

Bioinspired Living Coating System for Regenerative and Circular Architecture

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Surfaces of exposed materials are affected by biotic and abiotic degradation processes. They often are protected by architectural coatings that not only provide a decorative layer but also enhance integrity of the material structure. Common surface treatments often include mineral oil binders and other ingredients that are known to have a negative impact on the environment. To address these issues, an alternative bioinspired concept for materials protection based on engineered fungal biofilm is under development. This paper presents the first results related to the bioreceptivity of building materials and the initial steps of natural biofilm formation.

This research concluded that fungal colonisation and the variability of microorganisms is influenced by the type of material and climate condition at the exposure site. Fungal infestation was lower on protected materials (e.g., with commercial coatings). Samples from the eastern and western exposure exhibited the highest fungal colonisation, whereas samples from the northern and southern exposure exhibited the least growth. Furthermore, the samples in close spatial proximity were colonized by different fungal microbiota. It was determined that *Aureobasidium* sp. is the dominant species in the early phase of colonisation.

In the following steps, a bioactive protective coating system that works in synergy with nature will be developed. Based on the initial results *Aureobasidium* appears to be a viable candidate as an active, living component of a new nature-inspired coating system. The novel protection concept is based on three interrelated components – bioinspiration as a driving force for materials design, bio-based ingredients, and living fungal cells that will provide self-healing and bioremediation capacity. The living coating will be designed to protect various architectonic materials, including porous materials such as biomaterials, concrete, stone, and non-porous, as well as plastics, and metals. The ultimate goal is to advance the development of engineered living materials that interact, adapt, and respond to environmental changes.

Keywords: bioinspired coating system; bioreceptivity of building materials; early fungal colonisers; engineered living materials.

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Abstract



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Introduction

Functional and aesthetical durability of degradable materials is usually enhanced by architectonic coatings. Subsequently, paints and coatings applied on the exterior of buildings augments the aesthetics of the structure and protects it against adverse weather conditions, such as heat, UV, harsh winters, and soaking rain. The mainstream paints and varnishes are produced from synthetic chemicals. About 70% of raw materials used in coatings are based on petroleum and only 50-60% of paints are recycled (IMA Europe, 2023). The applied preservatives mostly rely on biocides as active ingredients (Gesthuizen, 2020). The use of toxic substances is increasingly restricted by legal regulations since there is an awareness of their negative environmental impact. Nevertheless, the use of renewable raw materials is still limited despite the government's aim to encourage the use of sustainably produced and processed materials (EU Regulation, 2006).

As an alternative to conventional coatings, the concept proposed by the ongoing ARCHI-SKIN project relies on implementing bioinspired protection mechanisms, active bio-based ingredients, and a living biofilm (Sandak, 2023). The project aims to both comprehensively evaluate bioinspired strategies of the chemical-structural properties of biofilms and develop a solid base for the development of a living coating system. The coating formulation will fully rely on bio-based components. The use of natural ingredients and environmentally sustainable processes for coating manufacturing will, therefore, contribute towards a reduction in greenhouse gas emissions. The toxicity and safety requirements will be assessed at different manufacturing steps and during the use phase. The bioremediation potential of living fungi is expected to contribute towards lowering air pollution. Moreover, the self-repair functionality is expected to enhance the performance and reduce the cost of frequent maintenance. This will ensure that the developed coating system is in line with regenerative and circular design in addition to creating a positive impact for society and the environment.

This paper presents results from the exploration phase of the project, which focused on the investigation of naturally occurring fungal biofilm formation on various architectonic surfaces. Bioreceptivity is defined as the ability of a material to be colonised by one or several groups of living organisms without necessarily undergoing any biodeterioration (Guillitte, 1995). It is an important intrinsic material property that facilitates the attachment of organisms, while the subsequent biocolonisation is modulated by environmental factors (Fuentes et al., 2021).

An understanding of kinetic microbial colonisation on various façade materials is indispensable for revealing organism-material interaction and developing a new concept for material protection. In this first phase of the ongoing research the morphological, functional, and social interactions in fungal communities that appear naturally on building materials were thoroughly investigated. The aim was to assess the variety of fungi that can colonise exposed surfaces and understand their interactions with various building materials. The goal was to identify fungal species with a protective potential that can be used as an active living ingredient of the ARCHI-SKIN coating.

Methods

Natural weathering process

This research investigated 33 wood-based façade materials, exposed in two replicas on the roof of the InnoRenew CoE building, Izola, Slovenia (45.5350, 13.6577) (Fig. 1). Experimental samples were exposed to four cardinal directions and included specimens representing seven categories described previously by Butina Ogorelec et al. (2023). The experiment is ongoing; this paper presents the results of the initial observations (up to 5 months of natural exposure).

Material characterisation

Multi-sensor and multi-scale measurements were performed to assess the influence of surface properties (wettability) and material structure (roughness) on fungal growth, species variability, and dominance. Samples were conditioned to reach equilibrium moisture content before measurements.

**Fig. 1**

Natural weathering stand used for the evaluation of materials bioreceptivity. Displayed configuration presents the southern exposure where duplicate samples were assessed in parallel

Roughness and wettability measurements were conducted before and after 2.5 months of exposure. The contact angle and topography were measured with the Attension Theta Flex Auto 4 optical tensiometer equipped with a 3D topography module (Biolin Scientific, Gothenburg, Sweden). On each sample, three contact angle measurements with distilled water as well three topography measurements, each covering an area of 3.2 mm × 2.8 mm, were performed. The mean ± SD of replicates was calculated. The Keyence VHX-6000 digital microscope (Keyence, Osaka, Japan) and EVOS M7000 (ThermoFisher Scientific, Massachusetts, United States) were used for microscopic observations.

Hyperspectral imaging (HI) measurements were conducted before and after 5 months of exposure. HI as a high-throughput method was used to understand the effect of fungal growth on material properties as well as to objectively quantify the infested area on the entire sample. Hyperspectral images were acquired with the FX17 camera (Specim, Oulu, Finland) and LumoScanner program (Middleton Spectral Vision, Wisconsin, United States). Data were analysed using Evince (Prediktera, Umeå, Sweden). Spectral data were pre-processed using Standard Normal Variate (SNV) before Principal Component Analysis (PCA). The loadings of the first principal components were plotted using Prism 9 (GraphPad Software, Massachusetts, United States).

Fungal colonisation

The surfaces were sampled with a wet swab and plated on DG-18 agar to evaluate microbial growth. Agar type was selected to prevent the growth of bacteria and limit the growth of fast-growing fungi. Pure cultures of the dominant species were then isolated on potato dextrose agar (PDA) plates (PDA, REF: 4019352, Biolife, Milan, Italy) and identified through polymerase chain reaction (PCR) amplification and Sanger sequencing of specific DNA regions/genes according to the protocol described by Butina Ogorelec et al. (2023). After obtaining sequences of isolates from Microsynth (Austria), the most similar sequences of type strains and other important taxonomical reference strains were retrieved from the GenBank nucleotide database with the BLAST algorithm (Altschul et al. 1990). All sequences were aligned and phylogenetically analysed using the maximum likelihood method as implemented in program Mega11 (Tamura et al. 2021). All DNA sequences from the representative isolates from this study were deposited in the GenBank database: OR054020-OR054066.

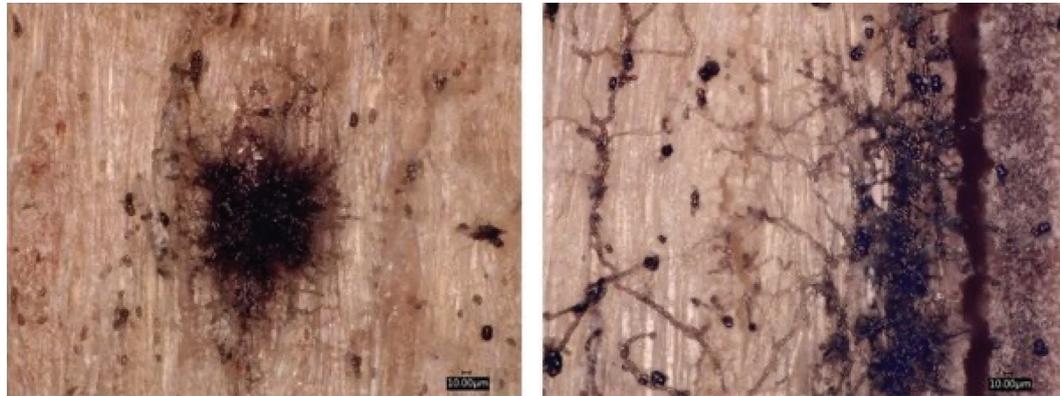
Results

The surface roughness increased for natural materials, compared to those protected by coatings. Samples protected by coatings exhibited higher contact angle measurements, which signifies poor spreading and physical affinity for liquids. Materials without surfaces protection were easily wetted, and the sessile drops disappeared within a few seconds of starting the test.

A digital microscope was used to identify zones infested by fungi. The initial infestation presented in Fig. 2 occurred in zones that had eroded due to natural weathering mainly in unprotected materials.

Fig. 2

Microscopic images acquired with Keyence microscope (500 magnification); natural beech (left) and acetylated beech (right) exposed to natural weathering (northern exposure)

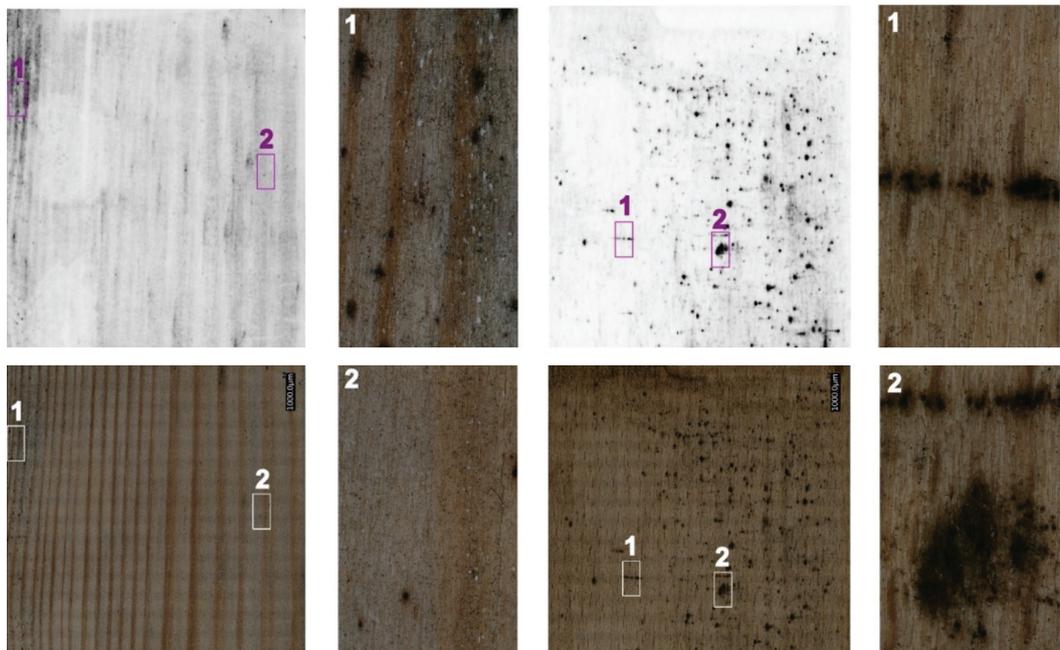


Hyperspectral imaging enabled a fast and reliable identification of zones colonised by microorganisms over the entire sample surface. Fig. 3 presents the PCA results of the hyperspectral images and corresponding colour images allowing good visualisation of the surface appearance.

Visual assessment was performed to investigate the occurrence of the dominant species in a more quantitative manner. The average number of colonies identified on samples exposed to different cardinal directions is presented in Fig. 4. In cases where $\geq 90\%$ of the colony forming units (CFUs) appeared to have the same morphology, a representative colony was isolated to a pure culture on PDA plates.

Fig. 3

PCA of HI of TO_2 impregnated and natural (without any treatment) samples exposed to the north (5 months)



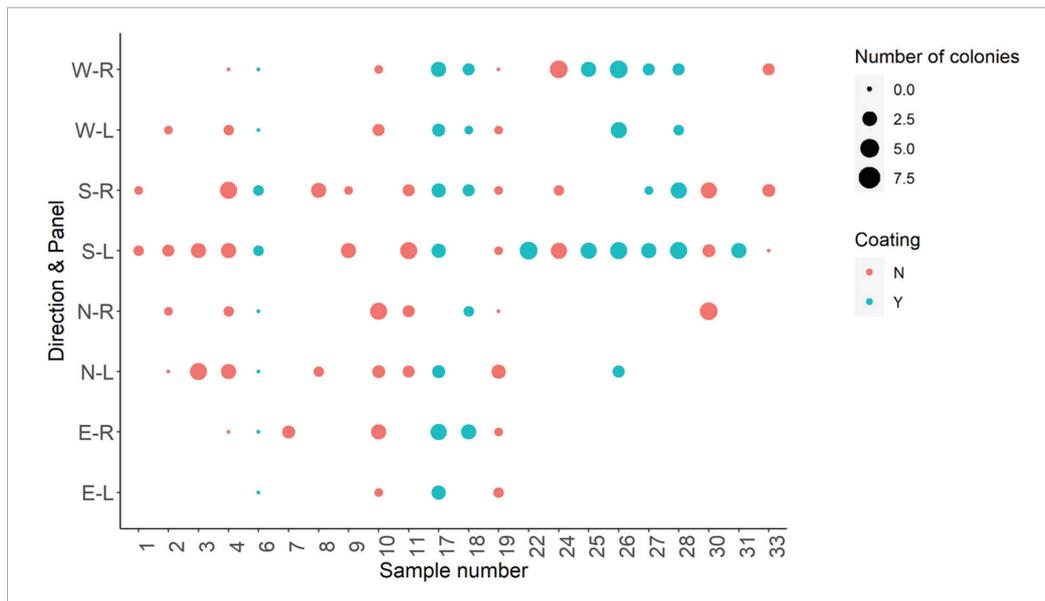


Fig. 4

Average number of colonies identified on samples exposed to different cardinal directions. Samples were divided into two categories: coated and unprotected. The abbreviations on Y-axis represent cardinal direction (W, S, E, N) and panel (left or right)

The pure cultures of fungi identified on the investigated materials and macroscopic images of selected strains are presented in Fig. 5 and 6 respectively. On most of the investigated materials, *Aureobasidium sp.* was the dominant genus. This signifies that in the early phases of colonisation this species can establish dominance.

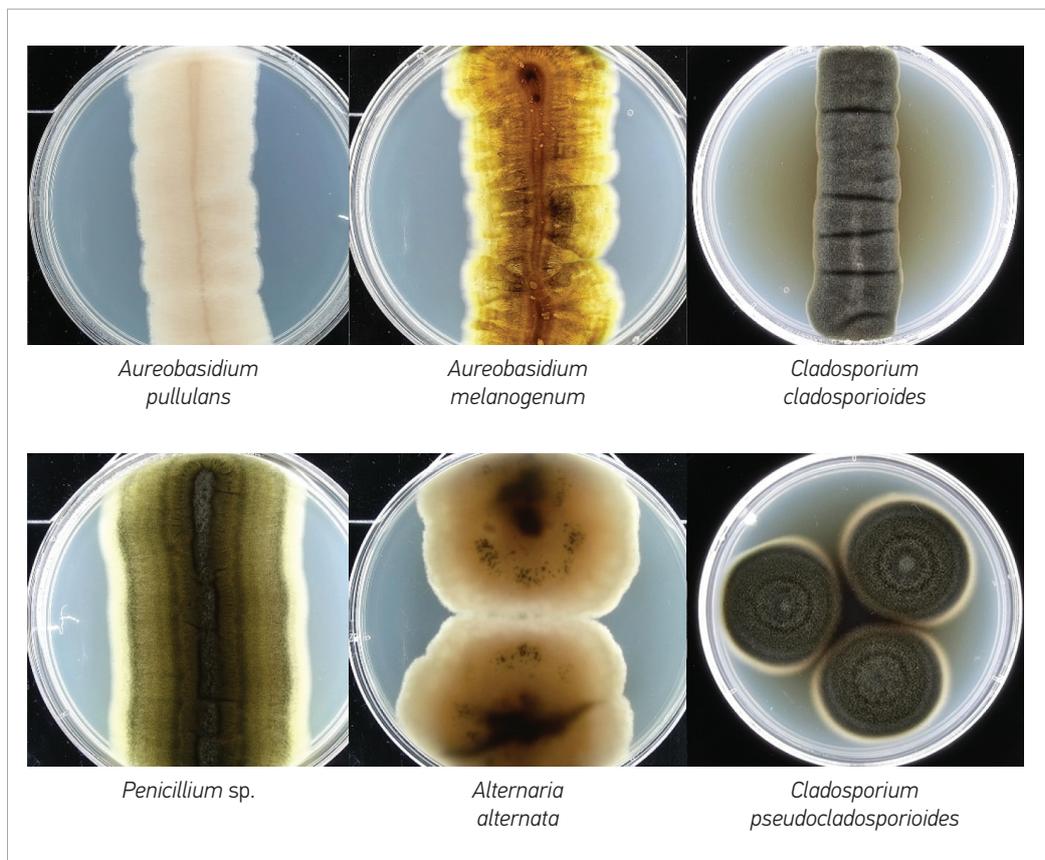
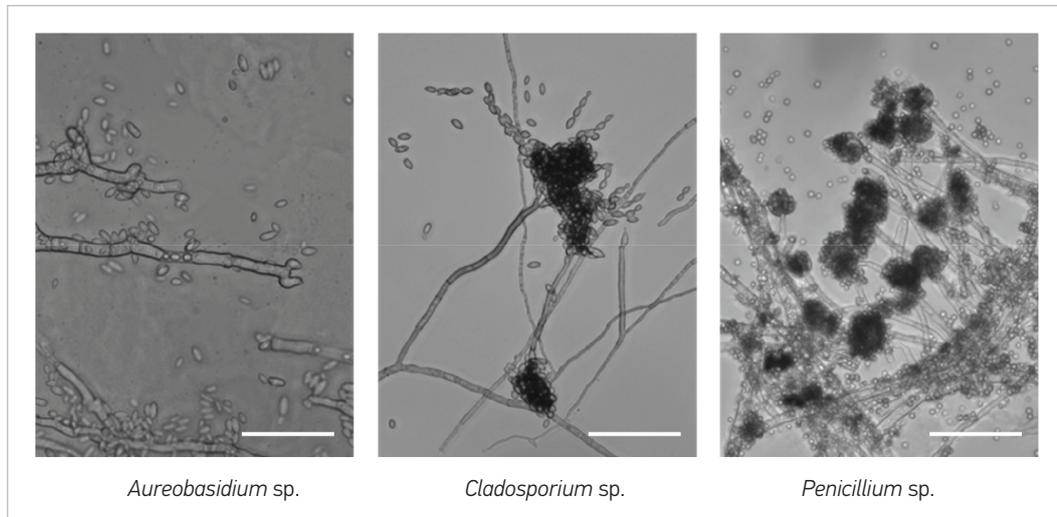


Fig. 5

Images of pure cultures

Fig. 6

Microscopic images of pure cultures obtained with a 60x objective (Evos microscope). Scale bar represents 50 μm



Discussion

ARCHI-SKIN implements biomimetic principles for the development of smart living surfaces that are intended to be used for active protection of various building materials. It is expected that learning from the evolution of living organisms and the use of natural resources in a more efficient, sustainable and environmentally friendly manner will facilitate the design of new bioinspired protection methods (Bhushan 2018). Fabrication of surfaces with properties similar to biological ones offers therefore an alternative approach for coatings design with significant commercial potential. The proposed concept relies on fungal biofilm engineering that aims to imitate biofilm formation occurring in nature. The initial phase of the project focused on understanding biofilm formation and its structure so that the process can be reproduced under laboratory conditions. The bioreceptivity of various biobased materials was assessed and the progress of microbial colonisation was simultaneously monitored. The increase in roughness that was observed in materials with an unprotected wood surface is due to erosion of the surface, removal of single fibres, and leaching of photodegraded components (Sandak et al., 2021). Such changes increased susceptibility to accumulation of dirt, moisture, and pollutants as well as enhanced spore attachment and germination (Gobakken et al., 2012a). Evidence of this phenomena is presented in Fig. 2, where spores were deposited in areas with high surface roughness. Wettability parameters exhibited a similar trend. Unprotected materials had lower contact angle measurements compared to coated ones. The observed results confirmed that high surface roughness, high open porosity, high capillary water content, and high wettability, considered as the most common material characteristics for improving bioreceptivity, also influenced the colonisation rate of the investigated biobased materials (Veeger et al., 2021). The intrinsic bioreceptivity of the material is, therefore, a crucial factor in the initial biocolonisation and long-term establishment of biofilms (Stohl et al., 2023).

Hyperspectral imaging enables simultaneous and non-destructive acquisition of spectral and spatial information. PCA was implemented to reduce data complexity. In this investigation the first principal component enhanced zones infested by fungi, allowing for an objective quantification of the infestation rate of materials where fungal presence is not yet visible with the naked eye (Fig. 3). Spectra can be averaged to show global characteristics or analysed at single pixels, emphasising local variability (e.g., zones particularly prone to infestation). As an imaging technique, hyperspectral imaging doesn't require contact with the measured object, which makes it a perfect candidate for non-destructive examination of various building materials. Considering the relatively high speed of measurement, HI has huge potential as a high throughput methodology that can be considered in parallel to the conventional microbial swabbing.

The previous study reported that the cardinal direction of exposure affects the colour and chemical changes in the wood due to weathering (Sandak et al., 2015; Sandak et al., 2021). However, there are fewer reports that assess the effects of cardinal direction on fungal growth (Rüther et al., 2013). The results reported in Fig. 4 present the average CFUs of samples exposed to all directions. Wilcoxon signed-rank test, $p < 0.05$ was used to prove that the distribution of CFUs on samples exposed in the four directions differ significantly. The southern direction exhibited the least growth, which can be explained by the longer exposure to UV radiation, leading to more dynamic changes in surface moisture (Sandak et al. 2021). The northern exposure showed slightly higher colonisation compared to the southern. Less direct sun on claddings exposed to the north means longer periods of high moisture and more ideal conditions for fungal growth (Gobakken et al., 2012b). The claddings on the eastern and western exposure showed the highest fungal burden due to the predominant wind direction at the test location. Despite the physical contact between the samples visible in Fig. 1 the fungal counts of samples located in the vicinity differed, which signifies that fungal growth is related to the bioreceptivity of the material.

Aureobasidium sp., a polyextremotolerant fungus (Gostinčar et al., 2010) was the dominant genus on wood-based materials exposed to the outdoors. The predominant species in the investigated case was *Aureobasidium melanogenum*, which was recently defined as a separate species from *A. pullulans* (Gostinčar et al. 2014). Early assessment of bioreceptivity and the prevalence of *Aureobasidium* sp. implies that the species become dominant during the early phases of colonisation. In addition to *Aureobasidium* sp., *Cladosporium* sp., *Alternaria* sp., and *Penicillium* sp., were also found on the surface of the tested material, though in much lower quantities. Due to its polyextremotolerance, oligotrophic nature, and broad enzymatic profile *Aureobasidium* sp. appears to be an optimal species for living coatings that are potentially suitable for protection of materials in various climates. These characteristics are also relevant while considering future predictive maintenance schedules that will be calculated based on the performance of ARCHI-SKIN coating during natural weathering campaign.

This study presents results from the first phase of the project where bioreceptivity of cladding materials was assessed. It is a starting point for the development of a new solution for the protection of architectonic materials based on engineered fungal biofilm formation. The features that enhance fungal colonisation related to bioreceptivity of materials and the preference of certain fungi for specific materials were identified. Intensity of fungal colonisation and the variability of microorganisms was influenced by the material type and the climate condition at the exposure site. Natural samples (materials without any treatments) were generally more susceptible to fungal growth. The distribution of CFUs on samples exposed to the four directions differed significantly. The highest fungal colonisation was observed in the east and west, while the least growth was observed in the south. Samples in close spatial proximity exhibited different fungal microbiota, demonstrating that fungal colonisation across samples is not random.

Based on initial results *Aureobasidium* sp. appears to be a viable candidate for an active and living component of a new nature-inspired coating system, designed to effectively protect architectonic surfaces. Although *Aureobasidium pullulans* is not known to be harmful to human health, the potential cytotoxic effect of selected strains will be tested on human cell lines (human skin fibroblasts and lung cells) in vitro. The developed coating system will be entirely biobased, bringing it in line with the Chemicals Strategy for Sustainability and the European Green Deal priorities. It is expected that the self-repair functionality will enhance coating performance, improve its environmental impact as well as reduce the cost and maintenance activity in service. Finally, the pioneering approach for protecting materials developed by the ARCHI-SKIN project aims to push the boundaries of traditional materials concepts towards the development of engineered living materials (ELMs) capable of interacting, adapting, and responding to environmental changes and advance their progress in the building sector.

Conclusions

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