



HAL
open science

Assessing the durability of bio-based materials with respect to microbial contamination: Microstructure and macroscopic properties

Mohamad El Hajjar, S. Bourgerie, Christian Défarge, Naima Belayachi

► To cite this version:

Mohamad El Hajjar, S. Bourgerie, Christian Défarge, Naima Belayachi. Assessing the durability of bio-based materials with respect to microbial contamination: Microstructure and macroscopic properties. *Case Studies in Construction Materials*, 2024, 20, pp.e03349. 10.1016/j.cscm.2024.e03349 . insu-04615920

HAL Id: insu-04615920

<https://insu.hal.science/insu-04615920>

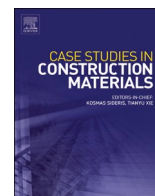
Submitted on 18 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Assessing the durability of bio-based materials with respect to microbial contamination: Microstructure and macroscopic properties

Mohamad El Hajjar^{a,*}, Sylvain Bourgerie^b, Christian Défarge^{c,d}, Naima Belayachi^a

^a Univ. Orléans, Univ. Tours, INSA-CVL, LaMé – EA794, 8 rue Léonard de Vinci, Orléans cedex 2 45072, France

^b Univ. Orléans LBLGC – EA1207, INRAE USC1328, rue de Chartres BP6759, rue de Chartres BP6759, Orléans cedex 2 45067, France

^c Univ. Orléans, Cellule R&D CETRAHE, Polytech Orléans, 8 rue Léonard de Vinci, Orléans cedex 2 45072, France

^d Institut des Sciences de la Terre d'Orléans (ISTO), UMR 7327, Université d'Orléans-CNRS/INSU-BRGM, Orléans 45071, France

ARTICLE INFO

Keywords:

Plant aggregates
Bio-based materials
Lime
Microbial growth
Bacteria
Fungi
Cryo-HRSEM

ABSTRACT

The aim of this paper was to assess the effect of the microorganism development on the thermal and mechanical properties of building insulation materials (rapeseed straw and lime (RL) and sunflower bark and pith and lime (SL)) and three plant aggregates (rapeseed straw (RS), sunflower bark (SB) and pith (SP)). The samples were exposed to accelerated ageing, in a static environment with a temperature of 30°C and a high humidity of 90% ($\pm 3\%$), to favor the proliferation of microorganisms. The paper presents the biological study of the cultivated bacteria and fungi at four ageing times (0, 3, 6, and 12 months) using a counting test. The results showed that these materials were more vulnerable to fungi than to bacteria. Cryo-HRSEM observations were carried out to follow the development of microorganisms at micro scales, at 0 and 6 months of exposure to microbial ageing. The observations showed that the aggregates (RS, SB, and SP) were colonized by microorganisms prior to microbial conditioning for 6 months, but not with the same tendency on the biocomposites (SL and RL) studied which presented weaker development of microorganisms by comparison to the aggregates. The lime has an immediate anti-microbial effect on the aggregates after storage conditions. After ageing, the microorganisms continued to grow on the aggregates, as did the biocomposites, which showed microbial growth of microorganisms of the same type as those found initially on the aggregates. Physical characterization was conducted in order to evaluate the effect of the microbial development. The thermal conductivity was measured with hot wire method for plant aggregates and heat flow meter for the biocomposites. Mechanical characterization was performed by uniaxial compressive test. The studied functional properties (thermal conductivity and compressive strength) of RS, SB, RL, and SL showed a decrease after 3 months, and after 6 months for SP.

1. Introduction

The use of biobased materials based on plant aggregates is an alternative that aims to reduce the impact of the construction sector on the environment, in terms of carbon dioxide emissions, energy used for the manufacture of materials, and the exploitation of

* Correspondence to: Laboratoire de Mécanique Gabriel Lamé Polytech Orléans, 8 rue Léonard De Vinci, Orléans cedex 2 45072, France.
E-mail address: Mohamad.el-hajjar@univ-orleans.fr (M. El Hajjar).

<https://doi.org/10.1016/j.cscm.2024.e03349>

Received 1 April 2024; Received in revised form 17 May 2024; Accepted 26 May 2024

Available online 31 May 2024

2214-5095/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

granular raw materials. These materials, which are durable, eco-friendly, and cost-effective, will also meet the global aim of the construction sector for thermal rehabilitation [1].

Recent research on biocomposite materials has shown that these materials have a high performance, making them usable in different civil engineering activities. These performances were achieved thanks to the use of one or some components of biomass origin. These biomasses are derived from three streams: recycled, animal, and plant [2]. (Nguyen, 2010) [3] developed hemp concrete for prefabricated building elements. The concrete had a structural or load-bearing role (Young modulus $E = [60; 120]$ MPa and compressive strength $\sigma = [0.5; 3]$ MPa) while preserving good thermal insulation properties (thermal conductivity $\lambda = [0.08; 0.12]$ W/m.K). (Limam et al., 2016) [4] evaluated the thermal conductivity and diffusivity of wood and cork bio-based materials with regard to humidity. They showed that the increase in mass of the materials due to water absorption led to an increase in the values of the evaluated parameters. (Delannoy, 2018) [5] studied the durability of hemp concrete by monitoring its thermal, hydric, acoustic, and mechanical properties, exposed to different environmental conditions for two years, in order to identify the ageing mechanisms of the material. The study showed a degradation of the properties of their materials regarding the different conditioning. (Belayachi et al., 2013) [6] and (Ismail et al., 2020) [7] developed biobased materials based on wheat straw, for the thermal insulation and energy rehabilitation of buildings. These biocomposites showed a very good thermal, mechanical and hydric behavior.

Despite these developments, the application of biocomposites is still limited to non-structural fields such as building insulation. In addition, the lack of specific standards and application rules does not yet guarantee the widespread use of these materials and a mastery of their implementation. Their presence in environmental and/or accidental conditions of use (use in structures with high humidity, water contact, etc.) can expose them to excess humidity. These materials can constitute storage for water, due to the high porosity of the plant aggregates. An increase in the moisture content of the material leads to a decrease in mechanical strength, thermal performance, and water diffusivity [7]. Moreover, this permanent contact with water or high humidity, and the biological nature of the materials, may be conducive to microbial development. (Rabii, 2017) [8] studied the microbiological ageing of composites reinforced with flax fibers. Ageing was performed at 30°C and 96% ($\pm 3\%$) of temperature and relative humidity respectively. The materials showed a trend of increasing microbial counts at different percentages of flax. (Delannoy, 2018) [5] conducted a laboratory study of the mechanism of biodeterioration of bio-sourced materials based on hemp shiv. This phenomenon was achieved by the colonization of materials by microorganisms after 100–120 days of incubation in a severe environment (90% of relative humidity), that resulted in the deterioration of the materials. (Maundrill et al., 2023) [9] studied the effect of fungal development on the functional properties of fibrous plaster materials over a period of up to 2 years. This development led to a 51% reduction in its bending strength.

The phenomenon of biodeterioration can affect different domains such as the oil industry, port, maritime and river facilities, electricity production plants, the aerospace industry, heritage materials, and above all, civil engineering and bridge structures/materials [10]. (Verdier et al. 2014) [11] reviewed different building material that presented an indoor microbial growth such as concrete attacked by *A. fumigatus* and *A. melleus*, gypsum board by *P. chrysogenum* and *S. chartarum*, wood, mortar and bricks by *A. versicolor* and *Actinobacteria*. In order to evaluate the risks of biodeterioration, some investigations were carried out on building materials. Microorganisms can be present in different environments such as air, water and soil, and/or associated with different living organisms on the surface as well as inside the material ([12,13]). (Lekounougou, 2008) [14] studied the durability of wood, which is naturally biodegradable by microorganisms and especially Xylophages fungi (also called wood-destroying organisms) such as *Basidiomycota* (cubic and fibrous decay), *Ascomycetes* (bluing and heating), and *Saprophytes* (soft decay). (Singh et al., 2011) [15] investigated the biodegradation of wheat straw by *Phanerochaete chrysosporium*, a species of lignolytic fungus, capable of degrading lignin, which is a major component of plant cell walls. The results showed a 30% loss of total lignin after three weeks of contact with *Phanerochaete chrysosporium*. (Čilerdžić et al. 2016) [16] also studied the degradation of wheat straw and oak sawdust by another type of fungi, *Ganoderma applanatum*, a saprophytic, woody basidiomycete fungus commonly found on dead or dying deciduous and coniferous trees in temperate regions worldwide. This fungus showed a significant potential, especially during the submerged fermentation of plant residues that stimulated the selectivity of degradation. (Warscheid et al., 1991) [17] showed that stone can be attacked by bacteria, leading to the degradation of its physio-chemical properties. (Loutz et al., 2000) [18] demonstrated that bacteria caused the loss of cohesion in the material by the dissolution of limestone, leading to the appearance of cracks. These cracks were present due to the corrosion of stone by surface-dwelling sulfide-oxidizing bacteria and deep-dwelling sulfate-reducing bacteria in the absence of air [19]. The proliferation of bacteria can modify the original bio-receptivity of facade coatings and also promote the development of other types of microorganisms [20]. (Delannoy, 2018) [5] noticed the presence of biofilms of bacteria on the surface of hemp concrete samples visible by ESEM (Environmental scanning electron microscope). These bacteria may impact the material properties by closing the porosity and decreasing the water permeability of hemp concrete. However, (Laborel-Préneron et al., 2016) [21] observed that microorganisms probably modify the microstructure of the material by creating pores, channels, and cavities, which enhance the thermal performance of the material.

The main objective of this paper was to study in controlled conditions the influence of the proliferation of microorganisms on two biobased materials and three types of plant aggregates used in the manufacture of these biobased materials developed in previous work ([6,22]). To our current knowledges, there have been no studies investigating the influence of microbial growth on the behavior of the plant aggregates examined in this research. Furthermore, exploring the effect of this microbial development after their incorporation into the biocomposite holds significant importance for assessing their durability in terms of biodegradation. The experimental study aims to understand the microstructural behavior and the evaluation of the macroscopic properties for the durability of these materials with respect to microorganism development. In order to do so, microbiological, and microstructural analyses were conducted to complete the study of the materials properties against the development of microorganisms. Firstly, the article presents the characterization of the studied aggregates (bulk density and water absorption), before their exposure to accelerated microbial growth. During a year of ageing monitoring, a biological study was carried out, consisting of the estimation of the microorganisms cultivated from

these materials, by a counting test. Cryo-HRSEM observations of the aggregates and the biocomposites were also carried out, in order to visualize at micro scales, the development of microorganisms after 6 months of microbial ageing. Finally, a physical characterization was performed to evaluate the effect of the microbial development on the bulk density and the thermal conductivity of the material investigated and the mechanical strength of the biocomposites. The results provide crucial databases regarding the microstructural behavior of the aggregates and biocomposites in relation to microbial development, as revealed by cryo-HRSEM. This offers a clear insight into the type and morphology of the microorganisms that have developed.

2. Materials and experimental methods

2.1. Plant aggregates studied

Three different aggregates (Fig. 1) were studied in this work: the rapeseed straw (RS), the sunflower bark (SB), and the sunflower pith (SP). They were harvested in the Region Center Val de Loire in France. Each material was dried until mass stability, and then stored in boxes to be conserved in a controlled environment favoring microbial growth.

Before placing the aggregates in the conservation conditions of accelerated ageing, a characterization of their bulk density, their water absorption coefficient, and their size distribution, was performed and showed in Table 1.

The sunflower pith presented the lowest density while the sunflower bark presented the highest one. In terms of water absorption, the three aggregates showed an important coefficient, with sunflower pith, known for its high porosity, displaying the highest coefficient of water absorption. Indeed, a sufficient quantity of the water available in the material makes it a favorable environment for the development of microorganisms [8] [23].

Regarding the size distribution, the rapeseed straw showed that 45 % of the aggregates fell between 0 and 0.25 cm, and 50 % between 0.25 and 1.5 cm. Sunflower bark predominantly consisted of aggregates between 0.5 and 2 cm, while sunflower pith mostly contained aggregates between 0.75 and 1.75 cm. All three types of aggregates displayed a spread of sizes depending on the grinding method of the rapeseed straw and bark, as well as the technique used for separating sunflower pith from its bark. The analysis of the size distribution of the aggregates' results enabled confirmation that the processing unit's grinding adheres to the specified requirements, ensuring that no aggregates exceed 2.5 cm in size.



Fig. 1. Photos of the aggregates studied: (a) rapeseed straw (RS), (b) sunflower bark (SB), and (c) sunflower pith (SP) at 0 month before conservation conditioning.

Table 1
Characteristics of the studied aggregates before microbial ageing (mean \pm std; n=3).

Aggregates	Bulk density (ρ) (kg/m ³)	Water Absorption (Ab) (%)	Size distribution (cm)
Rapeseed straw (RS)	82.5 (\pm 1)	455 (\pm 5)	45 % [0;0.25]; 50 % [0.25;1.5]; 5 %>1.5
Sunflower's bark (SB)	105 (\pm 3)	340 (\pm 5)	20 % [0;0.5]; 65 % [0.5;2]; 15 %>2
Sunflower's pith (SP)	28.5 (\pm 0.3)	500 (\pm 6)	80 % [0.75;1.75]; 20 %>1.75

2.2. Preparation of biocomposites

Two biocomposites were selected from our previous work ([6,22]): (1) the first one (RL) (Fig. 2a), was based on rapeseed straw and lime ((Tradical PF70) with a mass ratio of water/binder = 1.1 and rapeseed straw/binder = 0.3; The binder used for formulating biocomposites is the preformulated mixture Tradical PF70. It consists of 75 % air lime, 15 % hydraulic lime, and 10 % pozzolan, with a bulk density of 620 (\pm 20) kg/m³ and a particle size not exceeding 90 μ m. (2) the second one (SL) (Fig. 2b), was a mixture of sunflower's pith, and bark with percentages of mass 60 % and 40 % respectively. This mix was manufactured with these percentages in order to have the same bulk density as the rapeseed straw. It was prepared with the same mass ratios of water/binder and aggregates/binder as RL. These ratios were optimized in the previous work using cereal straw aggregates ([7]).

The preparation of the biocomposites was manufactured by following 6 steps ([7]): (1) weight the components according to the mass ratio; (2) mix the lime with water for 5 min to obtain homogeneous binder; (3) put vegetables aggregates in the mixer with 20 % of the quantity of water and mix for 2 min; (4) add the binder to aggregates and then mix for 5 min; (5) and finally mold the materials in frameworks of 200 \times 100 \times 100 mm for the mechanical test, and 200 \times 200 \times 25 mm for thermal test. The molding was performed by a compaction at a slow speed (0.5 mm/min) in order to avoid breaking plant fibers, using mechanical machine and special framework mold to guarantee the same density of the samples at fresh state; (6) conserve the samples at 50 % of humidity and 20 $^{\circ}$ C of temperature for 28 days for the cure before the accelerated microbial ageing.

2.3. Conservation conditions for accelerated microbial ageing

For this study, the conditions adopted for accelerated microbial growth were 30 $^{\circ}$ C and 90 % (\pm 3) of temperature and relative humidity respectively ([24–26]). The samples were stored in a box incubated in a specific saline solution of potassium nitrate KNO₃, to ensure a humidity of 92.5 % (\pm 0.66) according to (Greenspan, 1977) [27]. Then, this box was kept in an oven at 30 $^{\circ}$ C for a one-year follow-up at 0, 3, 6, and 12 months of monitoring.

2.4. Microbial analysis: counting of microorganisms and statistical study

The purpose of the counting technique was to enumerate the microorganisms cultivated on a specific quantity of material. The procedure was as follows: (1) 5 g of materials were infused in 50 mL of autoclaved peptone water (1 g of peptone in 1 L of distilled water) for 10 minutes at room temperature; (2) the infusion water was diluted to one-fifth to increase the volume of the solvent diluent (peptone water) while preserving the quantity of solute (number of microorganisms); (3) microorganisms were cultivated in culture media. The cultivation was carried out in two different ways depending on the culture medium used: (3.1) the medium used for bacteria growth was Yeast Extract Gel (YEG). YEG can propagate aerobic microorganisms, cultivated on the surface of the culture media, and anaerobic microorganisms, cultivated in the core and on the surface of the same media. 20 mL of supercooled YEG were deposited on 1 mL of infusion water already deposited at the bottom of a petri dish, then the deposits were homogenized by a simple rotation of the dish. (3.2) the medium used for fungi growth, was Potato Extract (PE): 20 mL of PE were placed in the petri dish until jellification. Then, 100 microliters of the infusion water were deposited on the surface of the solid PE. Sterile glass beads were placed in

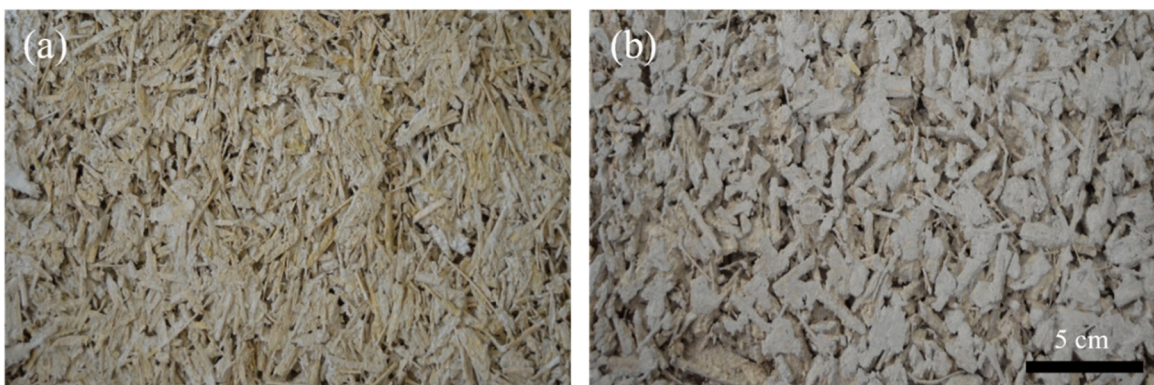


Fig. 2. Biocomposites studied: (a) rapeseed straw with lime (RL), and (b) sunflower with lime (SL), at 0 month of microbial ageing.

the petri dish to spread the infusion water evenly over the gelled surface. Finally, (4) the microorganisms were counted after incubating the petri dishes for 5 days at 25°C (Fig. 3).

After that, a statistical study was performed using the R software [28], in order to compare the counting results of the microorganisms (bacteria and fungi) in two ways: on the one hand, for each material, the effect of time on the microorganisms counted, and on the other hand, for each time, the effect of the type of aggregate. Firstly, two criteria were checked: the normality and the homoscedasticity of the data, and then the comparison was carried out with different methods of statistical analysis depending on these two criteria: an Anova then a TukeyHSD test if normality and homoscedasticity were respected, and if not, a Kruskal Wallis then a Dunn test.

2.5. Microstructural analysis by high-resolution cryo-scanning electron microscope (Cryo-HRSEM)

The Cryo-HRSEM (a Jeol JSM-IT800 SHL Field Emission SEM equipped with a Quorum PP3010T cryopreparation device) was used to visualize the micromorphological transformations occurring during accelerated ageing as regards microbial development on the aggregates and biocomposites studied in this article. For RS, and SB and RL, samples of a few millimeters long and wide were collected from the bulk material at 0 and 6 months. They were examined on their internal and external faces, and in cross-section. For SP and SL, the core of the pith was sampled, as well as the exterior (pith/cortex transition) that had been naturally detached, and that was examined on its internal and external faces; both were also observed in cross-sections.

The samples were first “cryofixed” by plunge-freezing in nitrogen slush at -210°C . The purpose of this ultra-fast freezing is to transform the water of the samples into “vitreous water”, i.e., without the formation of ice crystals that would disturb the morphology of the solid components at the observation scale of the microscope [29]. They were then transferred to the Quorum cryopreparation chamber, where cross-sections were cut on half of them with a razor blade. The superficial (~ 100 nm thick) part of this solid water was then sublimed by increasing the temperature up to -70°C during 20–30 min. The temperature of the samples was then lowered back to -140°C , allowing the stabilization of the sample’s ultrastructure close to its natural state [30]. After this step, the samples were metal coated by cathodic sputtering of Pt, then transferred without contact with air, into the microscope chamber, where observations were carried out at 1 kV. This low voltage preserves components that may be sensitive to electron beam irradiation, such as microorganisms, and, because the penetration depth of the beam is limited, enables the most superficial parts of the samples to be observed [30]. Energy Dispersive X-ray microanalyses (EDX) were performed using an Oxford Ultim Max 100 EDS.

2.6. Thermal conductivity

The thermal conductivity of the aggregates was measured using a NeoTIM conductivity meter-FP2c based on the hot wire technique, according to the recommendation of the RILEM TC 236-BBM [31]. This measurement was carried out in order to evaluate the effect of the biodegradation of the structure by microorganisms on the ability of the material to diffuse heat. The measurements consisted in (1) drying the aggregates at 60°C until mass stabilization, (2) placing the aggregates in a container, (3) placing the hot wire between the two layers of aggregates with a power between 0.1 and 0.2 W and a duration of the measurement between 60 and 120 s, (4) repeating the test 5 times.

Thermal conductivity of the biocomposites was measured using Heat flow meters (HFM 446 Lambda), developed by NETZSCH, and based on plate method for insulators, after drying the samples until mass stabilization. The sample ($200\times 200\times 25\text{mm}$) was placed between two plates. The energy was transported, in the form of heat, through the sample, as the result of a temperature gradient between the cold plate with the low temperature, and the hot one with the high temperature. According to the second law of thermodynamics, heats always flow in the direction of lower temperature. A preliminary test was carried out in order to choose these two temperatures. The thermal conductivity of a sample was measured, after varying the mean temperature (15°C , 20°C , 25°C and 30°C), with a same difference of 10°C . A difference of 1.4 % between the values was measured, which is negligible. For this study, the temperatures chosen were 10°C and 30°C which were equivalent to a mean value of 20°C and a difference of 10°C .

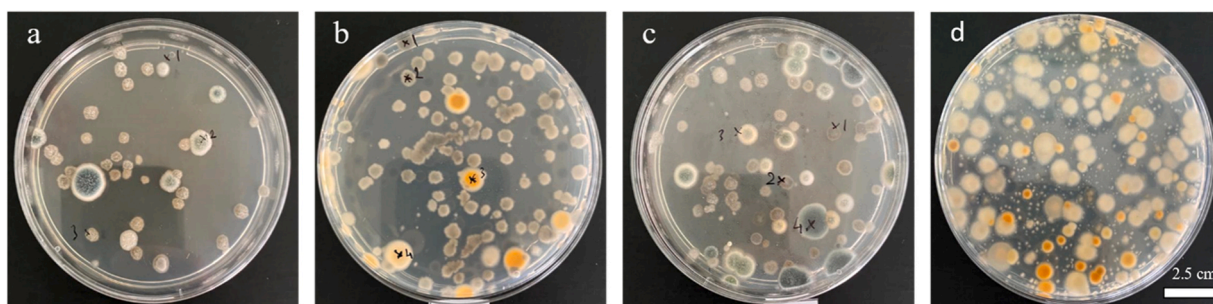


Fig. 3. Petri dishes of fungi cultivated with PE culture media from water infusions of (a) WS, (b) RS, (c) SB, and (d) SP at 12 months of microbial development.

2.7. Bulk density

The bulk density of plant aggregates was determined following the recommendations of RILEM TC 236-BBM [31]. Aggregates were placed in an oven at 50°C until mass stabilization and poured into a cylindrical tube 150 mm in diameter and 250 mm in height up to half its height. The cylinder was then capped and inverted 10 times. Following this step, the final volume was marked, and this volume was filled with water and weighed.

The bulk density was calculated using Eq. 1:

$$\rho = \frac{m}{m_w} * \rho_w \quad (1)$$

with: ρ : bulk density of aggregates (kg/m³)

m : mass of dry aggregates (kg)

m_w : mass of water corresponding to the volume of aggregates (kg)

ρ_w : density of water (1000 kg/m³)

2.8. Mechanical resistance

To measure the mechanical strength of the materials, uniaxial compression tests were conducted on biocomposite samples with a surface area of (10 cm×10 cm) and a height of 20 cm, using an Instron compression press, at a speed of 0.5 mm/min. The tests were carried out in the direction of the aggregate fiber casting and were performed on the samples after a slow drying process for 5–6 days at 60°C until mass stabilization.

2.9. pH and electrical conductivity in diluted media

Due to the importance of pH and the electrical conductivity as parameters that can influence the life cycle and development of microorganisms [32], these two parameters were studied. They were measured by a pH-meter, dual electrode HANNA HI 2020–02, during a year of monitoring at four different times (0, 3, 6 and 12 months). The measurement was done for the infused water from the studied materials. Five grams of each material were infused in 50 mL of distilled water at ambient temperature, for one hour without agitation.

3. Results and discussions

3.1. Visual observations of microorganisms' development

Figs. 4 and 5 showed the visual progress from the photo follow-up of the development of microorganisms on the three studied aggregates and the two biocomposites, during their exposure to the accelerated conditions of microbial growth, for the different time periods (0, 3, 6, and 12 months). Photos were taken by a commercial digital camera (Nikon D3200, AF-S NIKKOR 18–55 mm 1:3.5–5.6 GII). During each photographic session, the camera was fixed on a tripod stand. The tripod, in turn, was positioned on three points fixed on a white background table, where the box of aggregates or the biocomposites was positioned. The camera parameters such as the angle of positioning, the zoom and the flash-off were also fixed.

The development of microorganisms showed as a color change in the rape straw (RS) after 3 months of accelerated microbial conditioning. After 6 months, a layer of microorganisms had developed, covering the whole aggregate. For sunflower bark (SB), the color change was lighter compared to RS, indicating a weaker development of microorganisms during the first three months. At 6 months, a thin layer of microorganisms had developed on the aggregates. For sunflower pith, no change was noticed in the state of the aggregates between 0 and 3 months. At 6 and until 12 months, the aggregates showed a withered appearance and their color changed gradually from white beige to gray beige.

Concerning the visual appearance of RL and SL biocomposites after incubations (Fig. 5), only a few small spots of colored microorganisms appeared at 3 months. At 6 months, these spots became more spread out on the surface of the sample. At 12 months, in addition to the gray spots, black dots (indicated by the square boxes) were noticed, indicating probably the development of new type of microorganisms on the sample surface (Fig. 5). These points developed enhanced a color changing of the material. According to (Flemming, 1998) [33], indirect damage to a material by microorganisms is a color changing due to the excretion of lipophilic microbial pigments.

3.2. Counting of microorganisms

3.2.1. Counting of microorganisms on the aggregates

Figs. 6 and 7 showed the evolution of the number of bacteria and fungi, cultivated respectively on the three aggregates, as a function of the accelerated ageing time for one-year follow-up. The tables below the figures report the statistical study carried out on the counting test results. Indeed, lower case letters indicate the difference between the aggregates at the same time and capital letters indicate the time effect on each aggregate.



Fig. 4. Follow-up of microorganism development on rapeseed straw (RS), sunflower bark (SB), and sunflower pith (SP) at 0, 3, 6, and 12 months of microbial ageing.



Fig. 5. Follow-up of microorganism development on the biocomposites RL and SL at 0 and 12 months of microbial ageing. Black boxes indicate the black dots of microorganisms developed on the surface of biocomposites.

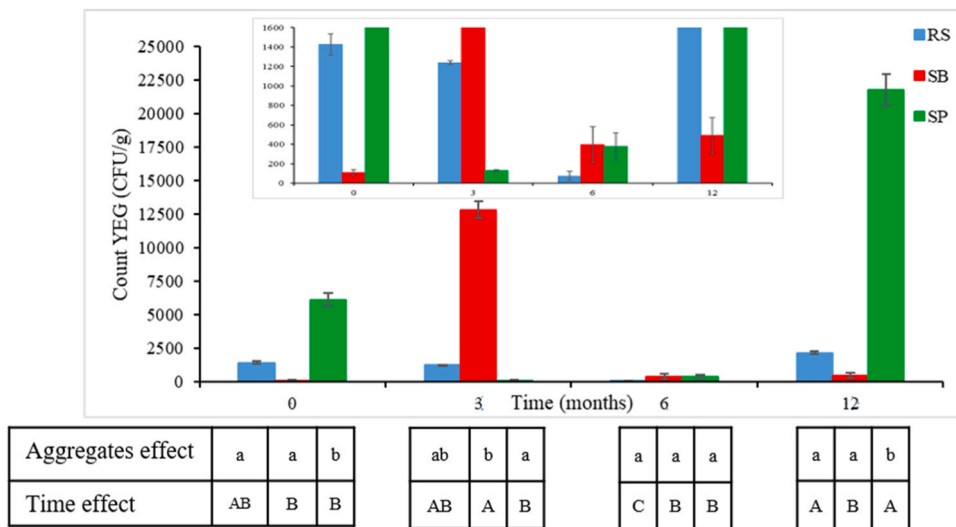


Fig. 6. Evolution of the number of bacteria (mean \pm std; n=3), cultivated on the aggregates studied, at 0, 3, 6, and 12 months at a dilution of 10^{-4} . Statistical studies (SS) are reported in the tables below the figure: lower case letters indicate the difference between the aggregates at the same time and capital letters indicate the time effect on each aggregate.

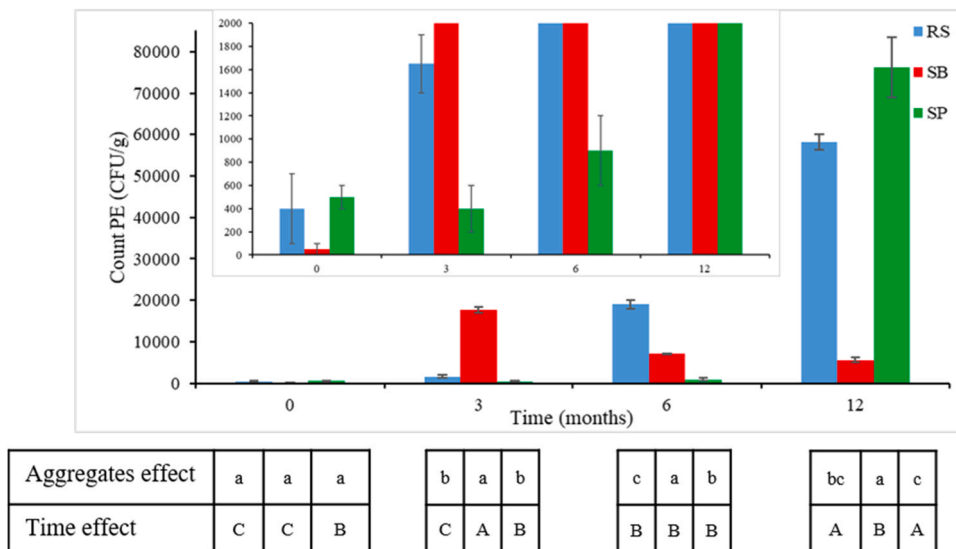


Fig. 7. Evolution of the number of fungi (mean \pm std; n=3), cultivated on the aggregates studied, at 0, 3, 6, and 12 months at a dilution of 10^{-1} for PE. Statistical studies (SS) are reported in the tables below the figure: lower case letters indicate the difference between the aggregates at the same time and capital letters indicate the time effect on each aggregate.

In Fig. 6, it can be seen that at 0 month, a large number of bacteria have developed, before the starting of the microbial conditioning, on RS and SP more than on SB. This means that these materials were already contaminated by bacteria that could come from the storage conditions. According to the statistical study (SS), SP has the behavior (b - b), as do RS and SB (a - a): this indicates that SP were strongly contaminated by bacteria while RS and SB were slightly contaminated. Between 0 and 3 months, SB showed a notable development compared to RS and SP. Three levels of the development can be distinguished by the statistical study: a weak development (a), a medium development (ab) and a strong one (b). These results show that RS and SB were contaminated by bacteria while SP was slightly contaminated. At 6 months, the number of cultivated bacteria had fallen almost to zero when compared with the values at 3 months. The statistical study showed that the behavior of the 3 aggregates was the same (a-a-a-a), the number of cultivated bacteria was negligible. At 12 months, a second phase of development of bacteria was observed. The number of bacteria cultivated increased on RS and SP while SB did not cultivate bacteria after 1 year of microbial conditioning. According to the SS, two ranges can be distinguished. In the first one, with RS and SB, an average quantity of cultivated bacteria was found (between 0 and 4000 CFU/g). In the second one, with SP, a high quantity of cultivated bacteria was observed (more than 20,000 CFU/g), compared to the other three materials.

The statistical study also considered the effect of exposure time to microbial ageing for each aggregate. It can be noticed that for RS (A B B C A), at 0, 3, and 12 months, the behavior was almost the same and the variation was observed at 6 months where the number of bacteria decreased to about 0 CFU/g. For SB (B A B B), the variation was detected at 3 months where the number of bacteria reached a maximum value of 12,820 CFU/g. Finally, for SP (B B B A), at 12 months, the number of cultivated bacteria was the highest, compared to 0, 3 and 6 months of accelerated microbial conditioning.

Fig. 7 represented the evolution of the number of cultivated fungi on the three aggregates investigated. At 0 month, for the other materials, no development was noticed. This was also demonstrated by the statistical study (SS) (a a a) showing the same behavior from the three materials. At 3 months, for RS and SP, no considerable development was observed while SB showed a development of fungi. These results were demonstrated by the SS (b a b) that present the same tendency near 0 CFU/g for RS and SP, and an increase for SB. At 6 months, SB showed a decrease while RS showed an increase. For SP, no significant development was observed. At 12 months, RS and SP showed a significant increase while SB showed a slight decrease compared to the other two aggregates.

Over time, the behavior of the aggregates was different. RS exhibited a gradual increase between 0 and 12 months. SB showed an increase between 0 and 3 months and then a gradual decrease until 12 months. Lastly, SP showed a very notable increase at 12 months.

The culture of bacteria using Yeast Extract Gel medium (YEG) and fungi using Potato Extract (PE) on different plant substrates showed that the proportion of fungal flora was greater than that of bacterial flora at all times: for rapeseed straw 70 fold, for bark 8 fold and two times for pith. The dynamic variation in the number of microorganisms over time, demonstrated by either increases or decreases or both together, could be linked to the maximum carrying capacity of microorganisms possible on these materials. The maximum carrying capacity refers to the maximum number of microorganisms that can be supported by a given environment [13].

Variations in microbial density can also be explained by the fact that microorganisms were subjected to various influences, particularly those of the physical-chemical conditions of the substrates (humidity level, pH, surface condition, etc.) and also to significant variations in biochemical factors (nutritional and energy) [34]. It should be mentioned that the samples were exposed to constant humidity and temperature throughout the monitoring period. Such conditions compensated the effect of these two parameters on the variation of the number of microorganisms. Fig. 8a and b show the evolution over time of the pH and the electrical conductivity respectively of the three aggregates RS, SB, and SP. The pH variation was similar for the 3 aggregates. Some microorganisms are known to be better adapted to a neutral pH (around 7), while others prefer a more acidic or alkaline environment [28]. (Rabii, 2017) [8] found that fungi prefer an acidic environment, with a pH of around 4–6. Microorganisms have different tolerances to salinity, and high levels of electrical conductivity can inhibit their growth by affecting their ability to maintain an optimal ionic balance between their internal and external environments [32]. (Dellal et al., 1992) [35] studied microbiological activities in saline conditions in some salt-affected soils in the Relizane region. They demonstrated that an increase in salinity from 6.4 mS/cm to 15.7 mS/cm and then to 22.3 mS/cm inhibited the growth of nitrous and nitric bacteria after 40 days of incubation. The dynamic variations in pH and electrical conductivity of the studied aggregates can have a significant impact on microbial proliferation by altering their environment and their ability to grow and survive.

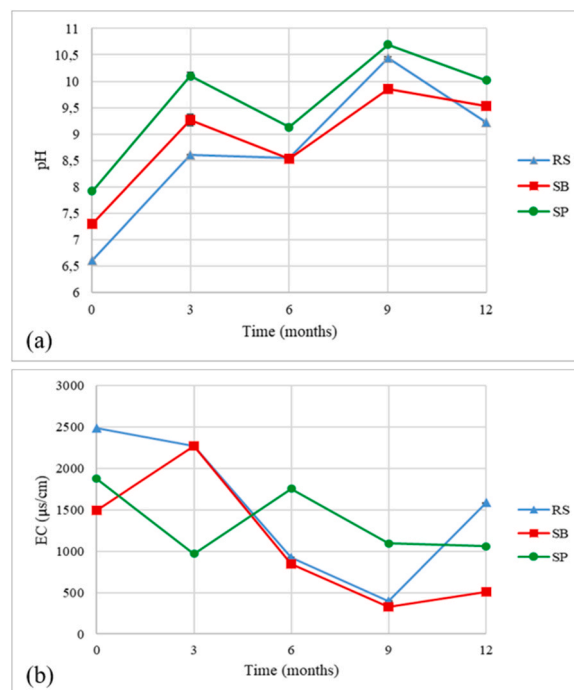


Fig. 8. Evolution of (a) the pH (mean ± std; n=3) and (b) the electrical conductivity (EC) (mean ± std; n=3) of the infusion water of the three aggregates RS, SB, and SP at 0, 3, 6, 9 and 12 months of microbial ageing.

3.2.2. Counting of microorganisms on the biocomposites

Fig. 9 illustrated the evolution of the enumeration of microorganisms, as bacteria cultivated by YEG, and fungi cultivated by PDA, on the two studied materials RL and SL respectively, at the four monitoring times 0, 3, 6, and 12 months.

This evolution showed that RL and SL were not contaminated by bacteria or fungi at 0 month. The development of microorganisms on RL presented a dynamic variation at 3, 6 and 12 months. On the other hand, SL didn't show a development of bacteria or fungi at 0 and 3 months. The material then developed gradually microorganisms at 6 and 12 months respectively. It is important to note that the immediate inhibiting effect of lime on microorganisms' growth on plant aggregates due to storage conditions after harvesting.

The investigated biocomposites had shown a gradual growth in terms of bacteria (YEG) and fungi (PDA), after the conservation of the samples in controlled conditions for microbial growth at 30°C of temperature and 90 % (± 3 %) of relative humidity. By comparison between the two biocomposites, RL was the most contaminated. Moreover, the variation of this development on the biocomposites, as the aggregates, may be due to the variation of the pH. For that, this parameter was monitored during the one-year of follow-up and presented in Fig. 10. The pH evolution exhibited some variations between RL and SL. At the start of the experiment (0 month), RL had a pH of 11.85, while SL had a pH of 11.7. Over time, RL experienced a gradual decrease in pH, reaching values of 10.77, 10.99, and 10.02 at 3, 6, and 12 months respectively. On the other hand, SL initially experienced a significant decrease in pH to 8.39 at 3 months, followed by an increase to 11.2 at 6 months. However, it then resumed its decline, reaching a pH of 9.98 at 12 months. The dynamic variation of the pH between 0 and 12 months, can lead to a change in the chemical environment that may influence the growth and the activity of microorganisms present in the material. In general, RL and SL showed a decrease of the pH between 0, 6 and 12 months. This decrease is related to the carbonation of the lime [36]. It is relevant to highlight the overlap of two phenomena: lime carbonation and microbial growth. However, it is difficult to determine which of these phenomena predominates after growth, as carbonation does not stop after 28 days.

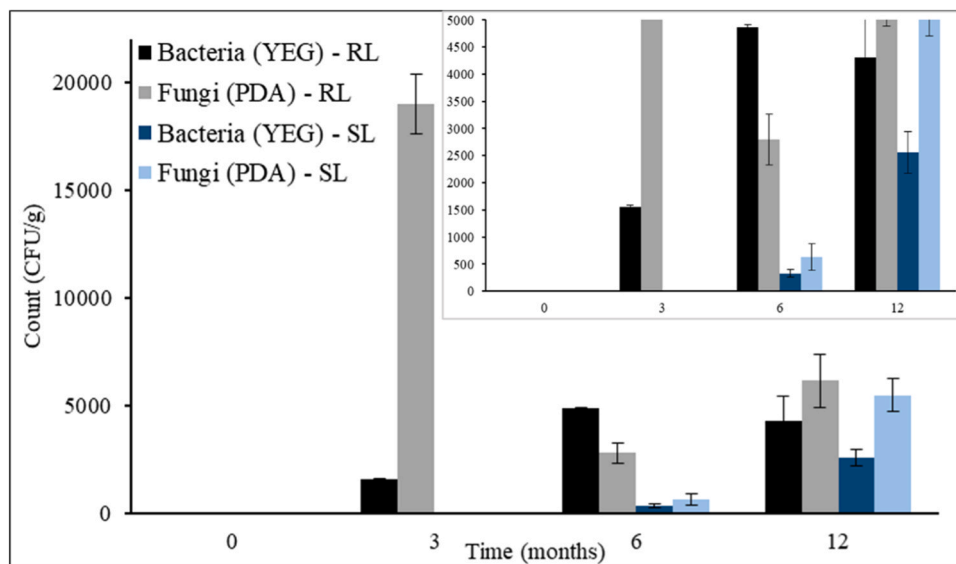


Fig. 9. Evolution of the number of microorganisms (mean \pm std, n=3): bacteria (YEG media culture) and fungi (PDA media culture) cultivated on RL and SL at 0, 3, 6, and 12 months.

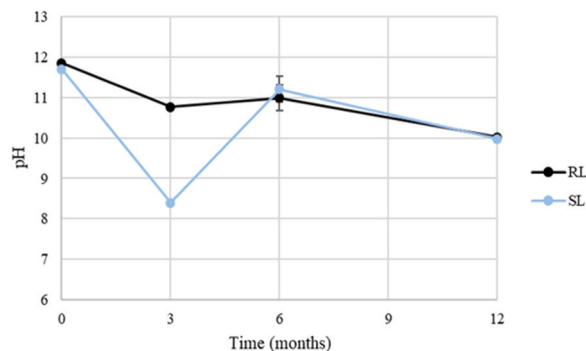


Fig. 10. Evolution of the pH (mean \pm std; n=3) of the infusion water of the two biocomposites RL and SL at 0, 3, 6, and 12 months of microbial ageing.

3.3. Cryo-HRSEM characterizations

3.3.1. Aggregates

Fig. 11, Fig. 12, and Fig. 13 are representative of Cryo-HRSEM views of the rapeseed straw (RS), the sunflower bark (SB), and sunflower pith (SP), respectively, at 0 and 6 months of accelerated microbial ageing.

Microorganisms were present on the rapeseed straw (RS) before exposing it to the microbial ageing (Fig. 11a). The 6 months of exposure also resulted in a proliferation of microorganisms (Fig. 11b, c, and d). Four types of microorganisms were mainly developed: (i) the first one with an arbuscular organization evocative of *Ascomycete conidia* (Fig. 11a compared to [37]); (ii) the second one with an ellipsoidal form, an average size of 5 μm and a smooth surface, evocative of bacteria organized in colonies developing around fungal hyphae (Fig. 11c); (iii) the third one appearing as single spheres a few micrometers in diameter, also evocative of bacteria (Fig. 11c); (iv) the fourth one also with a spherical shape, an average size of 1 μm or less, frequently organized in colonies connected by filaments (Fig. 11b, c, d and f), which are rather evocative of fungi. Cross-sections showed at 6 months some filaments seemed to pierce the material walls (Fig. 11f), to be compared to the cross-section at 0 month shown in Fig. 11e.

Like rapeseed straw, sunflower bark (SB) material was less contaminated with microorganisms at 0 month (Fig. 12a). However, at 6 months of microbial ageing, a considerable development of microorganisms was observed (Fig. 12b, c, and d). Several types of microorganisms were detected, among which some showed an arbuscular organization evocative of *Ascomycete conidia* (Fig. 12c compared to [37]). Other types were also present: (i) spheres of an average size of several μm , lined up in a chain, which are rather evocative of bacteria rather than of fungi (Fig. 12d); (ii) smaller spheres, with an average size of 2.5 μm and a spiny surface, which were organized in kinds of sheets (Fig. 12b and d); while the spiny surface of the latter is evocative of fungal spores, we did not find in the literature any similar images of fungal spores organized in sheets. The cross-section showed more microbial colonization in internal cavities of the material at 6 months (Fig. 12 f) than in the case of RS, whereas at 0 month cavities were devoid of any microorganisms (Fig. 12e).

The sunflower pith (SP) was significantly more colonized with microorganisms at 0 month than the other three materials. Exposed surfaces of pith as well as the outside of the cortex, were covered with organic mucilage wrapping both microbiotic filaments and fungal spores (Fig. 13a, and b). This mucilage retained water, which made sublimation more difficult than in the case of the other samples. Other organisms included short chains evocative of fungal hyphae, in particular because they are associated with spores (Fig. 13c), branched hyphae (Fig. 13d), and spheres a few micrometers in diameter (data not shown). Cross-sections also contained microorganisms already present at 0 month (Fig. 13e), contrarily to the other three samples, as well as minerals (not shown here) exhibiting calcium (Ca) and sulfur (S) by EDX analyses and whose shapes (and thus, composition) are evocative of gypsum or related species. As in the case of bark, the 6 months of exposure also resulted in a proliferation of microorganisms, but also filamentous microorganisms as well as spores (Fig. 13f).

The Cryo-HRSEM analysis demonstrated that the rapeseed straw (RS), sunflower bark (SB), and sunflower pith (SP), were contaminated by microorganisms in their initial state before the microbial conditioning and that these microorganisms continued to multiply and grow during the 6 months. The accelerated conditioning of microbial development provided a favorable environment for the microorganisms to develop and grow. Based on shape considerations, the microbial populations present at 6 months appeared to come mostly from the development of those already present before the beginning of ageing, evidencing that there was no external contamination of the samples during the manipulations. The types of microorganisms developed on the materials were different for each type of aggregate, which was further evidence that these organisms were present on the original samples. They seem to be probable bacteria in the form of colonies of ellipsoidal cells organized around fungal hyphae (Fig. 11c), or of individual spheres (Fig. 12d), and also by filaments producing mucilage (Fig. 13a, and b), whose attribution to fungi or bacteria remains to be done.

The sunflower pith appeared to be the most contaminated aggregate from the beginning, followed by rapeseed straw, and sunflower bark to a lesser degree. The pith, and the part of the cortex that remained attached to it, were covered with a mucilage produced by the filamentous microorganisms cited above (Fig. 13a, and b), which retained water. The presence of this mucilage may be responsible for the moisture content of these aggregates, which showed the highest water absorption of the four types studied (Table 1), or it may be the moisture content of the samples that favors the development of microorganisms and the associated mucilage. The high moisture content of the samples was also probably at the origin of the presence of gypsum or related minerals in the core of these aggregates, where they had precipitated; microbial filaments were also present from 0 month in the alveoli of the cross-section (Fig. 13e), unlike the other three aggregates. Rapeseed straw and sunflower bark were less covered with fungal mycelia at 0 month than sunflower pith, and underwent, up to 6 months, the proliferation of bacteria with these fungi, to a greater extent on rapeseed straw (Fig. 11c, and 9d). On the other hand, at 6 months, the alveoli observed in the cross-section of these two aggregates were more contaminated by microorganisms than the sunflower bark (Fig. 11f). Organisms in the material alveoli occasionally appeared to puncture their walls (e.g., Fig. 11f, Fig. 12 f, and Fig. 13e), but no other evidence of biodegradation at 6 months was observed on any of the three aggregates.

The cryo-HRSEM observations allowed us to provide more comprehensive information on the presence and the development of microorganisms on the aggregates at 0 and 6 months. On the one hand, the microorganisms may not be visible to the naked eye if they were micrometer-sized or not abundant like fungi. While visual observation (Fig. 4) allowed us to observe the development of fungi on the materials after 6 months of microbial ageing, these materials appeared to the naked eye to be uncontaminated at 0 month. On the other hand, the counting test may not reveal the same tendency of the results as that shown by the cryo-HRSEM, because the microorganisms counted were cultivated on media culture for bacteria and fungi, which was different from the microorganisms present or developed in the material and shown by the cryo-HRSEM. The counting test revealed that SP was the most contaminated by bacteria at 0 month (Fig. 6), and that was demonstrated by the presence of mucilage shown by the cryo-observation (Fig. 13a), whereas the other

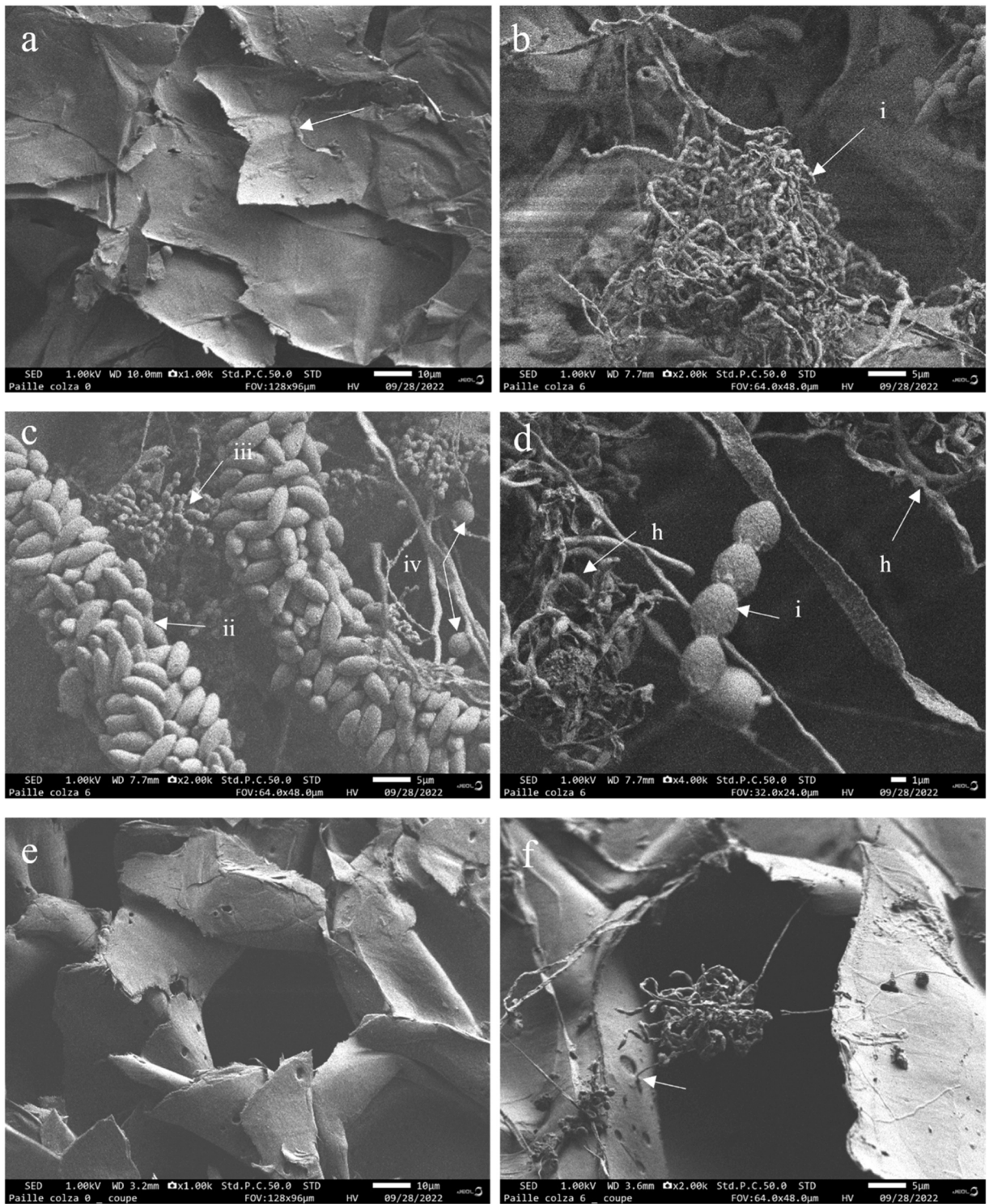


Fig. 11. Cryo-HRSEM micrographs of rapeseed straw (RS): (a) view of the surface at 0 month showing the presence of microorganisms (example of filament arrowed), (b) view of the surface at 6 months, showing microorganisms (i) whose arbuscular organization is evocative of ascomycete conidia (c) view of microorganisms at 6 months, including (ii) colonies of ellipsoidal organisms, probably bacterial, organized around fungal filaments, (iii) colonies of spherical organisms, evocative of ascomycete conidia, and (iv) individual, larger spheres, evocative of bacteria, (d) view of microorganisms present at 6 months including fungal hyphae (h) and chains of spheres (i), (e) cross-section at 0 month, (f) cross-section at 6 months, showing the presence of microorganisms inside alveoli whose filamentous parts seem to pierce the material walls (see arrow).

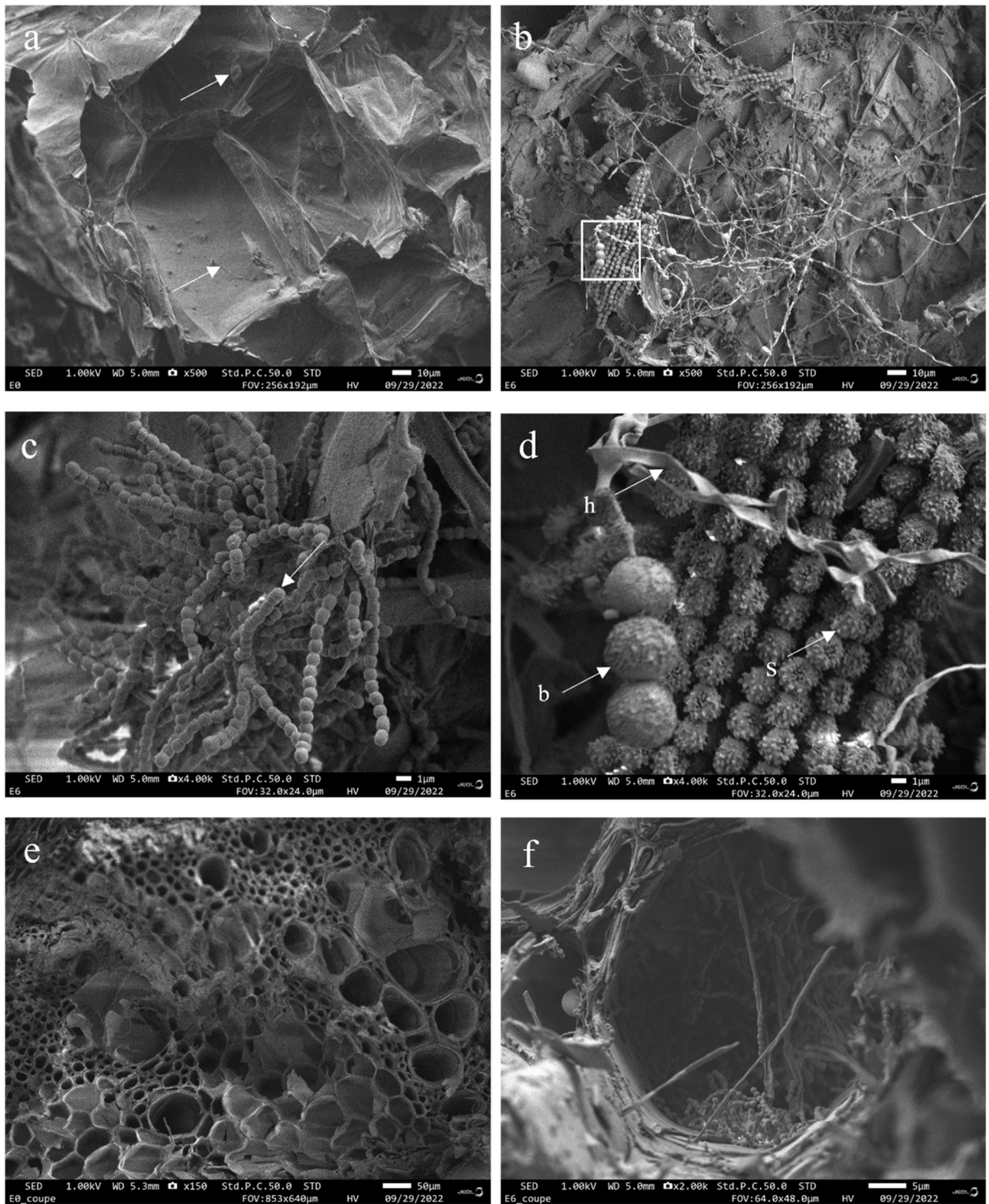


Fig. 12. Cryo-HRSEM micrographs of sunflower bark (SB): (a) view of the surface at 0 month, showing the presence of some microorganisms (arrowed), (b) view of the surface at 6 months, showing the proliferation of microorganisms (filaments and chains of spheres); the boxed area in enlarged in (d), (c) enlarged view of microorganisms present at 6 months, whose arbuscular organization is evocative of ascomycete conidia, (d) enlarged view of the boxed area in (b), showing filamentous structures evocative of fungal hyphae (h), spiny spheres evocative of fungal spores (s), and chains of larger spheres evocative of bacteria (b), (e) cross-section at 0 month, and (f) cross section at 6 months, showing alveoli invaded by microorganisms (filaments, and filamentous structures evocative of ascomycete conidia).

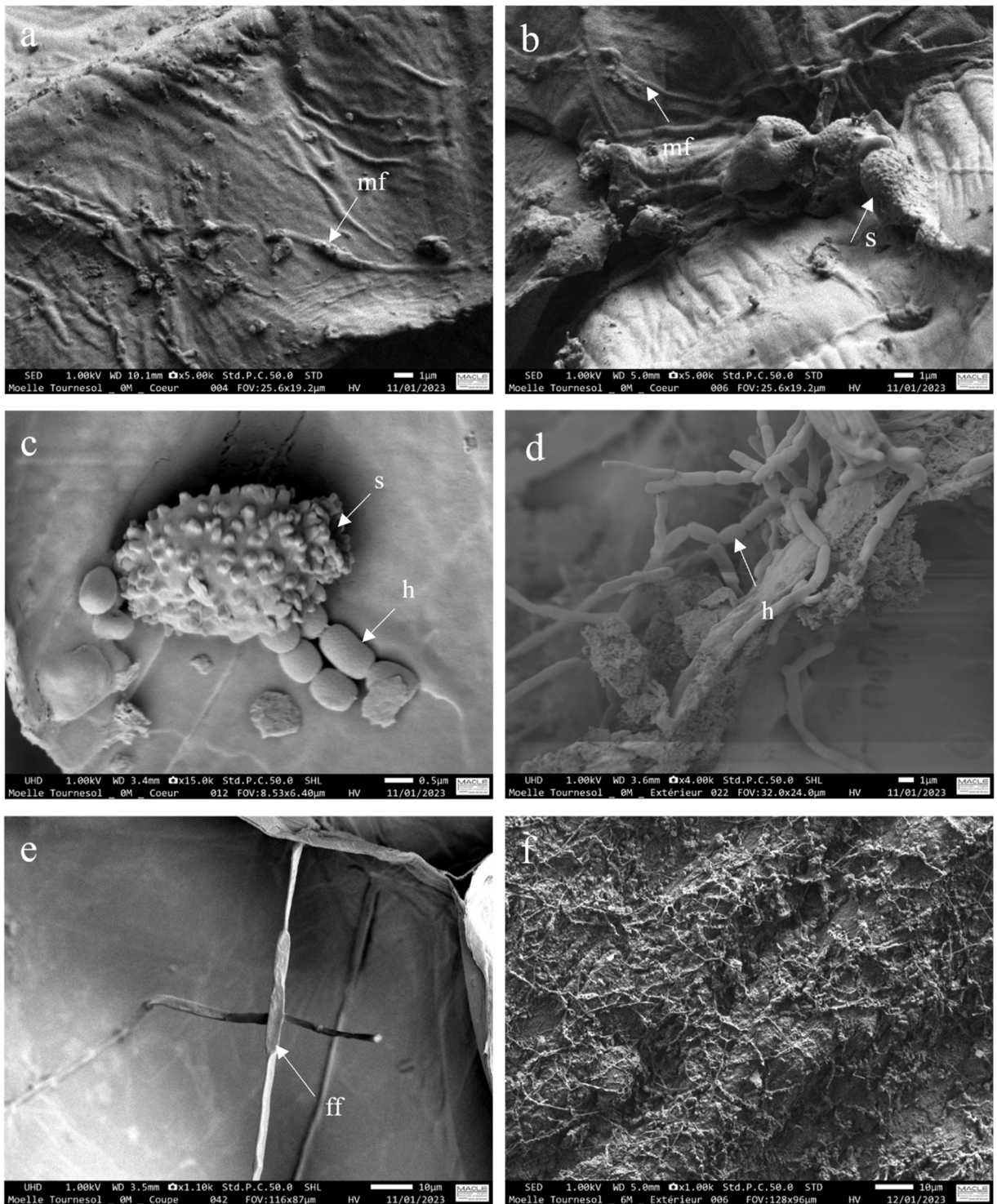


Fig. 13. Cryo-HRSEM micrographs of sunflower pith (SP): (a) view of the pith core at 0 month, showing the surface covered with mucilage notably wrapping microbial filaments (mf), (b) enlarged view of the core at 0 month, showing fungal spores (s) wrapped by mucilage as well as filaments (mf), (c) view of fungal spore (s) present at 0 month on the pith core, with probable emerging hyphae (h); (d) view of the exterior of the cortex at 0 month, showing well developed fungal hyphae (h, compare with c), (e) cross-section at 0 month, showing the presence in the alveoli of fungal filaments (ff), and (f) view of the exterior of the cortex at 6 months, showing the proliferation of filamentous microorganisms (mostly fungi).

two materials (RS and SB) did not show a culture of fungi at 0 month (Fig. 7), which was also in line with the cryo-observation (Fig. 11a and Fig. 12a).

3.3.2. Biocomposites

Figs. 14 and 15 are representative of the Cryo-HRSEM observations of the two biocomposites RL at 0 and 6 months and SL at 0 and 12 months of accelerated microbial ageing. It is worth noting that the 6 months of microbial ageing did not result in the development of microorganisms on SL.

The biocomposites based on rapeseed straw and lime (RL), showed no presence of microorganisms at 0 month, before exposing it to the microbial ageing (Fig. 14a). After 6 months of microbial exposure, a proliferation of microorganisms occurred (Fig. 14b). Three main types of microbial components were observed. Firstly, the fungal spores (Fig. 14b and d), some of which appeared to be damaged (Fig. 14d). The presence of the lime in the biocomposite caused a damage of the surface of microorganisms. Secondly, the hyphae are present (Fig. 14c), resembling those observed on the aggregate alone (compare Fig. 11a and b).

Fig. 14c also showed chains, some of which appear to be damaged, also resembling those observed on the aggregate (compare with Fig. 11b) evocative of bacterial strings or fungal conidia. At 6 months, cross sections showed filaments evocative of fungal filaments, as in the aggregate (Fig. 11e).

The biocomposite SL, based on sunflower aggregates, exhibited the presence of some microorganisms at 0 month, essentially on the bark part, and primarily in the form of spores, generally appear damaged (Fig. 15a). The surface of spores appeared to be damaged (Fig. 15b), in comparison with the spores associated with aggregates alone (Fig. 13). It is probably the addition of lime that seems to be the cause of this damage of the microorganisms. Moreover, the microbial contamination appeared minor when compared to the aggregates alone (sunflower bark and pith, which were abundantly contaminated as shown above in Fig. 12 and Fig. 13).

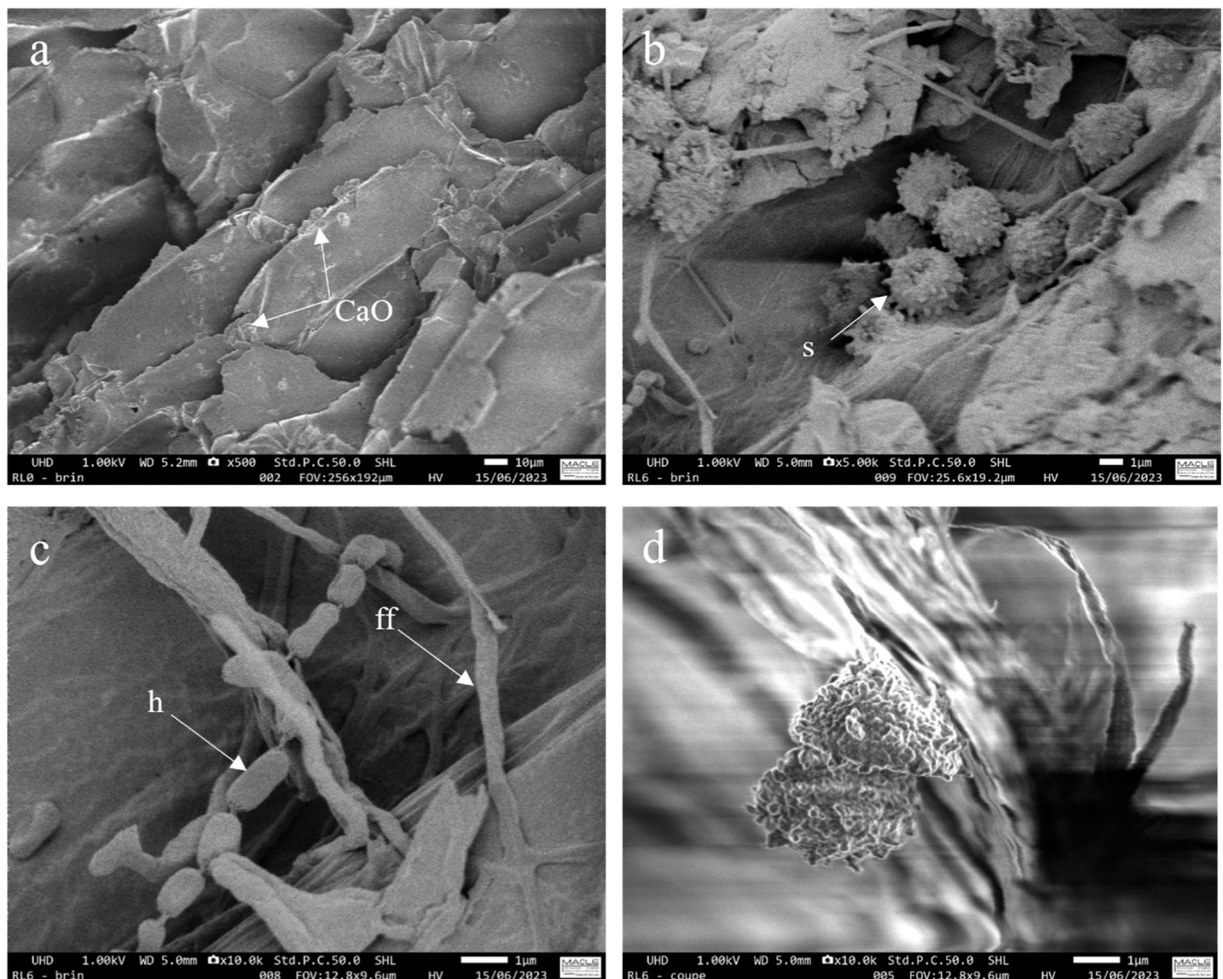


Fig. 14. Cryo-HRSEM micrographs of biocomposite (RL): (a) view of the surface at 0 month, showing the absence of microorganisms, and the presence of granular clusters of CaCO_3 (arrowed) (b) view of the surface at 6 months, showing spheres evocative of fungal spores (s), (c) enlarged view of microorganisms present at 6 months, with probable fungal hyphae (h) and fungal filaments (ff) (d) damaged microorganisms at 6 months of microbial ageing.

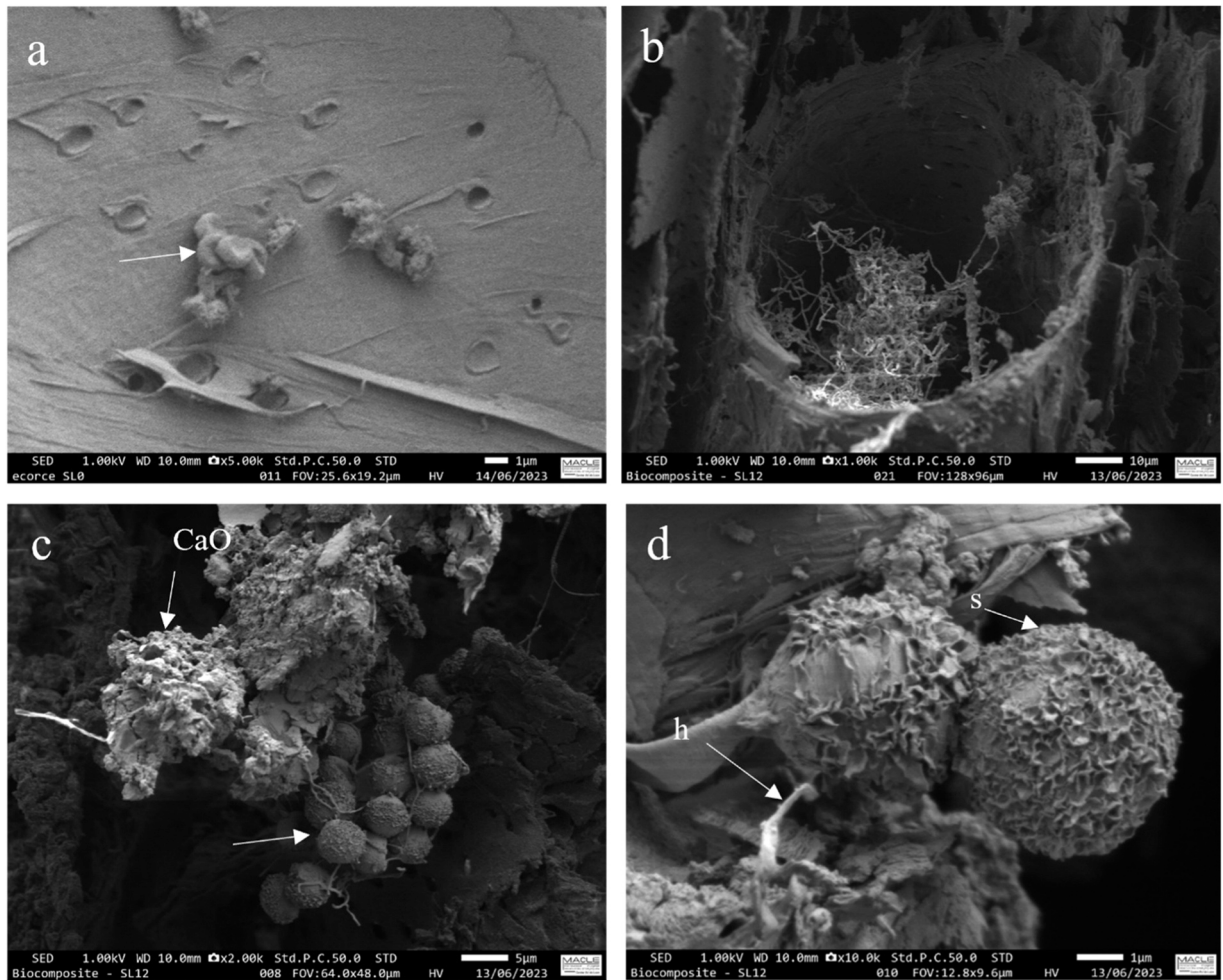


Fig. 15. Cryo-HRSEM micrographs of the bark of biocomposite (SL): (a) surface view showing the bark at 0 month, (b) cross section of the bark at 12 months, showing alveoli invaded by filaments, (c) view of the surface of the bark at 12 months, showing spores (s) and granular clusters of CaO, (d) enlarged view of the bark at 12 months, showing hyphae (h) and spores appeared to be damaged (s).

After 12 months of microbial ageing, a microbial development was observed under the form of bunches of spheres associated with filaments (Fig. 15c and d) resembling the fungal spores and hyphae present in the sunflower pith alone (by comparison with Fig. 13f). Cross sections at 12 months also revealed the presence of some filamentous organisms, probably fungi, in alveoli. Conversely, the pith part of the sunflower biocomposite didn't show any signs of microbial contamination at 0 month (figure not shown), even after 12 months of microbial ageing (figure not shown). In both cases, microbial development was not nearly as important as in the case of aggregates alone at 6 months (compare with Fig. 13c and d).

Cryo-HRSEM analysis revealed that the RL biocomposite was free from microbial contamination, unlike the SL biocomposite, which exhibited the presence of microorganisms at 0 month prior to microbial conditioning. When compared to the biocomposites, all aggregates, except the rapeseed straw, demonstrated higher levels of bacterial contamination at 0 month. The addition of lime in the biocomposites thus seemed to have a biocide effect on pre-existing microorganisms in the aggregates. However, this elimination was temporary, as the accelerated microbial conditioning provided an optimal environment for the growth and the development of microorganisms. Considering the shapes observed, the microbial populations present at 6 months appeared to mainly originate from the pre-existing microorganisms, indicating no external contamination during handling and manipulation of the samples. It's important to notice that the presence of microorganisms on the biocomposites RL and SL was relatively lower compared to the aggregates.

3.4. Physical properties of the investigated materials

3.4.1. Thermal conductivity and bulk density of the aggregates

Fig. 16 reports the evolution of the thermal conductivity (dark continuous line), and the bulk density (dark dashed line), of the three aggregates as a function of exposure time to accelerated microbial ageing. These two parameters were measured while the aggregates were in dry conditions at each time of microbial ageing (0, 3, 6 and 12 months). An experimental error range (light-colored

dashed line) was carried out on the results of the thermal conductivity in order to evaluate the tendency of the values that seems to be

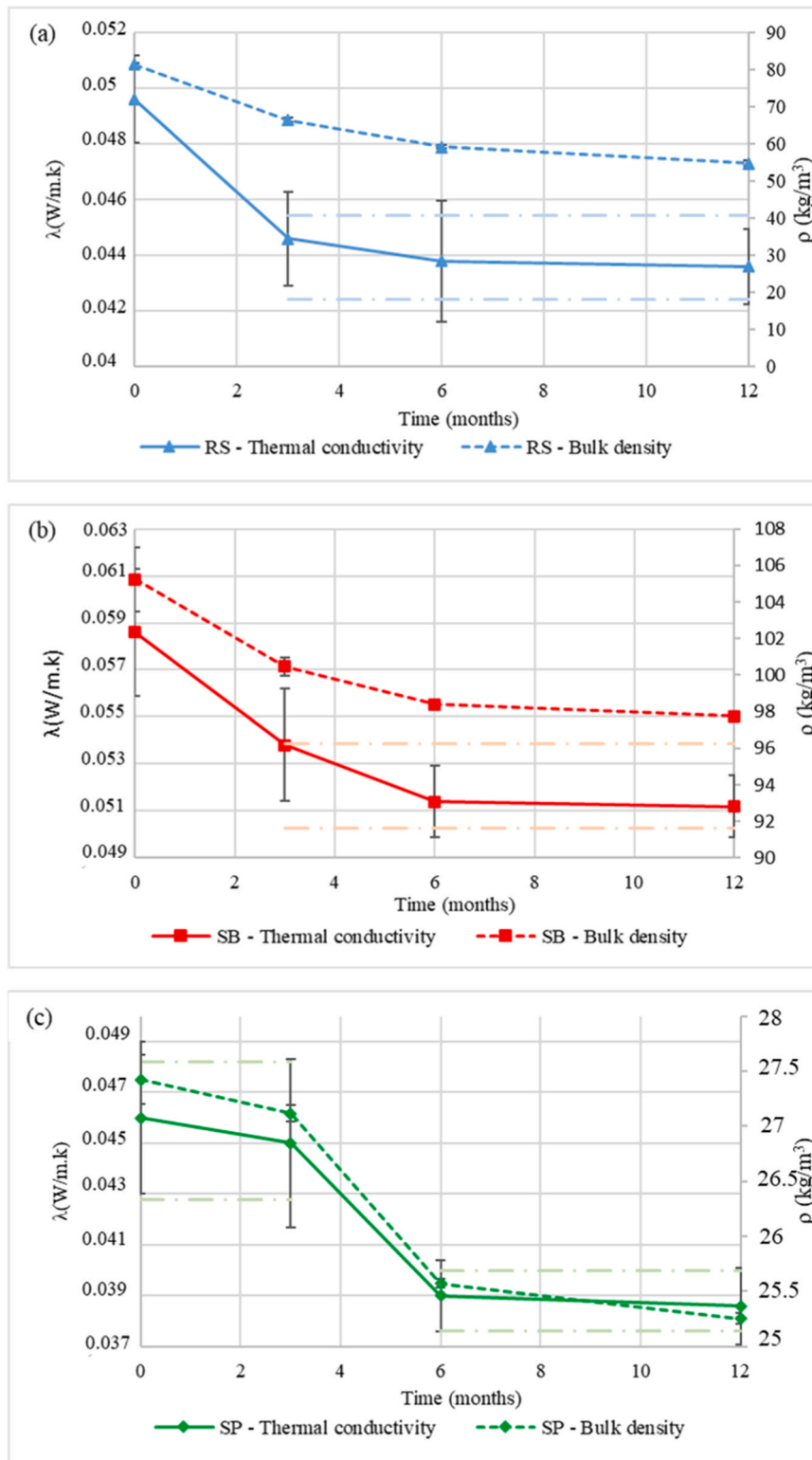


Fig. 16. Evolution of the thermal conductivity (mean \pm std; n=5) and the bulk density (mean \pm std; n=3) as function of time of accelerated conditioning for microbial development for (a) wheat straw (WS), (b) rapeseed straw (RS), (c) sunflower bark (SB), and (d) sunflower pith (SP).

constant. This study consisted of calculating the mean value (μ) of the thermal conductivity in the range that seems to be stable, then the corresponding standard deviation (std). If the measured values are in the interval $[\mu - \text{std}; \mu + \text{std}]$, these values can be considered as constant. If not, a variation should be taken into consideration.

Fig. 16.a and b, show a decrease in the thermal conductivity with a percentage of 10.08 % for the rapeseed straw (RS) and 8.03 % for the sunflower bark (SB). In parallel, the bulk density decreased, with the same tendency, 21 % for RS and 6.19 % for SB. Between 3 and 12 months, the thermal conductivity and the bulk density remained almost constant statistically, there was a negligible variation.

Fig. 16.c shows that between 0 and 3 months, the thermal conductivity of the sunflower pith (SP) remained constant with a mean value of 0.0455 W/m.k, while the bulk density showed an insignificant decrease to 27 kg/m³. After 3 months of microbial ageing, the thermal conductivity decreased to 0.0388 W/m.k (-14.72 %) and remained constant until 12 months. Similarly, the bulk density decreased moderately to 25 kg/m³ (-7.4 %) and remained constant until 12 months.

The thermal conductivity of a material is an important property that describes its ability to conduct heat. As the aggregates investigated are destined to be used in biocomposites for the insulation of buildings, a low thermal conductivity of the biocomposites is targeted.

For this study, the effect of the microbial development on the thermal properties of the aggregates, was carried out. Microbial growth in a porous material can have a significant effect on its thermal conductivity. (Laborel-Préneron et al., 2016) [21] mentioned that microorganisms can probably modify the physical properties of the material by creating pores, channels, and cavities, which reduce the density and effective thermal conductivity of the material. (Vasiliauskienė et al. 2023) [38] studied the impact of microorganisms on the performance of oil and Tung tree oil impregnated composites made of hemp shives and corn starch. After 6 months of incubation of the materials, their thermal conductivity had decreases on average 1.8 %, which is in line with our results.

On the other hand, microorganisms can also influence thermal conductivity through their metabolism. Most microorganisms produce heat when they metabolize nutrients in their environment [39], which can locally increase the thermal conductivity. However, (Delannoy, 2018) [5] noticed that the biofilm of bacteria present on the surface of hemp concrete had closed the porosity and consequently led to an increase in thermal conductivity. The fact that the thermal conductivity of the materials investigated decreased may mean that the types of microorganisms developed on these materials did not produce heat during their metabolism.

3.4.2. Mechanical strength, thermal conductivity, and bulk density of biocomposites

Fig. 17 shows the evolution of the mechanical strength (straight line on the left), the thermal conductivity (straight line on the right) and the bulk density (discontinued line), of the two studied biocomposites, as function of times of different period of the accelerated microbial ageing: 0, 3, 6, and 12 months. These parameters were measured after the samples were dried for 5–6 days at 60°C until mass stabilization in order to eliminate the effect of water content on the materials.

Fig. 17.a, and b show that the mechanical resistance (σ) of RL, and SL respectively in terms of times of microbial ageing: σ of RL decreased with a percentage of 11.27 % between 0 and 3 months. It maintained constant between 3 and 6 months and showed a decrease of 25.88 % at 12 months. For SL biocomposite, the strength decreased progressively between 0 and 12 months, with a percentage of 22.5 %. By comparing the behavior of the two materials, it appears that the decrease in mechanical strength was primarily attributed to biodeterioration, while not completely excluding changes in porosity associated with the carbonation phenomenon of the binder. The lime-based binder has a more healing effect due to its alkalinity [5]. It is worth mentioning that there is a distinct interface effect, albeit challenging to measure accurately. Furthermore, the roughness and absorption capacity differ among various organic aggregates, making it impractical to achieve a uniform interface.

(Herisson, 2013) [40] studied the process of biodeterioration of cementitious materials in sanitation structures, through a comparative study of calcium aluminate cement and Portland cement. This process led to loss of material properties, changed in its composition, or even a decrease in its mechanical characteristics. When the material will be used as a part of a structure, biodeterioration can present a risk to the structure's integrity. Concerning our materials, blocks made of bio-based materials will be used to cover the entire wall in a building to provide thermal insulation. Their use is non-structural. From a mechanical point of view, to let these materials usable, it must support its weight in a wall of 3 m height. The compressive resistance limit (σ_{lim}) was equal to 0.015 MPa for RL, and 0.014 MPa for SL, respectively. The lowest mechanical strengths were also achieved after 12 months of accelerated ageing. They were 0.355 MPa for RL and 0.243 MPa for SL. These values were significantly higher than the targeted resistance limits (σ_{lim}), both RL and SL materials were able to support their own weight.

(Rabii, 2016) [8] monitored the Young's modulus of the biocomposites in order to evaluate the effect of microbial growth. The 6-month fungal ageing did not seem to have affected the mechanical properties of the PE (the biocomposite non-reinforced by flax fibers), PE+11 %, and PE+29 % mass of flax composites. Only the PE+38 % mass of flax experienced a drop in its Young's modulus, attributed mainly to the humidity conditions rather than to microbial growth. Compared to the results at three months, where microbial colonization was more significant and the drop-in modulus was more pronounced, the development after six months can be associated with less microbial colonization than during the three-month tests. From this study, it can be noticed that the less in the mechanical properties of the material, may be due to the humidity conditions in a first step, and/or to the microbial ageing in a second step. Concerning the two materials studied in this article, the measurement of mechanical resistance was carried out, after drying the sample until mass stabilization. From that, we can conclude that the decrease of the mechanical resistance was due to the microbial development that ensure the degradation of the investigated materials, and not from the effect of the humidity.

On the other hand, different studies ((Grandclerc, 2017) [10], (Siddique et al., 2011) [41]) were carried out to evaluate the effect of microorganisms on the mechanical properties and they found that the development of microorganisms enhanced the mechanical properties of the materials. According to (Grandclerc, 2017) [10], microorganisms developed in the form of a biofilm, leading to higher resistance to certain particular conditions of the surrounding environment. Indeed, the different microorganisms will form a structured

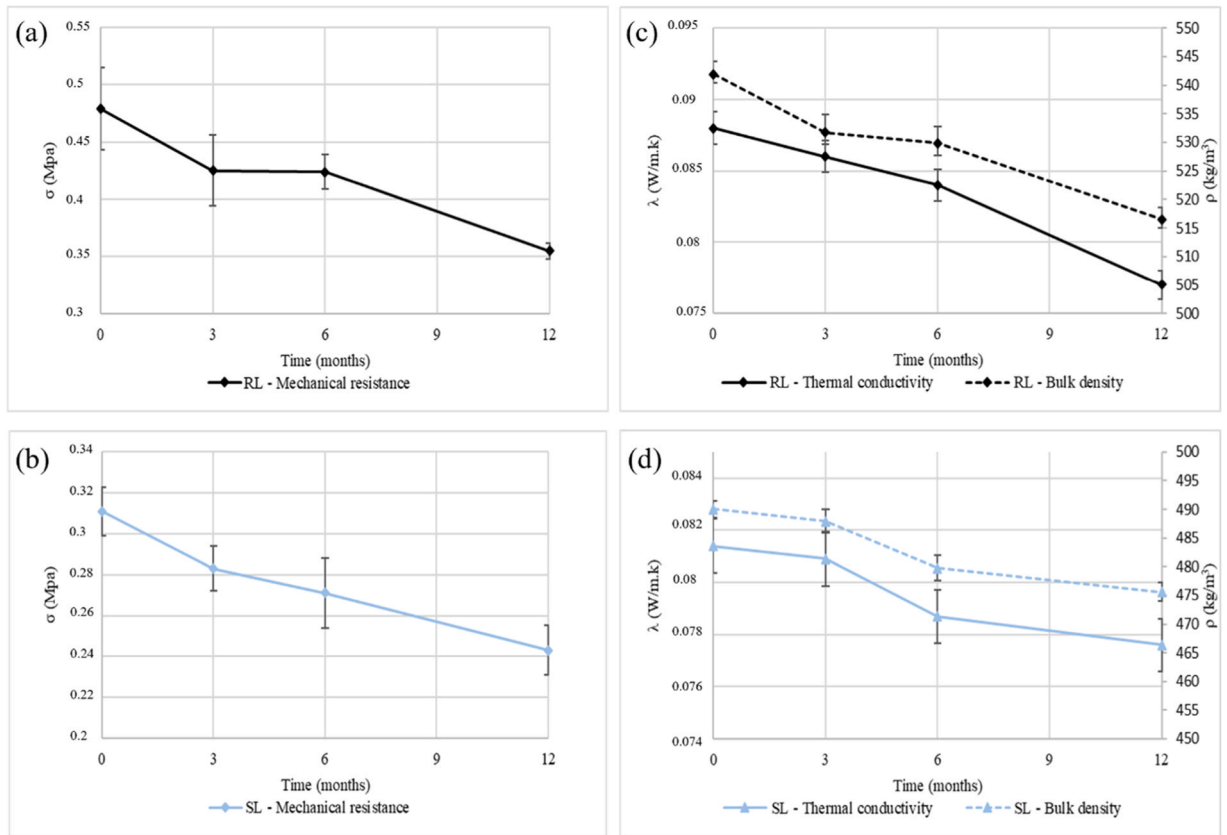


Fig. 17. Evolution of the mechanical resistance (mean \pm std, n=3) of (a) RL, and (b) SL and the thermal conductivity (mean \pm std, n=3) of (c) RL, and (d) SL as function of time of accelerated microbial development.

community of microbial cells enveloped in a self-produced polymeric matrix and adherent to each other to enhance the mechanical resistance of the material. (Siddique et al., 2011) [41] demonstrated that the incubation of anaerobic microorganisms of *Shewanella* species, increased the strength of the mortar. They reported that the strength had shown an increase of 25 % after 28 days of the addition of about 100,000 cells /mL of mixing water. For the case of our investigated materials, the development of microorganisms ensured the decrease of mechanical performance. These organisms didn't form structures of adherent biofilms, able to improve the mechanical strength. For RL and SL, a few microorganisms in the form of black dots were developed on the edges of the sample. This limited development, has not shown a significant loss in mechanical strength on RL (-25 %) and SL (-22.5 %) even after 12 months on conservation in accelerated microbial ageing, without exceeding a targeted resistance limit corresponding to the dead weight of the material used in a wall of 3 m height.

Fig. 17c and d show that the thermal conductivity (λ) of tested biocomposites gradually decreased from 0, 3, 6, and 12 months. λ decreased with a percentage of 12.5 % and 4.6 % for RL and SL respectively. In parallel, the bulk density (ρ) of the two biocomposites decreased with the similar trend of the thermal conductivity (λ). Between 0 and 12 months, ρ showed a decrease of 4.68 % for RL, and 2.93 % for SL. The same reasoning as the mechanical resistance, the thermal conductivity has decreased due to the phenomenon of biodeterioration inducing pores and cracks in the materials. These pores are susceptible to filling with air, which is a good thermal insulator. (Flemming, 1998) [42] studied the deterioration of polymeric materials by colonizing microorganisms on their surface. These microorganisms can cause direct and indirect damages to the material in different ways and can attack the material, leading to both embrittlement and loss of mechanical stability. Moreover, microbial filaments can penetrate in the polymer matrix, cause swelling that leads to cracks in the structure.

According to (Monroy, 2020) [43], who studied the potentiality of the development of microorganisms on biobased material destined for insulation uses, the thermal conductivity increased with the decrease of the relative humidity for the biobased materials (except for fiberglass insulation). In this case, there was also the effect of water content absorbed by the materials in high humidity environment, which is different in our work where we dried the samples until mass stabilization. The results of Delannoy [5] showed an increase of about 19 % of thermal conductivity for hemp concrete after exposure of 24 months to accelerated microbial ageing. He noticed also that the increase is only 7 % in the case of cement-base binders. This is in opposition to our results which showed a decrease of 12.5 % and 4.66 % in thermal conductivity at the dry state for RL and SL materials respectively.

4. Conclusion

The objective of this experimental study was to evaluate the effect of microbial development on the physical and mechanical properties of bio-based materials and to understand the relationship between microstructure and macroscopic behavior. Based on the various results and comparisons, the main conclusions are outlined below:

- Bulk density and thermal conductivity of the aggregates decreased, with the same trend, except for sunflower pith, during the first three months, and then remained constant until 12 months. This decrease is due to the phenomenon of biodegradation induced by microorganisms that have altered the properties of the materials.
- The mechanical strength of the two biocomposites studied, decreased progressively between 0 and 12 months, without exceeding a targeted resistance limit.
- As in the case of the aggregates, bulk density, and thermal conductivity of the two biocomposites decreased, with the same trend. The decrease in physical characteristics of these materials may be due to the degradation of the microstructure of the material by microorganisms.
- Microbial counting tests were manufactured using two culture medias, Yeast Extract Gel (YEG) specific for bacteria growth and Potato Extract (PE) specific for fungi growth. The results showed that the aggregates and the biocomposites were mainly contaminated by fungi, more than by bacteria. The three aggregates were contaminated by bacteria at 0 month before the exposition to microbial ageing, due to the storage conditions at the farmer's or their suppliers' premises. In order to preserve them from microbial attack during post-harvest storage, it is recommended to store them in a well-ventilated area, without exposing them to high humidity, which can promote the growth of microorganisms. In addition, the microorganisms continue to develop until 12 months except for sunflower pith. It is important to mention that under normal conditions and with correct installation, these materials do not have problems with the proliferation of microorganism. In fact, the mechanisms are long under real conditions. The aim of using accelerated conditions is to promote microbial development on the materials studied, in a relatively short time, in order to understand their behavior and their impact on the materiel's properties.
- Biocomposites, both RL biocomposite based on rapeseed straw and lime and SL biocomposite based on sunflower aggregates and lime, did not exhibit any microbial growth at 0 month, indicating that the presence of lime, derived from the binder, inhibited the growth of microorganisms immediately, due to its high alkalinity.
- Significant growth after 3 months for RL biocomposite based on rapeseed straw and lime, whereas SL biocomposite based on sunflower aggregates and lime, exhibited a delayed onset of microbial development, occurring after 6 months and persisting until the 12-month for both biocomposites.
- Cryo-observations confirmed and showed that the aggregates were affected by microorganisms prior to the exposure conditions with low abundance for sunflower pith. However, all materials showed growth of microorganisms after being exposed for six months to microbial ageing.

CRedit authorship contribution statement

Christian Défarge: Writing – review & editing. **Naima BELAYACHI:** Writing – review & editing, Validation, Supervision. **Mohamad EL HAJJAR:** Writing – original draft, Methodology, Investigation, Data curation. **Sylvain BOURGERIE:** Writing – review & editing, Validation, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mohamad EL HAJJAR reports statistical analysis and writing assistance were provided by University of Orléans. Naima Belayachi reports a relationship with Centre-Val de Loire Region that includes: board membership, funding grants, and travel reimbursement.

Data availability

Data will be made available on request.

Acknowledgements

The authors gratefully acknowledge the “REGION CENTRE VAL DE LOIRE” for the financial support of the research program MATBIO. The authors thank Virginie BAZIN for help in Cryo-HRSEM of the platform MACLE-CVL which was funded by the European Union and the Centre Val De Loire Region (FEDER). Thanks also to Dr. Manhattan LEBRUN for the statistical tests and to Dr. Fabienne BRULE-MORABITO for help in counting test.

References

- [1] T. Gurunathan, S. Mohanty, S.K. Nayak, et al., A review of the recent developments in biocomposites based on natural fibres and their application perspectives, *Compos. Part Appl. Sci. Manuf.* vol. 77 (2015) 1–25, <https://doi.org/10.1016/j.compositesa.2015.06.007>.
- [2] F. Bennai, Étude des mécanismes de transferts couplés de chaleur et d'humidité dans les matériaux poreux de construction en régime insaturé, Université Abderrahmane Mira-Bejaïa (Bejaïa, Algérie), 2017.
- [3] T.T. Nguyen, Contribution à l'étude de la formulation et du procédé de fabrication d'éléments de construction en béton de chanvre, 2010.
- [4] A. Limam, A. Zerizer, D. Quenard, H. Sallee, A. Chenak, et al., Experimental thermal characterization of bio-based materials (Aleppo Pine wood, cork and their composites) for building insulation (mars), *Energy Build.* vol. 116 (2016) 89–95, <https://doi.org/10.1016/j.enbuild.2016.01.007>.
- [5] G. Delannoy, Durabilité d'isolants à base de granulats végétaux, Université Paris-Est, 2018.
- [6] N. Belayachi, M. Bouasker, D. Hoxha, M. Al-Mukhtar, et al., Thermo-mechanical behaviour of an innovant straw lime composite for thermal insulation applications, août, *Appl. Mech. Mater.* vol. 390 (2013) 542–546, <https://doi.org/10.4028/www.scientific.net/AMM.390.542>.
- [7] B. Ismail, et al., Optimizing performance of insulation materials based on wheat straw, lime and gypsum plaster composites using natural additives, *Constr. Build. Mater.* vol. 254 (2020) 118959, <https://doi.org/10.1016/j.conbuildmat.2020.118959>.
- [8] H. Rabii, Étude du vieillissement de composites renforcés par des fibres naturelles: application bâtiment, Paris Est, 2016.
- [9] Z.C. Maundrill, et al., Moisture and fungal degradation in fibrous plaster, *Constr. Build. Mater.* vol. 369 (2023) 130604.
- [10] A. Grandclerc, Compréhension des mécanismes de biodétérioration des matériaux cimentaires dans les réseaux d'assainissement: étude expérimentale et modélisation, Université Paris-Est, 2017.
- [11] T. Verdier, M. Coutand, A. Bertron, C. Roques, et al., A review of indoor microbial activity across building materials and sampling and analysis methods, *Build. Environ.* vol. 80 (p. 136) (2014) 149, <https://doi.org/10.1016/j.buildenv.2014.05.030>.
- [12] A. Simons, Caractérisation et maîtrise de la prolifération microbienne dans des produits biosourcés pour des bâtiments sains et durables, Univ. é Paul. Sabatier-Toulouse III (2018).
- [13] M.G. Minerbe, et al., Impact d'un vieillissement fongique sur la durabilité de composites PE/ fibre de lin, *Acad. J. Civ. Eng.* vol. 34 (n° 1) (2016).
- [14] S.-T. Lekounoungou, Evaluation et Compréhension des Mécanismes fongiques impliqués dans la dégradation du bois, 2008.
- [15] D. Singh, J. Zeng, D.D. Laskar, L. Deobald, W.C. Hiscox, S. Chen, et al., Investigation of wheat straw biodegradation by *Phanerochaete chrysosporium* (mars), *Biomass Bioenergy* vol. 35 (n° 3) (2011) 1030–1040, <https://doi.org/10.1016/j.biombioe.2010.11.021>.
- [16] J. Čilerdžić, M. Stajić, J. Vukojević, et al., Degradation of wheat straw and oak sawdust by *Ganoderma applanatum*, *Int. Biodeterior. Biodegrad.* vol. 114 (2016) 39–44, <https://doi.org/10.1016/j.ibiod.2016.05.024>.
- [17] T. Warscheid, M. Oelting, W.E. Krumbein et al., Physico-chemical aspects of biodeterioration processes on rocks with special regard to organic pollutants, 1991.
- [18] S. Loutz et al., Salissures et altérations des matériaux pierreux par les micro-organismes, CSTC - Bruxelles, 2000.
- [19] A. Perrichet, Développement de microorganismes à la surface des bétons et enduits, *Mat.ériaux Et. Constr.* (1984) 173–177.
- [20] O. Guillitte, Bioreceptivity: a new concept for building ecology studies, *Sci. Total Environ.* (1995).
- [21] A. Laborel-Préneron, J.E. Aubert, C. Magniont, C. Tribout, A. Bertron, et al., Plant aggregates and fibers in earth construction materials: a review (mai), *Constr. Build. Mater.* vol. 111 (2016) 719–734, <https://doi.org/10.1016/j.conbuildmat.2016.02.119>.
- [22] Y. Brouard, N. Belayachi, D. Hoxha, N. Ranganathan, S. Méo, et al., Mechanical and hygrothermal behavior of clay – sunflower (*Helianthus annuus*) and rape straw (*Brassica napus*) plaster bio-composites for building insulation, févr. *Constr. Build. Mater.* vol. 161 (2018) 196–207, <https://doi.org/10.1016/j.conbuildmat.2017.11.140>.
- [23] M. Viel, 2018, Développement de composites bio-sourcés destinés à l'isolation des bâtiments1, Université Rennes.
- [24] K.F. Nielsen, G. Holm, L.P. Uttrup, P.A. Nielsen, et al., Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism, déc. *Int. Biodeterior. Biodegrad.* vol. 54 (n° 4) (2004) 325–336, <https://doi.org/10.1016/j.ibiod.2004.05.002>.
- [25] P. Krijghsheld, et al., Development in *Aspergillus* (mars), *Stud. Mycol.* vol. 74 (2013) 1–29, <https://doi.org/10.3114/sim0006>.
- [26] J.L.P. Santos, Estimation of growth parameters of six different fungal species for selection of strains to be used in challenge tests of bakery products, déc. *Food Biosci.* vol. 20 (2017) 62–66, <https://doi.org/10.1016/j.fbio.2017.08.006>.
- [27] L. Greenspan, Humidity fixed points of binary saturated aqueous solutions (janv), *J. Res. Natl. Bur. Stand. Sect. Phys. Chem.* vol. 81A (n° 1) (1977) 89, <https://doi.org/10.6028/jres.081A.011>.
- [28] B.D. Ripley, The R Project in Statistical Computing, févr, *MSOR Connect* vol. 1 (n° 1) (2001) 23–25, <https://doi.org/10.11120/msor.2001.01010023>.
- [29] R. Apkarian, Cryo-temperature stages in nanostructural research, *Scanning Microsc. Nanotechnol.* (2006).
- [30] C. Défarge, et al., Apports du cryo-microscopie électronique à balayage à émission de champ à l'étude des matières organiques et des relations organo-minérales naturelles. Application aux croûtes microbiotiques des sols, *Comptes Rendus Acad. émie Sci. -Ser. IIA-Earth Planet. Sci.* (1999) 7.
- [31] S. Amziane, F. Collet, M. Lawrence, C. Magniont, V. Picandet, M. Sonebi, et al., Recommendation of the RILEM TC 236-BBM: characterisation testing of hemp shiv to determine the initial water content, water absorption, dry density, particle size distribution and thermal conductivity (juin), *Mater. Struct.* vol. 50 (n° 3) (2017) 167, <https://doi.org/10.1617/s11527-017-1029-3>.
- [32] G. Mainelis, et al., Electrical charges on airborne microorganisms, *J. Aerosol Sci.* vol. 32 (n° 9) (sept. 2001) 1087–1110, [https://doi.org/10.1016/S0021-8502\(01\)00039-8](https://doi.org/10.1016/S0021-8502(01)00039-8).
- [33] H.C. Flemming, Relevance of biofilms for the biodeterioration of surfaces of polymeric materials (janv), *Polym. Degrad. Stab.* vol. 59 (n° 1-3) (1998) 309–315, [https://doi.org/10.1016/S0141-3910\(97\)00189-4](https://doi.org/10.1016/S0141-3910(97)00189-4).
- [34] K. Chaiche, M.K.M. Abdelmalek, M.H.-A. Baelhadj, M.K. Mokhtar et al., Caractérisation microbiologique des poussières sahariennes transportées en surface du sol du bas Sahara, 2018.
- [35] A. Dellal, A. Halitim, et al., Activités microbiologiques en conditions salines: cas de quelques sols salés de la région de Relizane (Algérie), *Cah. Agric. vol. 1* (n° 5) (1992) 335–340.
- [36] L. Xu, F. Zha, C. Liu, B. Kang, J. Liu, C. Yu, et al., Experimental Investigation on Carbonation Behavior in Lime-Stabilized Expansive Soil, févr. *Adv. Civ. Eng.* vol. 2020 (2020) 1–14, <https://doi.org/10.1155/2020/7865469>.
- [37] I. Saeed, et al., Assessment of mold and yeast in some bakery products of Lahore, Pakistan based on LM and SEM, févr, *Microsc. Res. Tech.* vol. 82 (n° 2) (2019) 85–91, <https://doi.org/10.1002/jemt.23103>.
- [38] D. Vasiliauskienė, G. Balčiūnas, R. Boris, A. Kairytė, J. Urbonavičius, et al., The impact of microorganisms on the performance of linseed oil and tung tree oil impregnated composites made of hemp shives and corn starch, févr. *Microorganisms* vol. 11 (n° 2) (2023) 477, <https://doi.org/10.3390/microorganisms11020477>.
- [39] N. Lago, J.L. Legido, M.I. Paz Andrade, I. Arias, L.M. Casás, et al., Microcalorimetric study on the growth and metabolism of *Pseudomonas aeruginosa*, août, *J. Therm. Anal. Calorim.* vol. 105 (n° 2) (2011) 651–655, <https://doi.org/10.1007/s10973-010-1175-0>.
- [40] J. Herisson, Biodétérioration des matériaux cimentaires dans les ouvrages d'assainissement – Etude comparative du ciment d'aluminate de calcium et du ciment Portland, Université Paris Est, 2013.
- [41] R. Siddique, et al., Effect of ureolytic bacteria on concrete properties, *Constr. Build. Mater.* vol. 25 (n° 10) (2011) 3791–3801, <https://doi.org/10.1016/j.conbuildmat.2011.04.010>.
- [42] H.C. Flemming, Relevance of biofilms for the biodeterioration of surfaces of polymeric materials, *Polym. Degrad. Stab.* (1998) 309–315.
- [43] A.M.T. Monroy, Etude de la potentialité du développement de micro-organismes sur des matériaux d'isolation bio-sourcés et conventionnels utilisés dans la rénovation de batiments: impacts sur la qualité de l'air intérieur, *Éc. Natl. supérieure Mines-TéléCom. Atl.* (2020).