fixed were vortexed for 5 or 10 minutes. The results are presented in Figure 7. When only homogenization by vortex was used, as few as $3.65x10^5$ conidia were recovered even though $8.7x10^6$ conidia were deposited. With a longer vortexing time (5 or 10 minutes), recovery of conidia increased significantly by around 1 log10, and $2.8x10^6$ conidia were recovered. No significant difference was observed between 5 or 10 minutes of vortexing, so a 5 minute vortexing time seemed enough to release fixed conidia, although the recovery of conidia was about 30%.

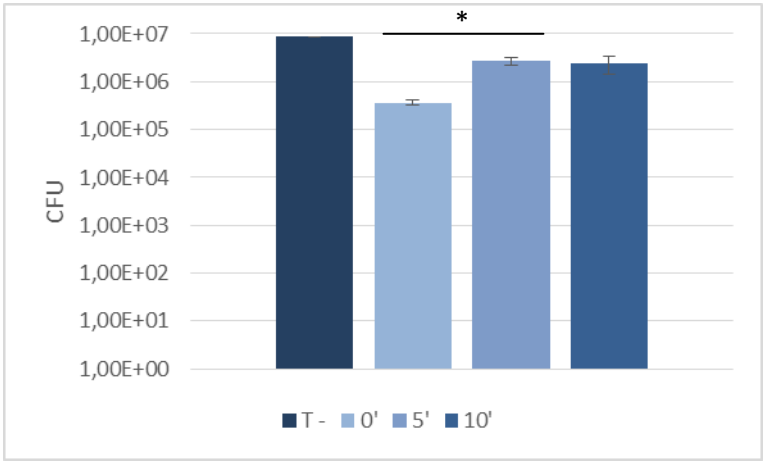

Figure 7. Colony-forming units (average ± standard deviation; 2 independent assays in triplicate) of *A. brazilensis* recovered according to vortexing time

4.3. Sampling on manufactured specimens

Figure 8 presents CFU enumerated after sampling of cylindrical specimens made of FWAS only or sampling on S3 specimens surfaces before or after the drying stage. Undried S3 specimens contained 1 log10 more bacteria and fungi than undried FWAS specimens. Although undried S3 specimens contained only 3% of straw, the addition of vegetable fibers led to detection of a significant quantity of microorganisms on the surface of the manufactured specimens. Molds could not be detected with FWAS specimens. The initial concentration of fungi in raw materials ($2x10^2$ CFU/g) was too low to be determined with this sampling method. Moreover, as presented above, the release of fixed conidia was about 30%, so all sampled microorganisms might not be observed.

Dried specimens contained 3 log10 less bacteria than undried specimens. Same difference of counted bacteria between FWAS and S3 specimens was observed after the drying stage. No enumeration was presented for fungi on dried specimens because too few isolates were obtained.

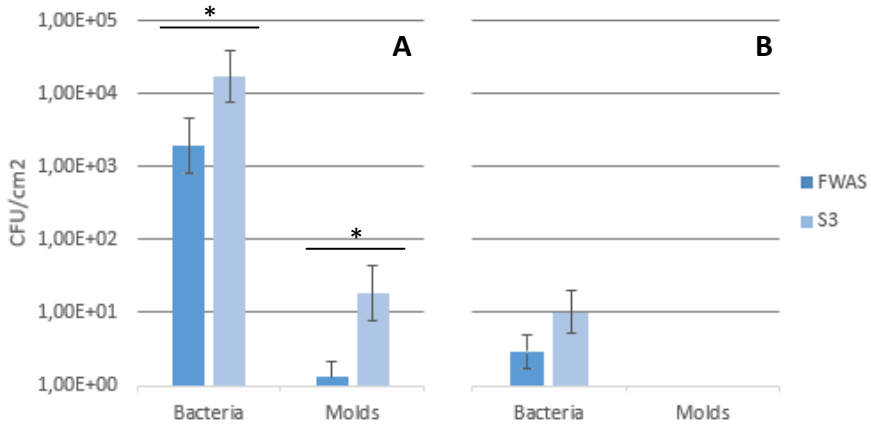

Figure 8. Colony-forming units (average ± standard deviation; 2 independent assays in triplicate) of microorganisms recovered by adhesive dressing sampling on undried (A) and dried (B) specimens

4.4. Characterization of isolates

The isolates obtained during tests were characterized by macroscopic and microscopic phenotypes. On FWAS, most of the bacterial isolates (more than 85%) were Gram + sporulated bacilli able to grow in aerobic conditions and so considered as *Bacillus* sp. Fungal genera observed were mainly *Penicillium* (around 40%), *Aspergillus* (35%), *Cladosporium* (10%) and more rarely *Rhizopus* (2%) and *Ulocladium* (2%) (Figure 9). As for FWAS, bacterial isolates from straw were mainly Gram + sporulated bacilli (more than 85%), and mold isolates mainly belonged to *Aspergillus* (around 95%) genera, with some *Penicillium* (2%) and *Rhizopus* (2%) isolated. The bacterial and fungal phenotypes observed on straw showed only half the diversity of those obtained on FWAS. Before packaging in plastic bags, FWAS were stored outdoors, which implied potential contamination by the ambient air or by rain. Moreover, FWAS could offer a more varied medium for the growth of microorganisms.

Finally, isolates obtained on cylindrical specimens were compared with those obtained on raw materials. Although few mycelia were sampled, they were the same as those observed on raw materials.

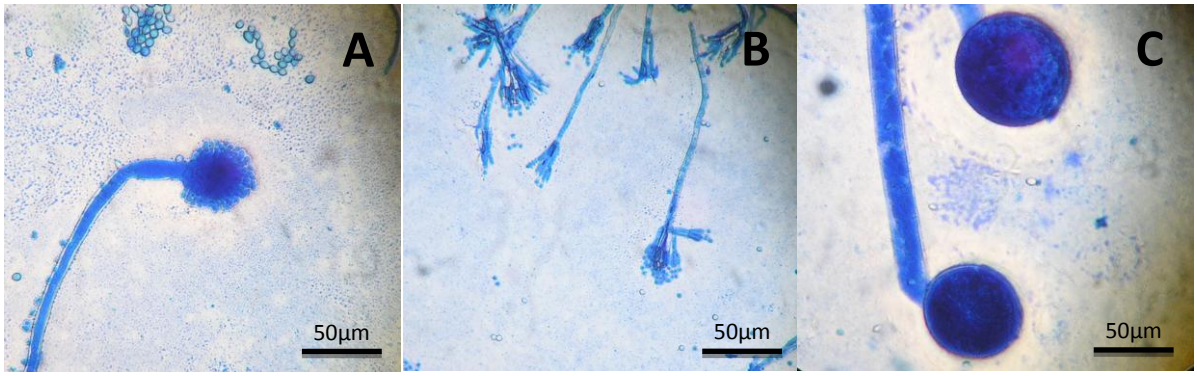

Figure 9. Observations by optical microscopy of *Aspergillus* sp. (A), *Penicillium* sp. (B) and *Rhizopus* sp. (C) isolates after staining with cotton blue.

5. Conclusion

The effects of additions of 3% and 6% of barley straw on the compressive strength, water vapor permeability and thermal insulation of earth bricks were investigated. The compressive test results showed that the specimen containing only FWAS had the highest strength. Adding straw decreased the compressive strength, by 7% for the S6 specimens, but improved the ductility. The tests carried out with reduced friction generated lower resistances than the other test. The effect of barley straw was not significant for the water vapor permeability factor but, when the straw content was increased by 6% in mass, the thermal insulation was increased by 75%. These results confirm the interest of using light plant aggregates in earth bricks to improve the thermal insulation of these materials and thus to make significant savings in the energy used for heating buildings. The interest of adding such plant aggregates in the earth has been demonstrated and the compressive strength still seems to be compatible with the use as a building material. Moreover, high ductility is an advantage in case of a use as a filling material inside a wooden-frame for example, which can deform itself with climate conditions. The effect of the addition of other types of plant aggregates will be studied in further experiments. Moreover, it will be necessary to study the hygroscopic properties of bio-based earth products in greater depth because these properties will strongly influence the possible growth of microorganisms.

The optimization of techniques to sample microorganisms showed that a longer shaking or blending time increased the recovery of microorganisms on straw, and the use of a blender improved the sampling. An addition of detergent also appeared to be very important in the recovery of fungal conidia. As expected, most fungal isolates on raw materials belonged to the *Aspergillus* or *Pencillium* genera, molds which are common in the environment. Most of the bacterial isolates were *Bacillus* sp. but isolation of anaerobic bacteria will be carried out in further work to extend the types of microorganism explored. First sampling using adhesive dressing did not recover enough conidia to ensure the characterization of the microflora of manufactured specimens. Despite this limit of quantification, this technique is a non-destructive sampling method that enables direct sampling in houses or constructions. The purpose of using this method was to detect microorganisms at a contamination level where moulds were visible on the material. Sampling on specimens surface revealed that dried step removed almost all microorganisms. Considering our preliminary results, one of the most important tool will be to define if visible contamination of specimens surfaces occurs after proliferation of microorganisms deposited on surfaces by the environment or initially present in the interior of the specimens.

On the basis of these sampling methods, the next steps of this work will be the identification and characterization of microbial diversity and proliferation on earth constructions and bio-based earthen products. Specimens will be collected *in-situ*, on existing earth buildings. Then, microflora profiles will be obtained by using microbial isolations and genomic

approaches (high-throughput DNA sequencing). The proliferation and adhesion of mycelia and biofilms on bio-based manufactured materials will also be studied to determine the environmental conditions favorable to their growth (temperature, relative humidity, etc.).

The first results concerning the hygroscopic properties of earthen bio-based product (vapor permeability) have to be completed in further experiments (especially regarding the sorption-desorption properties) but they confirm the high capacity of these materials to exchange quickly water vapor with the indoor air. The ultimate goal of this work will be to identify the links between the hygroscopic properties of earthen bio-based products, the environmental conditions (temperature and relative humidity) of indoor climate and the ability for microorganisms to proliferate at their surface. Finally, conditions of use of these types of materials in the construction and renovation of healthy, comfortable and sustainable buildings should be defined.

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