JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2021, 22(4), 296–313
https://doi.org/10.12911/22998993/134033
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Received: 2021.01.16
Accepted: 2021.02.24
Published: 2021.03.27

Microbiological Stability of Bio-Based Building Materials

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ABSTRACT

The aim of this paper was to study the microbiological stability of bio-based composite building materials, which are made using organic-rich lake sediments (further – sapropel) with lime and magnesium cement as binders and hemp shives as filler. The microbial stability properties of the obtained composite materials were investigated and compared to similar composites. Because of their high organic content, these materials are prone to biodegradation; therefore, they were coated with ALINA LIFE TM organoclay coating, which helps to extend the product life, reducing the rate of biodegradation compared to the biocides used in industry. The effect of the coating on the resistance to decay by the *Aspergillus versicolor*, *Penicillium chrysogenum*, *Alternaria alternata*, *Cladosporium herbarum*, *Chaetomium* sp. and *Trichoderma asperellum* fungi was investigated under different conditions: relative humidity modes of 75% and 99% at 20°C for 45 days and four months. The results indicated that the composites made of sapropel and lime have similar microbial stability properties as lime and magnesium cement binder composite materials. ALINA LIFE TM organoclay coating showed advanced resistance to biodegradation; sapropel-lime composites have shown several good properties that make them suitable to be considered for use in the construction material industry.

Keywords: sapropel; lime-hemp materials; magnesium cement; microbial stability; biocides; organoclay additive

INTRODUCTION

There is a growing need to replace synthetic and fossil (mostly oil- and coal-based) materials with natural ones (Fava et al., 2015) to reduce the impact of exhaustion of non-renewable resources and the adverse influence of the greenhouse gas emissions. Considering the requirements for the application of materials in different industries, composite materials are especially valuable, combining different properties such as high compressive strength, flexibility and low weight. In order to advance the use of natural materials, the properties of these composites should not be inferior to synthetic ones. One of the approaches is to develop the natural material composites that combine binding and structure-forming properties in one product. Organogenic lake sediments (sapropel) are a good natural binder that can replace synthetic binders; sapropel is abundant in lakes in the northern hemisphere, being a waste material after the lake recultivation process.

Sapropel is a partially fine-grained renewable resource: it is an organic-rich lake sediment or sedimentary rock, and it appears in lacustrine environments of inland waters (Kurzo et al., 2004). With its high content of organic matter, sapropel can be used in agriculture and horticulture as a soil fertiliser and soil amendment (i.e. binder for granules). It also has the potential to be used in construction materials as a binder or adhesive substance (Balčiūnas et al., 2016; Obuka, et al., 2015). It is a valuable and available resource of natural origin, and it is estimated that in Latvia the reserves of sapropel in inland waters are around 700–800 million m^3; furthermore 1.5 billion m^3 underlie the peat layer – in total, over 2 billion m^3. Sapropel itself has a high ability to bind, as well as shape-holding capacity, adhesive properties and plasticity (Obuka et al., 2015). Therefore, in this research, it was used as a binder, as has been demonstrated in previous studies (Obuka et al., 2015, Obuka et al., 2017; Balčiūnas et al., 2016b). As its use as a binder does not require thermal processing, it has low embodied energy and low CO_2 emissions, making it very appropriate for use as an ecological insulation material with low environmental impact.

Different natural fibres can be used as structure-forming materials. In this research, hemp shives were used as filler materials in the development of composites. These filler materials are agricultural by-products that need to be recycled. One of the materials that incorporate hemp shives is hemp-lime self-bearing insulation material. It has a positive environmental impact that is directly related to the CO_2 emissions, as both components of the composite have sequestered CO_2: lime during hardening (carbonation) and hemp during growth (Shea et al., 2012). Sapropel, in its formation, has also absorbed CO_2 in the form of organic substances, thus being similar to hemp-lime materials.

In previous studies, sapropel composite materials with various fillers (e.g. wood fibre, hemp shives, or wood sanding dust) have shown the compressive strength from 0.15 to 0.73 MPa, with a density ranging from 200 to 600 kg/m^3, and thermal conductivity from 0.063 to 0.080 W/m×K. These properties are similar to other bio-based building materials, such as hemp-lime concrete (Sinka et al., 2015) or magnesium-hemp concrete (Sinka et al., 2018), thus proving the potential of sapropel composite as a self-bearing insulation material used in construction.

One of the major problems in relation to the development of bio-based building materials is their microbial stability with respect to fungi and other microorganisms. The main cause of microbial growth is excess moisture and dampness from inadequate ventilation or leaking roofs; the concerns regarding this growth are worsening appearance (which leads to material replacement or reinstatement) and loss of mechanical properties, as well as sick building syndrome, which is caused by spores and metabolites of microorganisms and seriously impacts human health. These problems show that the reasons for protection are not simply economical but related to the health issues of the environment at home and in the workplace (Falkiewicz-Dulik et al., 2015; Klamer et al., 2004).

In a study that investigated the biodegradability of sapropel composite materials depending on the filler used, it was concluded that sapropel composites are biodegradable at the end of their life cycle (Obuka et al., 2019). This biodegradability leads to concerns about the microbiological stability of sapropel composites when they are used as construction materials in buildings. Thus, in this study the microbiological resistance of these materials was tested under different humidity and temperature conditions as well as compared to other bio-based building materials: commercially available (such as wood fibre cement boards and hemp-lime composites), experimental (magnesium-hemp composites) and filler only (hemp and flax shives).

Producing the materials with microbiological resistance would be economically beneficial because improving indoor environments could lower the health care costs and increase the worker productivity, resulting in an estimated savings of \$30 billion to \$150 billion annually (Dean & Doris, 2014). It is safe to say that this field of research is important, and the benefits are not only more durable materials, but human health in general.

The indoor concentration of microorganisms depends on various factors, such as the number of bacteria and fungi in the air, ventilation systems (natural, mechanical or mixed), building conservation conditions and indoor climate. The main factor influencing the growth of moulds is the moisture level; it is also influenced by temperature and the properties of the building materials. The optimal relative humidity for human health is 40–60%, at room temperature. The critical level for fungal growth was to be above 75% moisture. Excessive indoor humidity can be caused by such factors as natural disasters, construction failures, improper maintenance of buildings, as well as the initial moisture levels in the materials used in building construction (Apine et al., 2015).

One of the ways to improve the microbiological resistance is to use biocides; in this study, two different biocides were used as additives to composite material mass. The first was ACTICIDE FD (THOR Ltd.), which contains a combination of active substances CIT (chloromethylisothiazolinone, also known as 5-chloro-2-methyl-2H-isothiazol-3-one) and MIT (methylisothiazolinone also 2-methyl-2H-isothiazol-3-one) and is effective against *Aspergillus* spp., *Cladosporium* spp., *Penicillium funiculosum* and others. The second was BACTERICIDE (Elvi Ltd.), containing quaternary ammonium compounds as active substances. However, it must be noted that, according to the EU legislation, the use of active substances in biocides must be lowered several times; as of 1st of May 2020, MIT is classified as allergenic from concentrations as low as 0.0015% (15 ppm), according to the European commission's 13th ATP to CLP (*Commision Regulation (EU) 2018/1480*, 2018).

Biocide leaching from bio-based materials can be considered a problem, as it can lead to decreased efficacy of microbial protection and causes environmental pollution (Paijens et al., 2020). Therefore, it is important to consider and also to test alternatives to traditional biocides that are available in the industry. The chosen alternative to traditional biocides is organoclay technology, which is novel, environmentally friendly, non-leaching and easy to use. As an alternative, montmorillonite mineral material can be used: it is specifically surface treated to change a hydrophilic surface into a hydrophobic, as a result changing the structure and functionality of the mineral. The organoclays are a new type of additive for finishing materials and paints that prevent the negative effects of the external environment on materials, thereby prolonging their longevity and reducing the use of biocides to protect materials (Kostjukova et al., 2017). Organoclay additive material was used as an alternative to composite material mass protection for the sapropel composites and other bio-based building materials in this research.

MATERIALS AND METHODS

Materials

Sapropel

The sapropel sediments were sampled from Lake Pikstere, located in Jekabpils District, Selonia, Latvia. The properties of the sapropel used have been determined in previous research using various methods (Obuka et al., 2017). The sapropel samples differ from each other in terms of organic matter, moisture and carbonate, as well as the ash content. The properties of the sapropel that was used are the following: sapropel type – green algae; moisture – 96.45%; organic matter content in dry matter – 82.67%; ash content of dry matter – 17.33%; pH (water extract) – 6.89; electric conductivity – 124.75 mS/cm^2; non-H density – 1.028 g/m^3; H density – 1.069 g/m^3; and colour – greenish brown.

Mineral binders

In this research, four different binders were used – formulated hydraulic lime, hydraulic lime, magnesium phosphate and magnesium oxychloride cement. The sample designation can be seen in Table 2. Formulated hydraulic lime (FHL) binder consists of 60% hydrated lime CL90 (produced by Lhoist Poland Ltd.) and 40% metakaolin that was a by-product of porous glass production (by Stiklaporas Ltd. in Lithuania). The hydraulic lime (HL) binder used in hemp-lime construction in Latvia consists of 70% hydrated lime, 20% hydraulic lime and 10% additives. Hydraulic lime is used as a stand-alone binder and as a binder with sapropel, at a ratio by mass of 1:1.

Magnesium phosphate cement (MPC) consists of 55% (by mass) dead-burned magnesium oxide M-76 burned at 1700 ºC (Integra Ltd, Slovakia) and 45% mono-potassium phosphate 0–52–35 (N-P-K proportion) with P_2O_5 content at least 52.1% (Praton SA Ltd). Magnesium oxychloride cement (MOC) consists of caustic magnesium oxide CCM RKMH-F burned at 800 ºC (RHI AG Ltd, Austria) and magnesium chloride hexahydrate $MgCl_2 \cdot 6H_2O$ (Germany) dissolved in water (brine 1:1).

Table 1. Designation of materials and methods used

Designation	
Sample types	
Sapropel-lime-hemp composite	SLHC
Magnesium oxychloride cement	MOC
Formulated hydraulic lime	FHL
Hydraulic lime	HL
Magnesium phosphate cement	MPC
Hemp shives	HS
Flax shives	FS
Wood wool	WW
Wood fibre cement board	WC
Sample designations	
ALINA additive	A
Biocide additive	B
Fungi mixture	F
Control without additive	K

Sample preparation

Mixtures of the samples can be seen in Table 2. Sample preparation and the properties of hemp shives are described in previous research (Obuka et al., 2017); only the sample dimensions differ. For the first stage of the experiment, the samples were prepared in 70×70×70 mm cube moulds, wood wool (WW) was cut with a similar surface area. For the second stage, the samples were prepared in 40 mm diameter and 10 mm high cylindrical forms, with wood fibre cement board (WF) and WW cut with similar surface area; for the second stage, additional samples with lowered mineral binder amount were also produced, using 50% and 20% of the binder amount of the first stage with the same amount of shives, producing the samples with less binder coverage and microbiological protection.

Microbiological stability tests

Two experiments were conducted to determine the microbiological stability. In both experiment stages, the material samples were artificially inoculated with six fungal strains:

1. *Aspergillus versicolor* MSCL 1346;
2. *Penicillium chrysogenum* MSCL 281;
3. *Alternaria alternata* MSCL 280;
4. *Cladosporium herbarum* MSCL 258;
5. *Chaetomium* sp. MSCL 851;
6. *Trichoderma asperellum* MSCL 309.

Aspergillus versicolor and *Penicillium chrysogenum* belong to primary colonisers, *Alternaria alternata* and *Cladosporium herbarum* to secondary, and *Chaetomium* sp. and *Trichoderma asperellum* to tertiary colonisers. Primary colonisers can develop at a relative humidity <80%, secondary at 80–90%, and tertiary at a relative humidity >90% (Stefanowski et al., 2017).

For the experiments, the fungi were grown in Petri dishes, and a suspension of mycelial fragments and spores was prepared from each fungus in sterile distilled water (autoclaved at 121 ºC for 15 min) to obtain OD_{545} 0.16. All six fungal suspensions were mixed in equal amounts. The analysed samples of materials were inoculated with 3 ml or 5 ml of the mixed fungal suspension (depending on the size of the sample).

When the fungal growth was observed, the fungi were identified by macroscopic and microscopic (Leica DM 2000, Leica Microsystems) features, at least to the generic level. The intensity of fungal growth was assessed based on the scale (shown in Table 3) according to the ASTM C1338–96 *Standard test method for determining the fungi resistance of insulation materials and facings* (Klamer et al., 2004).

After visual evaluation, 1.0 ± 0.5 g material samples were removed from the composite material where fungal overgrowth was observed. The samples were divided into smaller fractions with a knife, scissors or tweezers, and placed in plastic tubes. Afterwards, they were poured into 1 ml of sterile distilled water (autoclaved at 121 ºC for 15 min), then shaken vigorously for 5 min. The sample suspension (0.1 mL) was then plated on Malt Extract Agar medium (Oxoid) and the samples incubated at room temperature (20 ± 2 °C) for 5–7 days. The fungal genera were determined with microscopic and macroscopic methods (Klamer et al., 2004).

Incubation conditions

In the first stage of the experiment, the analysed material samples were incubated in two relative humidity modes –75% and 99% RH – at a temperature of 20 °C: 75% RH was found to be the typical operating conditions of bio-based building materials measured during the in-situ tests (Sinka et al., 2018), while 99% RH represents elevated moisture that can occur during drying or improper building

Table 2. Mixtures of the samples

Type	Hemp shives	Water for shives	Sapropel	Hydraulic lime binder	Formulated hydraulic lime binder	Magnesium phosphate cement	Magnesium oxide	$MgCl_2$ brine 1:1	Water for binder
SLHC	1	-	2.5	2.5	-	-	-	-	-
MOC	1	1.25	-	-	-	-	2	1.33	-
FHL	1	1.25	-	-	2.5	-	-	-	1.25
HL	1	1.25	-	2.5	-	-	-	-	1.25
MPC	1	1.25	-	-	-	2.7	-	-	0.90

Table 3. Evaluation of fungal growth on materials (average values)

Evaluation/ Intensity of growth	Characteristics	Colour scale
0	No growth detected microscopically	🟥
1	Microscopically detected growth	🟥
2	Microscopically detected growth covering the whole surface	🟥
3	Macroscopic (visible to naked eye) growth present	🟥
4	Macroscopic growth covering >80% surface	🟥

maintenance. The moisture levels and temperature were monitored with digital sensors. In order to ensure 75% RH, a sodium chloride salt solution was kept in the chamber with the samples. In turn, to ensure 99% RH, the samples were kept in separate sealed boxes with sensors inside, and 3 ml of sterile water was poured on the samples when a decrease in RH level was detected. The visual evaluation of composite materials was performed after 4 months of incubation.

In the second stage of the experiment, the samples were kept only at an RH of 99% and a temperature of 20 ± 2°C; the visual assessment was performed after 45 days. In order to ensure 99% RH, the samples were poured with 3 ml of sterile water twice a week, thus maintaining a humid environment simulating rain condition.

Additional antimicrobial protection

For the first stage of the experiment, ACTICIDE FD – a combination of CIT and MIT – was used for additional biological protection tests. It was added to the composite materials in the amount of 1% of the total mass. It is effective against *Aspergillus* spp., *Cladosporium* spp., *Penicillium funiculosum* and others (Thor, 2020).

In the second stage of the experiment, the BACTERICIDE biocide was used as an active substance containing quaternary ammonium compounds. It was added to the composite materials in the amount of 1% of the total mass.

In both the experiments, the *ALINA Ltd.* product ALINA LIFE TM organoclay coating was used for additional biological protection. It was added to the composite materials in the amount of 4% of the total mass.

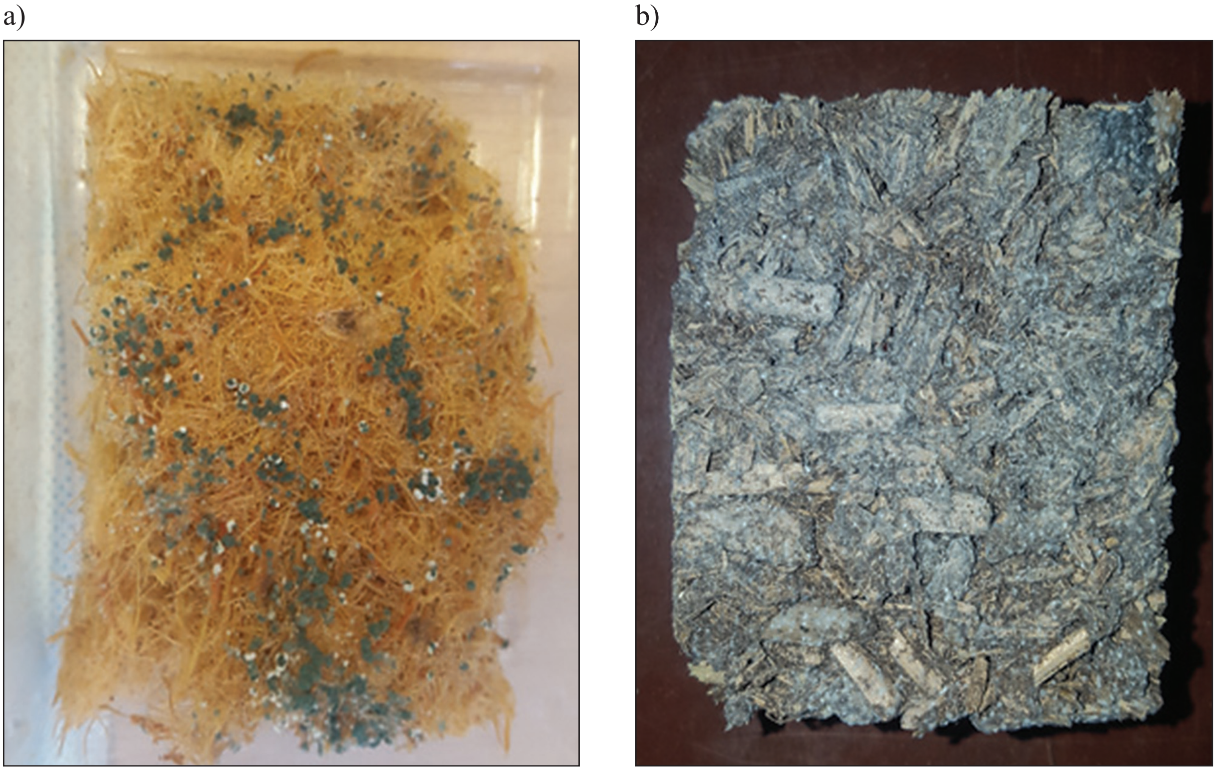

Fig.1. Control samples of wood wool covered with *Trichoderma* (A) and MPC composite covered with *Penicillium* and *Aspergillus* (B)

RESULTS

The first stage of the experiment

Out of the 75% RH material samples, only MPC and WW showed an overgrowth (Figure 1); no fungal overgrowth was observed on other samples. Under such humidity conditions, only fungi that are in the category of primary colonisers can grow and they have low activity, which results in small overgrowth.

At 99% RH, MPC showed intensity of growth level 4, wood wool showed intensity of growth level 3, and both showed macroscopic fouling with fungi (Fig. 1); the remaining specimens showed intensity of growth level 1 or an increase in microscopically detected fungi. The low microbiological stability of wood wool can be explained by a low pH level of 4.28. Although magnesium phosphate cement has a high pH level of 10.45 that develops with time as the cement hardens (Jia et al., 2019), it has low microbiological stability, which is related to the monopotassium phosphate that is used as a hardener for the binder. The monopotassium phosphate water solution has a pH lower than 7 and can also be used as a concentrated mineral fertiliser (Hegedűs et al., 2017; Shen et al., 2017); thus, the undissolved part of the hardener can serve as a nutrient for fungal growth.

The fungal species found in the samples of the materials are summarised in Table 4, where it can be seen that the main fungi that developed in the samples were those that were inoculated with the suspension, but others – such as *Verticillium*, *Simplicillium*, *Mucor* and *Aspergillus niger* – were also found.

As the first stage of the experiment did not show enough fungal growth to be able to fully compare the different materials, it was necessary to perform the second stage of the experiment. In the first stage, humidity was increased only when a decrease in air RH was detected in the samples with humidity sensors. Although microscopic fungal growth was observed in most of the samples, it can be concluded that such humidity conditions were not sufficient to produce the fungal growth large enough to be compared by visual inspection. Therefore, in the second round of experiments, the control of humidity conditions was significantly increased by adding 3 ml of sterile water twice a week, and the binder amounts for mineral binders were decreased to 50% and 20% of those used in the first stage.

The second stage of the experiment

From analysing the changes in the microbiological stability of composites depending on the concentration of the binder (Fig. 2), it can be concluded that a decrease in the amount of mineral binder decreased the microbiological stability. For MOC, FHL, and HL biocomposites at 100% concentration, the fouling assessment was 0–1.5, at 50% concentration it was 1–3, and at 20% it was 2.5–4 (Fig. 2).

The decrease in microbiological stability correlated with the lowering of pH in the specimens. The lime-based binder biocomposites (FHL and HL) showed higher microbiological stability than the MOC biocomposites, since on the 100% binder specimens fouling was not observed, while on MOC the fouling corresponded to the levels 1–2. At 50% and 20% specimens, this difference disappeared. This can be attributed to the pH level, which for the lime-based specimens was around 12 at 100% but for the MOC was 9.76, while the reduction of binder in 50% and 20% specimens

Table 4. Detected fungi at the first stage of the experiment

Type	Inoc.	C	A	B	pH
MOC	F	*Penicillium, Aspergillus, Cladosporium herbarum*	*Penicillium, Aspergillus*	0	9.76
	K	*Aspergillus, Scopulariopsis*	*Chaetomium*	0	
FHL	F	0	*Simplicillium*	*Verticillium*	11.99
	K	0	0	0	
MPC	F	*Aspergillus, Penicillium*	-	-	10.45
	K	0	-	-	
WW	F	*Trichoderma*	-	-	4.28
	K	*Aspergillus niger, Trichoderma*	-	-	
SLHC	F	*Aspergillus*	*Scopulariopsis*	0	12.18
	K	*Aspergillus, Chaetomium*	0	0	12.16

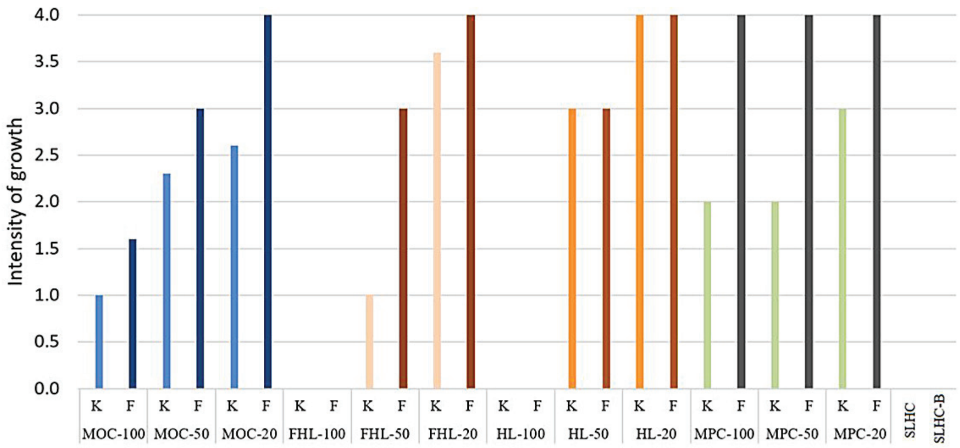

Fig. 2. The second stage of the experiment*:* fouling depending on binder.

resulted in a similar pH and microbiological stability (Table 5).

The decrease in microbiological stability in the MPC biocomposites was less pronounced, as the intensity of growth at 100% concentration was 2–4, while at 20% it was 3–4. Such an increased intensity of growth in MPC was similar to the results of the first stage of the experiment and can be explained by the impact of the hardener, potassium phosphate. Although the growth was found on local spots, it was evaluated as level 4; this can be seen as a drawback of the visual assessment method and scale used as, for example, there was no distinction between the HL-20 (Fig. 5) and MPC-20 samples (Fig. 6).

A comparison of the control (K) and fungal inoculated (F) samples (Figure 2) showed that the artificially inoculated samples had an increase of between 20% and 50%. Table 5 shows the diversity of fungal colonies in the samples, as determined by microscopic examination of the fungi. It can be seen from the table that the fungi found in the inoculated samples, mainly *Aspergillus versicolor*, *Penicillium chrysogenum* and *Cladosporium herbarum*, were almost absent in the control samples.

The comparison of the control samples and the samples with improved microbial stability by organoclay additive or biocide coating (Table 5) showed that both types of coating generally improved the microbial stability. The organoclay-added samples showed 13.8% lower overgrowth and those with the biocide product had 9.1% lower. However, this effect was not the same for all formulations. The organoclay additive in HL binder showed no improvement; neither did biocide for the MOC and FHL binders.

The microbiological stability of the biocomposite aggregate hemp shives (HS) was low – the intensity of fungal growth was 3.2–4 (Fig. 3). Some literature sources tend to attribute the antibacterial properties to the hemp shives (Ali, Almagboul, Khogali, & Gergeir, 2012), but the experiments showed fungi fouling on them. However, when compared with the aggregate of similar origin, flax shives (FS), it can be observed that the flax shives were completely covered with fouling (Fig. 3) and fungal growth started much earlier than for the hemp shives. Thus, hemp shives have somewhat better microbiological resistance than flax shives, but with the methods used in this research, they cannot be distinguished as both had macroscopic growth covering >80% of the surface and no evaluation according to the speed of fungal growth has been made, which limits a full interpretation of the research results.

The microbial resistance (Fig. 3) of the reference building materials – wood wool (WW) and wood fibreboard (WF) – was also experimentally tested. The fastest growth of *Trichoderma* on wood wool was due to the low pH 3.63 (Table 4), similar to the first stage of the experiment (Fig. 1). However, the WF samples showed very high microbial resistance and a high pH of 11.8. Only a small amount of *Paecilomyces* was detected in most samples (Table 5).

In the second stage of the experiment, it was discovered that the fungi belonging to the species of *Paecilomyces* and *Stachybotrys* were the most common on the materials included in the research. In some cases, *Penicillium, Acremonium, Cladosporium, Aspergillus, Trichoderma* and *Mucor* were also observed, indicating that the substrates

Fig. 3. Materials after microbiological stability tests: A) hemp shives; B) flax shives, C) wood wool; D) wood fibre cement board.

contained sufficient amounts of moisture and nutrients for the fungal development. Most of these fungi feed by cellulose; therefore, they can be found on cellulose-based materials (Bech-Andersen, 2004; Klamer et al., 2004).

Stachybotrys also feeds by lignin and for this reason, it is often found on wood and its products (Vance et al., 2016), and it is also known as black mould (Ding et al., 2018). Since hemp shives contain high levels of lignin and cellulose, this type of fungi can be found on a large number of specimens (Fig. 4–7). The mycotoxins produced by these fungi cause allergic reactions, and they are often associated with various health problems caused by inappropriate indoor microclimate (Hossain et al., 2004).

The environmental reaction (pH) plays an important role in the spread of fungi and bacteria in building materials and was measured in both the first and second stages of the experiment. The composite materials with pH levels up to 8 are more susceptible to colonisation by microorganisms than alkaline cement materials, which have a pH of about 12–14 and are therefore relatively insensitive to colonisation in the early state of the composite. However, over time, the carbonation process lowers the pH of cementitious alkaline materials to about 9, allowing the microorganisms to develop on the materials. Some

Table 5. Assessment of fungal colonies growth in the second stage of experiment

Type	Inoc.	C	A	B	pH
MOC-100	K	1	0	2	9.76
	F	1.6	1	1	
MOC-50	K	2,3	1	1	9.55
	F	3	1	3	
MOC-20	K	2.6	2.3	3	9.55
	F	4	3	4	
FHL-100	K	0	0	0	11.99
	F	0	0	0	
FHL-50	K	1	0	1	9.24
	F	3	3	3	
FHL-20	K	3.6	1	3	9.17
	F	4	4	4	
HL-100	K	0	0	0	12.40
	F	0	0	0	
HL-50	K	3	3	0	8.68
	F	3	3	1.5	
HL-20	K	4	4	4	8.61
	F	4	4	3	
MPC-100	K	2	2	2	10.49
	F	4	4	3	
MPC-50	K	2	2	3.5	10.47
	F	4	3	4	
MPC-20	K	3	3	3	10.32
	F	4	1	1	
HS	K	3.8	4	1	8.50
	F	4	4	4	
FS	K	4			7.25
	F	4			
WW	K	3			3.63
	F	3			
WF	K	1			11.80
	F	0			
SLHC	K	0	0	0	11.99
	F	0	0	0	
SLHC – B	K	0	0	0	12.1
	F	0	0	0	

Fig. 4. Magnesium oxychloride biocomposites with varying binder amount: A) MOC-100, B) MOC-50, C) MOC-20

studies have examined the accelerated carbonate contamination of mortars and show that their bioavailability is significantly increased. Thus, such composites can be a significant source of indoor air pollution (Verdier et al., 2014).

DISCUSSION

Composite materials are especially valuable, as they reflect the requirements for the application of materials in different industries, by combining

Fig. 5. Hydraulic lime biocomposites with varying binder amount: A) HL-100; B) HL-50; C) HL-20

various properties such as carbon neutrality and renewable raw material source. However, they need to gain microbial resistance and suitable strength properties to further develop their uses in the market. In order to develop the use of natural materials, the properties of these composites

Fig. 6. Magnesium phosphate biocomposites with varying binder amount: A) MPC-100; B) MPC-50; C) MPC-20

should not be inferior to the artificial ones. This study, which is a continuation of previous studies on the microbiological stability of materials, uses a new binder – organogenic lake sediments (sapropel) – that shows its effectiveness in comparison with the building materials currently used in the industry. Sapropel is a good natural binder that can replace synthetic and mineral binders and is a waste material after the lake recultivation process that should be reused in high value-added products. With its high substance of organic matter, it can be used in construction materials as a binder or adhesive substance (Balčiūnas et al., 2016; Obuka et al., 2015). Sapropel is a valuable and available resource of natural origin.

The same humidity conditions, i.e. 99% RH, were used in both test stages, but they were created in different ways. In the first stage, the humidity level was monitored by sensors and, when it dropped below 99%, it was manually increased

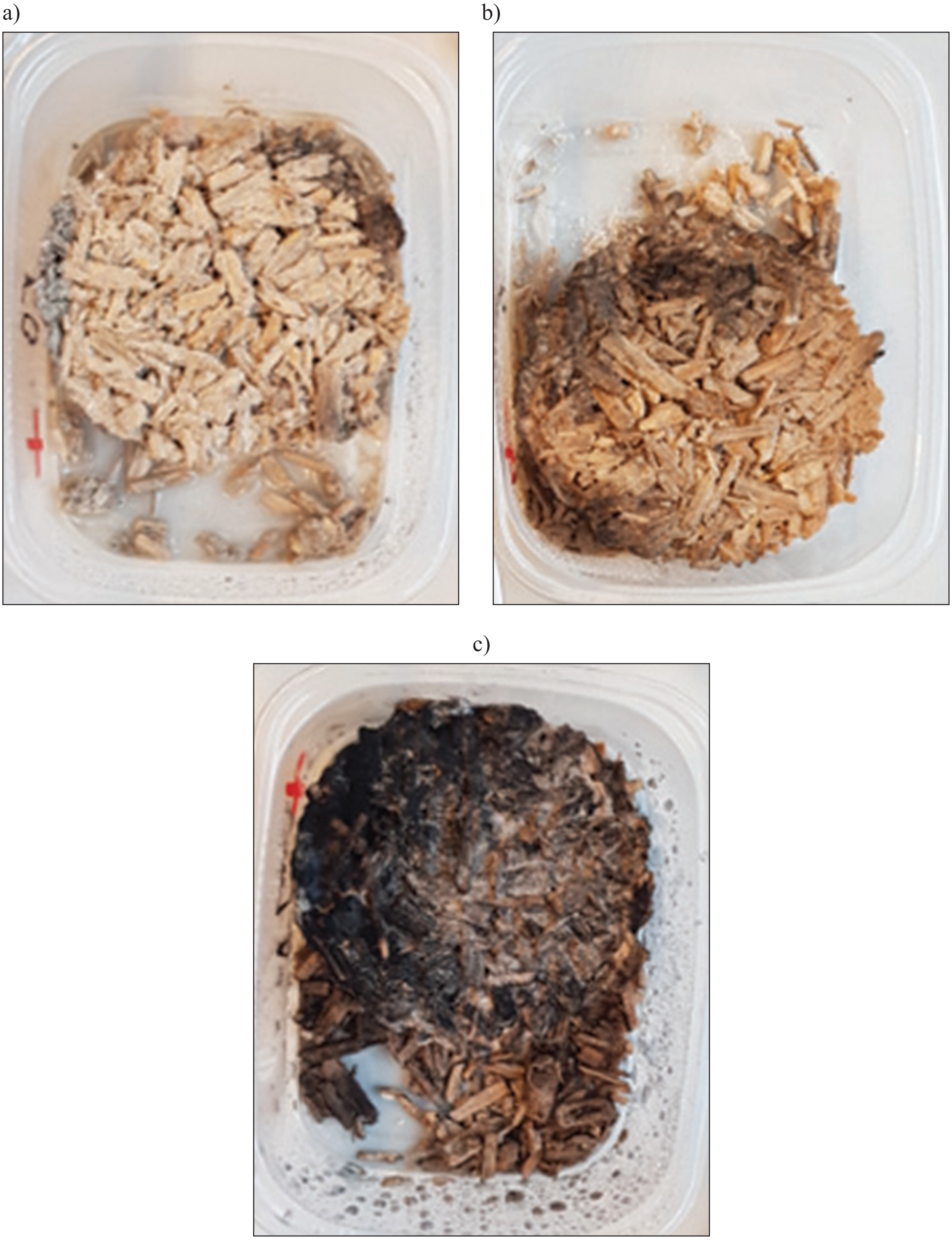

Fig. 7. Formulated hydraulic lime biocomposites with varying binder amount: A) FHL-100; B) FHL-50; C) FHL-20

by adding 3 ml of water to the samples. In the second stage, the humidity level was maintained by sprinkling the samples twice a week with 3 ml of sterile water, so that the samples were significantly wetter in this part of the test. Although microscopic fungal growth was observed in most of the samples of the first stage, it can be concluded that such humidity conditions were not sufficient to produce the fungal growth large enough to be compared by visual inspection. On

Table 6. Detected fungi and other organisms on samples

Type	Inoc.	C	A	B
MOC-100	K	*Paecilomyces*	*0*	*Paecilomyces*
	F	*Paecilomyces*	*Paecilomyces*	*Paecilomyces*
MOC-50	K	*Paecilomyces*	*Paecilomyces*	*Paecilomyces*
	F	*Paecilomyces, Scopulariopsis*	*Paecilomyces*	*Paecilomyces, Aspergillus, Cladosporium*
MOC-20	K	*Paecilomyces*	*Paecilomyces*	*Paecilomyces*
	F	*Cladosporium, Paecilomyces, Scopulariopsis*	*Aspergillus, Paecilomyces, Scopulariopsis*	*Paecilomyces, Scopulariopsis*
FHL-100	K	0	0	0
	F	0	0	0
FHL-50	K	0	0	0
	F	*Scopulariopsis, Cladosporium, Aspergillus, Paecilomyces*	0	*Scopulariopsis*
FHL-20	K	*Acremonium, Paecilomyces*	*Paecilomyces*	*Scopulariopsis, Paecilomyces*
	F	*Cladosporium, Scopulariopsis*	*Paecilomyces, Scopulariopsis, Stachybotrys*	*Paecilomyces, Scopulariopsis, Cladosporium*
HL-100	K	*0*	0	0
	F	*0*	0	0
HL-50	K	*0*	0	0
	F	*Aspergillus, Cladosporium, Paecilomyces*	*Paecilomyces, Chaetomium*	*Paecilomyces*
HL-20	K	*Scopulariopsis, Paecilomyces*	*Paecilomyces*	*Paecilomyces*
	F	*Paecilomyces, Chaetomium, Penicillium, Trichoderma, Cladosporium, Coprinus comatus, Scopulariopsis, Stachybotrys*	*Paecilomyces, Coprinus comatus, Scopulariopsis*	*Paecilomyces, Scopulariopsis*
MPC-100	K	*Scopulariopsis*	*Paecilomyces*	*Paecilomyces, Scopulariopsis*
	F	*Paecilomyces, Scopulariopsis,* Actinobacteria, *Readeriella*	*Paecilomyces, Scopulariopsis*	*Paecilomyces*
MPC-50	K	*Paecilomyces, Scopulariopsis*	*Paecilomyces*	*Paecilomyces*
	F	*Readeriella, Paecilomyces, Scopulariopsis*	*Readeriella, Paecilomyces*	*Readeriella, Paecilomyces*
MPC-20	K	*Paecilomyces, Scopulariopsis*	*Paecilomyces*	*Paecilomyces*
	F	*Paecilomyces, Scopulariopsis, nematodes, Readeriella*	*nematodes, Paecilomyces, Mucor, Trichoderma*	*Paecilomyces*
HS	K	*Coprinus comatus, Paecilomyces, Geotrichum*	0	*Chaetomium*
	F	*Mucor, Cladosporium, Chaetomium, Coprinus comatus, Stachybotrys*	*Mucor, Stachybotrys, Coprinus comatus*	*Mucor, Cladosporium, Chaetomium, Stachybotrys, Alternaria*
FS	K	*Scopulariopsis, Acremonium, Coprinus comatus, Paecilomyces*	-	-
	F	Nematodes, *Paramecium, Paecilomyces, Coprinus comatus, Alternaria*	-	-
WW	K	*Paecilomyces,*	-	-
	F	*Trichoderma*	-	-
WF	K	*Paecilomyces*	-	-
	F	-	-	-
SLHC	K	0	0	0
	F	0	0	0
SLHC – B	K	0	0	0
	F	0	0	0

the other hand, in the second part, increasing the moisture level resulted in a significantly higher level of fungal growth, which fully allowed the samples to be visually compared with each other. This variation in the results suggests that there was a difference in the conditions under which the sample was kept, because although the RH was 99% in both test stages, the regularly moistened samples were more favourable for fungal growth. Such elevated humidity is useful to assess the differences between various materials, but it should be noted that it represents very aggressive environmental conditions – the exposure to precipitation moisture – in which the tested materials would not be used. Therefore, the test results cannot be interpreted as material inability to resist biodegradation, but only to be used for comparison with each other and with other commercially available materials. Other researchers found that using high RH (93%) and increasing the temperature to 30ºC yielded the conditions favourable for mould growth and for the comparison of different materials (Laborel-Préneron et al., 2018).

Comparing the materials, it can be seen that the biocomposites of sapropel, lime and magnesium oxychloride cement binders have an equally high level of protection against biodegradation because at a medium level of binder they do not show fungal growth even under high humidity conditions. This result is equivalent to the results of a commercially used building material with biomass filler – wood fibreboard – and is even better than the result of wood wool, which shows growth even with reduced air humidity. This leads to the conclusion that these materials have the potential to be used as construction materials under the conditions where they are protected from the effects of external moisture. Magnesium phosphate biocomposites show lower microbial resistance, so their use in construction is possible only in combination with additional additives that would provide the protection against fungal exposure.

The organoclay-coated samples showed 13.8% lower overgrowth and biocide products showed 9.1% lower overgrowth. The observed improvement is not significant, but at the same time, it demonstrates that adding supportive coating with appropriate biocides or their alternatives can prolong the life of natural composite materials. However, this effect was not the same for all formulations.

Considering that the organoclay additive in HL binder showed no improvement, it could be due to a pH level decrease that leads to more active microbial growth, or it may be that the concentration of organoclay additives needs to be increased or that there is organoclay incompatibility with the HL binder. When using organoclay as a biocide alternative, it needs to be considered that it is not possible to substitute biocides in a ratio of 1:1. Current testing demonstrates that the biocides could be substituted with organoclay additive in a ratio of 1:4, which leads to the discussion of cost-effectiveness of the used alternatives available in the industry. The finding that the biocide ACTICIDE FD BACTERICIDE with MOC and FHL binders showed no improvement leads to the consideration that either the concentrations are too low or there is biocide incompatibility with MOC and FHL binder formulations.

The visual assessment method can be used effectively, but only in combination with microscopic methods. Studies often estimate the distribution of fungi on a plot as a percentage or points (Table 5) and assume that if the percentage is high, the surface of the composite will be discoloured. However, even if nothing can be seen with the naked eye, the whole sample can be completely covered with mould, which was also proven in this study (in the first stage of the experiment); therefore, microscopic methods were also used in this study. Using only visual expert judgment in a study can give poor insight into the microbiological resistance and stability of the analysed composite materials. It may also be that a fungus with a strong effect on the material can give a low percentage share; strong, but heterogeneous, well-developed growth would yield a low percentage (Johansson et al., 2014)

Fungi vary in their water requirements and represent different groups in the successional colonisation order (Johansson, 2014). Fungi are a series of micro-fungi belonging to various systematic categories. In their natural cycle, they act as decomposers and their spores are found in the air and on various types of surfaces. Under appropriate conditions, spores germinate, and hyphae grow to form mycelium. This process can occur in parts of the building structure and on internal surfaces, with the risk of adversely affecting the indoor environment and human health. The development of such fungi entails significant costs due to the need for renovation, so both economic reasons and health protection are important grounds for reducing the mould risk of buildings. The conditions for fungi growth include appropriate nutrient availability, temperature, pH and humidity. In general, the availability of water in the material is considered to be the decisive condition for the growth of the fungi.

Ultimately the best way to determine the susceptibility of materials to fungi is to physically test the subject material. One of the aims of this study was to evaluate the susceptibility of the materials used to mould growth under varying conditions and methods of inoculation, using the visual assessment and microscopic methods, and the set aim has been fulfilled. Because the materials used in the study are of natural origin and contain wood and fibre plant materials, processing is required to ensure the antimicrobial activity and protection (Stefanowski et al., 2017).

CONCLUSIONS

The following conclusions can be drawn from both stages of the experiment and the analysis of their results:

1. Lime-based binder biobased composite material shows the highest microbiological stability. This is attributed to the pH level, which for the lime-based specimens is around pH 12 at high binder concentrations. However, as the amount of binder decreases, the pH level and microbiological stability of biocomposites also decreases and they become more similar to biocomposites of other binders.
2. Magnesium oxychloride cement biobased composite material show slightly lower microbiological stability at high binder content, as their pH is around 10, but as the binder decreases, the pH does not fall as fast as lime binder biocomposites; thus, at lower binder amounts, the lime-based and magnesium oxychloride cement biocomposites have similar microbiological stability.
3. Magnesium phosphate cement biobased composite material have the lowest microbiological stability among mineral binders because, although the pH of the binder increases above 10 during curing, it does occur over time and the monopotassium phosphate water solution that is used as a hardener for the binder has a pH lower than 7; it can also be used as a concentrated mineral fertiliser; thus, the undissolved part of the hardener can serve as a nutrient for fungal growth.
4. Bio-based composite material, where sapropel was used as a binder, shows one of the highest microbiological stability results. In addition, fungi and other organisms were not detected on the samples.
5. The tested sapropel, lime and magnesium oxychloride cement biobased composite materials have a higher microbiological resistance than the commercially used wood wool insulation; therefore, they have the potential to be used in construction under similar conditions, i.e. in structures protected from external moisture.
6. Using visual expert conclusions, as in this study, can give a modest insight into the microbiological resistance and stability of the studied composite materials. It may also be that a fungus with a robust effect on the material can give a low growth percentage share.
7. The organoclay-added samples showed 13.8% lower overgrowth and those with the biocide product had 9.1% lower; however, incompatibility was observed with formulated hydraulic lime (20%), magnesium oxychloride cement (20%), hydraulic lime (20%) and magnesium phosphate cement (100%) binders.

Acknowledgments

This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 “Post-doctoral Research Aid” of the Specific Aid Objective 1.1.1 “To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure” of the Operational Programme “Growth and Employment” (No.1.1.1.2/VIAA/3/19/394).

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