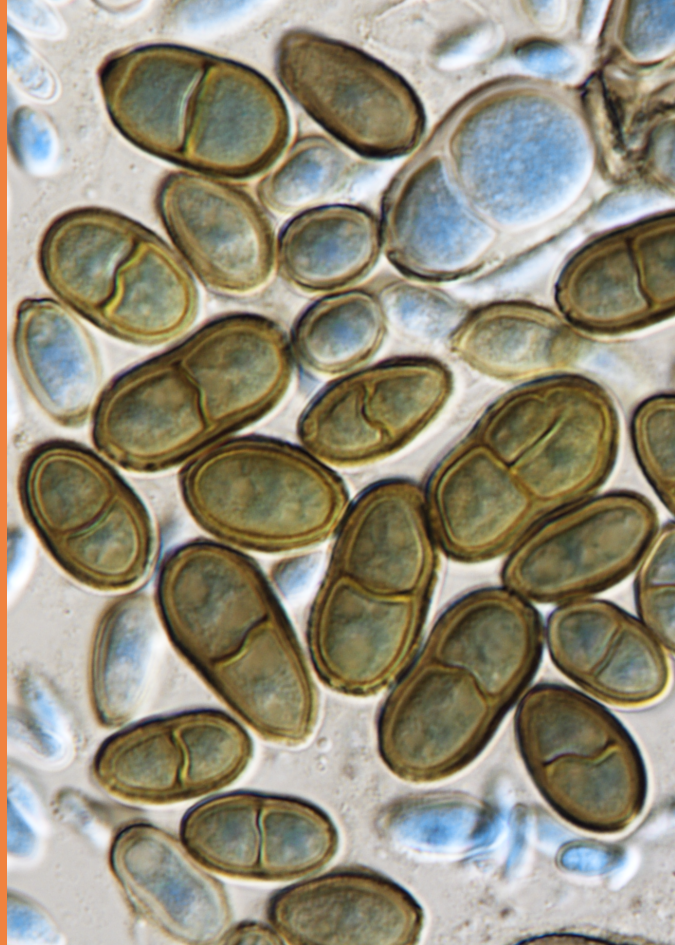


Bioinspired living coating system for wood protection

Faksawat Poohphajai



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Bioinspired living coating system for wood protection

Author name
Faksawat Poohphajai

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The bioinspired living coating system offers an innovative, sustainable approach to wood protection, relying on natural substances with minimal environmental impact and low maintenance requirements. While promising as an alternative to conventional coatings, key aspects remain poorly understood. Although *Aureobasidium pullulans* (*A. pullulans*) has been identified as the optimal fungal species, it is essential to further validate that this fungus meets crucial criteria for effective protection. Additionally, the impacts of natural weathering on substrate properties, bioreceptivity, microbial colonization rates, and the survival of fungal cells within the coating under varied conditions require further investigation. This thesis aims to 1) explore *A. pullulans*' resilience in biofilm formation across different wood substrates and environmental conditions, 2) assess the performance of wood treated with this biofilm-based coating, and 3) examine fungal cell survival throughout its service life.

The evaluation of fungal colonisation on wood surfaces exposed to diverse climate conditions and a range of coated and non-coated biobased façade materials revealed that specific species, notably *A. pullulans*, emerged as predominant primary colonisers on weathered wood surfaces, regardless of geographical location, cardinal direction, and surface treatment. The adaptability and capacity to thrive in a relatively broad range of ecological conditions make this fungal strain suitable as a protective layer for building materials.

The assessment of fungal colonisation on wood surfaces coated with Biofinish following a 9-month exposure period revealed that the majority of the detected species belonged to the genera *Aureobasidium*, specifically *A. pullulans*. These results indicate the survival and effective antagonistic action of *A. pullulans*, the living and active ingredient of the coating, against other wood-decaying fungi.

The performance of Scots pine (*Pinus sylvestris* L.) wood treated with Biofinish was evaluated against uncoated reference wood following a 12-month natural weathering trial. Biofinish exhibited superior performance across all examined aspects compared to the uncoated reference. The entirely bio-based composition of the Biofinish coating enhances its sustainability and compatibility with natural environments, rendering it an appealing alternative to contemporary wood surface protection solutions.

The results from this thesis will facilitate the control and optimisation of fungal biofilm and contribute to the development of novel bioinspired protection coatings based on optimised fungal biofilm working in synergy and not against nature.

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List of Publications

This doctoral thesis is based on the following publications:

- 1.** Poohphajai, F.; Myronycheva, O.; Karlsson, O.; Belt, T.; Rautkari, L.; Sandak, J.; Gubenšek, A.; Zalar, P.; Gunde-Cimerman, N.; Sandak, A. Fungal Colonisation on Wood Surfaces Weathered at Diverse Climatic Conditions. *Heliyon* 2023, 9, doi: 10.1016/j.heliyon. 2023.e17355.
- 2.** Butina Ogorelec, K.; Gubenšek, A.; Poohphajai, F.; Sandak, A. Assessing the Bioreceptivity of Biobased Cladding Materials. *Coatings* 2023, 13, 1–15, doi:10.3390/coatings13081413.
- 3.** Poohphajai, F.; Sandak, J.; Sailer, M.; Rautkari, L.; Belt, T.; Sandak, A. Bioinspired Living Coating System in Service: Evaluation of the Wood Protected with Biofinish during One-Year Natural Weathering. *Coatings* 2021, 11, 701, doi:10.3390/coatings11060701.
- 4.** Poohphajai, F.; Gubenšek, A.; Anjačernoša, A.A.; Ogorelec, K.B.; Rautkari, L.; Sandak, J.; Sandak, A. Bioinspired Living Coating System for Wood Protection: Exploring Fungal Species on Wood Surfaces Coated with Biofinish during Its Service Life. *Coatings* 2024, 14, Page 430 2024, 14, 430, doi:10.3390/coatings14040430.

Author's Contribution

Article 1: Fungal colonisation on wood surfaces weathered at diverse climatic conditions

FP had primary responsibility for conceiving and designing the experiments, conducting majority of the experimental work under the supervision of OM, AS, and JS, and carrying out the main part of the analysis and interpretation of the data together with OM, AS, JS, PZ and AG. FP was responsible for contributing reagents, materials, analysis tools, or data in conjunction with OM, AS, JS, PZ and NGC. Additionally, FP had primary responsibility for writing and revising the manuscript under the supervision of OM, AS, JS, OK, and LR.

Article 2: Assessing the bioreceptivity of biobased cladding materials

Publication 2: FP was responsible for conceptualisation, methodology, validation, formal analysis, and investigation in collaboration with KBO, AG, and AS. KBO contributed to software, visualisation, data curation, and preparation of the original draft. FP, KBO, and AG were responsible for reviewing, and editing the manuscript under the supervision of AS. AS was responsible for resources, project administration, and funding acquisition.

Article 3: Bioinspired living coating system in service: evaluation of the wood protected with biofinish during one-year natural weathering

FP was responsible for conceptualisation and investigation together with the co-authors, performing the main part of data validation and formal analysis. FP was responsible for writing - the original draft preparation under the supervision of JS, AS, TB, and LR. JS contributed visualisation. AS and JS were responsible for resources, data curation, and funding acquisition. FP had primary responsibility for reviewing and editing the manuscript together with the co-authors.

Article 4: Bioinspired living coating system for wood protection: exploring fungal species on wood surfaces coated with biofinish during its service life

FP was responsible for conceptualization and original draft preparation, performed the main part of the experimental work and methodology together with AG, KBO and AC. Additionally, FP carried out the main part of software development, validation, formal analysis, investigation, and data curation with AG under supervision of AS. FP had primary responsibility for writing, reviewing, and editing the manuscript together with the co-authors. AS had responsibility for resources, project administration, and funding acquisition

List of Abbreviations

ACE	Angiotensin-converting enzyme
Cfa	Warm temperate climate zone
CFU	Colony forming units
CTAB	Cetyltrimethyl ammonium bromide
Dfc	Subarctic or boreal climate zone
DG-18	Dichloran glycerol agar
IR	Infrared radiation
MC	Moisture content
MEA	Malt extract agar
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RH	Relative humidity
Sa	Arithmetical mean height
Skt	Kurtosis
Ssk	Skewness
UV	Ultraviolet radiation

1 Introduction

Microbial colonisation of wood in outdoor applications is undesirable and represents a critical aspect of the degradation process. It causes aesthetic issues and accelerates degradation of wood, leading to economic losses from maintenance costs and a significant reduction in the service life of wood (Schoeman and Dickinson 1997, Evans et al. 2005, Zabel and Morrell 2020). Various coatings and surface treatments have been developed to protect wood from fungal infestations. However, many of these treatments contain synthetic chemicals and toxic compounds that can negatively affect the environment and pose a risk to living organisms, including humans. Nature's solutions inspire advanced applications across scientific and research fields, utilising the concept of biomimicry and bioinspiration to address challenges. Drawing from nature's solutions regarding the growth of microorganisms on wood surfaces offers inspiration for an innovative living coating system with protective and self-healing properties. The concept of the bioinspired living coating system, comprised solely of natural substances, represents a fully sustainable wood treatment solution. This approach offers low environmental impact and reduced maintenance requirements, making it a promising substitute for conventional wood coating products. However, several aspects related to fungal strains, protection mechanisms, and coating performance are yet poorly understood.

This study is a part of the “Bioinspired living skin for architecture” project (ERC CoG #101044468 – ARCHI-SKIN). By mimicking the natural process, the "ARCHI-SKIN" project is developing an innovative bioinspired living coating system based on fungal biofilm, specifically *Aureobasidium pullulans* (*A. pullulans*), for protection of various building materials. However, critical knowledge gaps remain. Firstly, despite *A. pullulans* has been selected as the most suitable candidate for the project the identification of appropriate fungal strains for efficient protection is still ongoing. Furthermore, it is essential to further validate that this fungus meets all the critical criteria for effective bioinspired coatings. These criteria include efficacy in wood protection, compatibility with wood, environmental resistance, non-pathogenicity, viability for production and application, sustainability, potential for regeneration. Secondly, the effects of natural weathering on substrate properties, bioreceptivity, and microbial colonisation rates remain inadequately explored. Thirdly, the survival of fungal cells within the coating under various environmental

conditions require further investigation. Additionally, the performance of bioinspired living coatings throughout its service life have not been adequately researched. Therefore, the main objectives of this thesis were to:

1. Explore the resilience of *Aureobasidium pullulans* in biofilm formation across different wood substrates and environmental conditions.
2. Investigate the performance of wood treated with a bioinspired fungal biofilm-based coating.
3. Assess the survival of fungal cells, the living ingredient, in the coating under various conditions.

This thesis addresses significant knowledge gaps in bioinspired living coatings for material protection and aims to enhance our understanding and development of these innovative protective solutions. It comprises four papers, each delving into distinct aspects of colonisation of *A. pullulans* under diverse climate conditions, as well as on wood with different surface treatments and different species. Consequently, it evaluates whether *A. pullulans* adheres to the selection criteria essential for effective bioinspired coatings, such as environmental resistance and compatibility with wood surfaces. Furthermore, it investigates the survival of *A. pullulans* and assesses the protective potential and performance of bioinspired living coatings throughout their service life. **Paper 1** explored the colonisation of fungal species on wood surfaces exposed to varying climate conditions. **Paper 2** examined the primary colonisers on various bio-based façade materials, including both uncoated surfaces and those treated with different coating systems. The presence of fungi on wood is a recognised issue and most studies focus on protecting wood from fungal attacks. Nevertheless, some fungal strains naturally colonise wood materials and can produce a fungal biofilm with protective functionalities. Assessing fungal species that can thrive on weathered wood surfaces without causing significant degradation of the wood structure and comprehending their interactions with materials and climate conditions are crucial steps in identifying optimal fungal strains with the highest protective potential.

Paper 3 explored the performance and protective potential of commercial Biofinish coatings during natural weathering. Finally, **Paper 4** examined the colonisation of fungi along with assessing the survival and persistence of *A. pullulans* within the coating matrix on wooden facades coated with Biofinish during their service life. These investigations provide deep insights into the coating's performance, protective functionality, and longevity of fungal cells in the coating under practical application. By comprehensively examining *A. pullulans*' resilience, performance, and survival across various conditions and substrates, this thesis lays the groundwork for further development of bioinspired living coatings. The findings contribute to our understanding of how these innovative protective systems can be optimised for sustained effectiveness in wood protection applications. Additionally, it not only addresses crucial knowledge gaps but also

establishes a foundation for the practical application of fungal biofilm-based coatings in architectural and construction contexts. The research gaps, topics of this thesis and their relationship are summarised in Figure 1.

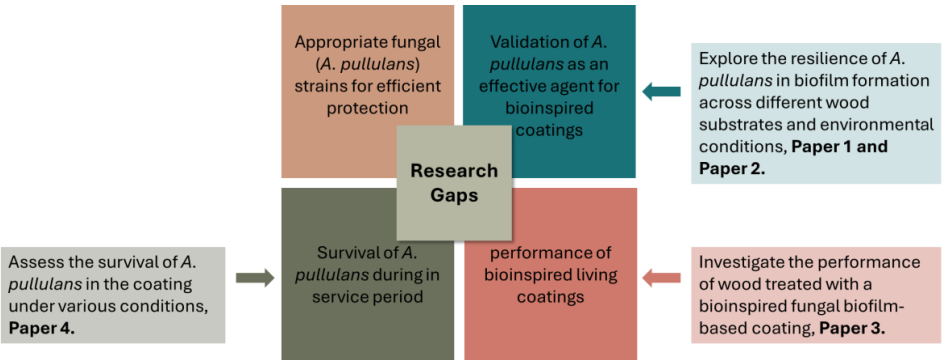


Figure 1. Research gaps, topics covered in this thesis and their relationship

2 Background

This section describes three key aspects that influenced this research. Weathering is a primary factor contributing to the deterioration of wood exposed to out-door conditions. However, fungal colonisation of wood during weathering was the inspiration for investigating innovative and sustainable wood protection methods. The concept of bioinspiration led to the pursuit of alternative wood protection strategies. Specifically, the fungal strain *Aureobasidium pullulans* exhibited potential as a protective layer in bioinspired living coating. Finally, an alternative method for wood protection based on controlled engineered biofilm was investigated.

2.1 Weathering of wood

Under outdoor conditions, wood undergoes a complex degradation process known as weathering, caused by a combination of environmental (abiotic) factors and biological attacks (Evans et al. 2005). This process results in surface discolourations, changes in gloss and brightness, the presence of loose fibers and raised grain, formation of checks and cracks. Additionally, it leads to surface roughening and structural deformations such as cupping and warping of the wooden boards. Over time, the surface of weathered wood is inhabited by fungi and lichens (Feist and Hon 1984, Rowell 2005). Weathering is considered a superficial phenomenon that primarily affects the surface of wood and does not significantly impact the wood's mechanical properties (Williams 2005, Evans 2015). Wood weathering is primarily influenced by the combined effects of solar radiation and wind-driven rain. Other influential factors include temperature fluctuations (heat and freezing), relative humidity, wind, pollutants and particulates, oxygen (O₂), human activities, and biological organisms. All of these play significant roles in the weathering process and should not be overlooked (Rowell 2005).

2.1.1 Factors and Mechanisms Associated with Wood Weathering

Solar radiation

Photodegradation is a process that is initiated by light and can occur rapidly on wood surfaces exposed to solar radiation (Feist and Hon 1984). In particular, the

critical wavelengths that can cleave carbon-carbon, carbon-oxygen, and carbon-hydrogen single bonds that connect the basic structural units of major wood constituent polymers are in the range of UV light (346, 334, and 289 nm, respectively) (Hon and Shiraishi 2000, Evans et al. 2005). Wood is an excellent light absorber due to the sensitivity of the chemical constituents in wood such as lignin, hemicelluloses, cellulose, and extractives (Gellerstedt and Petterson 1977, Chang, Hon, and Feist 1982, Feist and Hon 1984). Among these major chemical constituents of wood, lignin is the most sensitive component that can absorb approximately 80-95% of UV light followed by holocellulose (5-20%) and extractives (2%) respectively (Norrstrom 1969, Hon and Shiraishi 2000). The photodegradation of wood is complicated due to its composite nature. Wood consists of multiple polymers—primarily lignin, cellulose, and hemicellulose—as well as low molecular weight extractives. Each of these components responds differently to solar radiation, complicating the overall degradation process (Evans et al. 2005, Evans 2015). Generally, photodegradation is related to two main processes: 1) the degradation reaction that is carried out under irradiation in the absence of oxygen (photolysis) and 2) the degradation process that occurs in the presence of oxygen (photooxidation) (Feist and Hon 1984). Photooxidation is, commonly, recognised as the major reaction in discolouration and photodegradation of wood (Chang 1982).

Water

Water significantly contributes to the weathering of wood, especially when combined with solar radiation. Weathering with light or water alone has effectively less influence in comparison to the combined effects of light and water (Anderson et al. 1991). Rainwater contributes to the greying of weathered wood surfaces by leaching out of photodegraded lignin, hemicellulose, and extractives (Feist and Hon 1984, Evans et al. 2005, Evans 2015). The grey layer is mainly composed of partially degraded cellulose (Rowell 2005). Moisture causes the wood to swell, opening inaccessible areas within the cell wall, thereby triggering further degradation by light (Feist and Hon 1984). Likewise, cyclic changes in moisture content during weathering generate mechanical stresses leading to consecutive swelling and shrinkage of wood. Subsequently, this results in the formation of micro cracks and checks. Enlargement of micro checks can later lead to the development of visible checks and cracks on the wood surface, eventually increasing surface roughness (Feist and Hon 1984, Evans and Banks 1988, Rowell 2005, Evans, Urban, and Chowdhury 2008). Roughening can also be caused by erosion of the wood surface due to variations in moisture during weathering (Rowell 2005). Water absorption of wood during weathering establishes moisture conditions and enhances the colonisation of microorganisms (Carey 1982). Wood exposed to shaded, wet climate creates favourable conditions for the growth of algae and may also develop

symbiotic relationships with fungi in the form of lichens (Rowell 2005). In addition to causing swelling and shrinking stresses in the weathered wood, high moisture content allows hydrolysis reactions to take place or promote other reactions that are carried on by ionic mechanisms (Kalnins 1966).

Temperature (heat/freezing)

Temperature (heat) is not considered a critical factor in the surface degradation of wood as solar radiation and water. Instead, it accelerates chemical reactions that contribute to weathering processes, such as photo-oxidation and hydrolysis, which are primarily driven by light and water (Evans et al. 2005, Rowell 2005). Temperature is also responsible for cyclic changes in wood's moisture content which leads to repeated swelling and shrinking of wood. This process results in the development of micro cracks and checks on the wood surface (Feist and Hon 1984, Rowell 2005).

Microorganisms

Discolouration of wood exposed outdoors in the presence of moisture is due to colonisation of fungi on the wood surface (Evans et al. 2005, Rowell 2005). The major fungal species colonising the weathered wood surfaces are classified under the subphylum ascomycotina e.g. fungi in the genera *Cladosporium*, *Aureobasidium*, *Alternaria*, *Stemphylium*, *Phoma*, *Hormonema*, *Penicillium*, *Aspergillus*, and *Trichoderma* (Rowell 2005). One of the most common fungal species that have been isolated from weathered wood surfaces is the black yeast-like fungus *Aureobasidium pullulans* (DE BARY) ARNAUD (Feist 1989, Evans et al. 2005, Rowell 2005, Evans 2015). Under favourable conditions, it can grow on the surface of various organic and inorganic materials and/or coatings (Bardage 1998). Moreover, *A. pullulans* is capable of utilising lignin and lignin degradation products as nutrition sources (Schoeman and Dickinson 1996, Schoeman and Dickinson 1997). According to its relatively resistant and adaptable nature, *A. pullulans* is highly compatible with the micro-environment of weathered wood (Rowell 2005).

Other factors

The presence of atmospheric pollutants (i.e. gaseous sulphur compounds, ammonia, nitrogen oxides, carbon monoxide, and saturated/unsaturated aliphatic and aromatic hydrocarbons and their derivatives) and particulates (i.e. dust and smoke particles) have been reported as additional weathering agents that can accelerate degradation rate of wood surfaces (Anderson et al. 1991, Evans et al. 2005, Rowell 2005, Evans 2015). Abrasion or mechanical action by wind, sand, and dirt also participate in the degradation and removal of particles from wood surfaces (Feist 1989). The abrasion rate becomes more intense in cold and dry environments (Terry and Buras 2019). Air pollutants can be deposited or transferred to material

surfaces by two mechanisms: 1) when the pollutants are dissolved and brought down by rainwater (wet deposition) and 2) when the pollutant, in the form of gases or particles, directly impacts or deposits on to soil, water, or vegetation at the Earth's surface (dry deposition) (Grossi and Brimblecombe 2002). Particularly, sulphur dioxide and nitrogen oxide in the atmosphere can dissolve in rainwater and form acid rain (Terry and Buras 2019). Later, through the complex oxidation reactions in the atmosphere, sulfuric acid and nitric acid are formed (Grossi and Brimblecombe 2002). When acid rain comes in contact with the wood surface, the cellulose structure can degrade (Spedding 1970, Grossi and Brimblecombe 2002, Evans et al. 2005). It has been reported that higher atmospheric deposition of nitrogen compounds can provide nutrients that promote the growth of microorganisms on wood surfaces (Grossi and Brimblecombe 2002). The key elements involved in the weathering process, inspired by Feist (1989) are summarised in Figure 2 (Feist 1989).

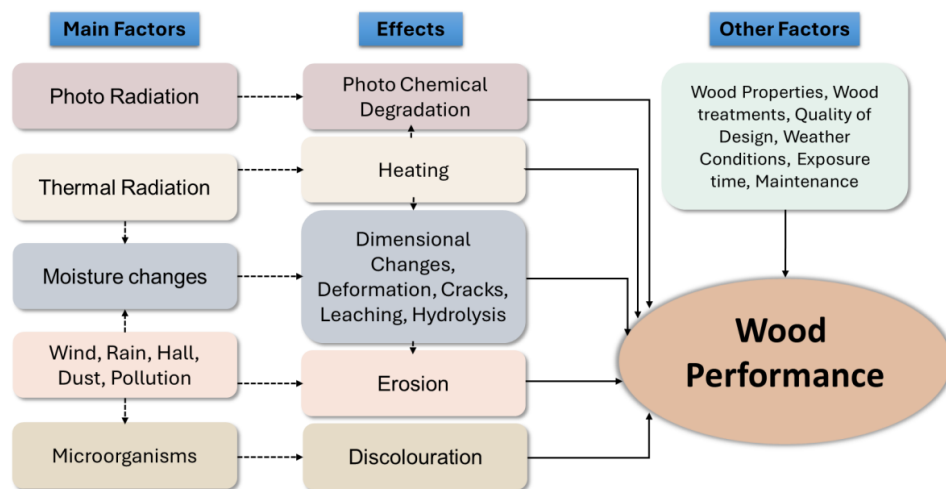


Figure 2. Key elements involved in the intricate weathering process.

2.1.2 Effects of weathering on wood properties

Chemical changes

When wood is exposed outdoors all major chemical components of wood are degraded and various chemical reactions take place (Evans et al. 2005). Photo-degradation is the major mechanism involved in wood weathering. It is initiated by the formation of free radicals and primarily initiated by the oxidation of phenolic hydroxyl. As the degradation progresses, the methoxyl and lignin content in the wood decreases, while acidity and carboxyl concentration increase. These processes are caused by the degradation of lignin that induces various degradation products

such as vanillin, syringaldehyde, high-molecular-weight compounds, and organic (carboxylic) acids (Kalnins 1966, Leary 1967, Leary 1968, Heitner 1993). Furthermore, additional chemical compounds such as formaldehyde, carbon monoxide, carbon dioxide, hydrogen, water, and methanol were observed (Kalnins 1966). Hemicelluloses can be degraded and leached from wood surfaces during weathering (Kalnins 1966, Evans, Michell, and Schmalzl 1992, Sudiyani et al. 1999). Cellulose is generally considered as the wood chemical constituent that is less affected by weathering than lignin and hemicelluloses (Evans et al. 2005, Rowell 2005). Hence, weathered wood surfaces tend to have a high cellulose content, as evidenced by the increased relative content of glucose observed after natural and artificial weathering (Feist, Rowell, and Ellis 1991, Sudiyani et al. 1999).

Micro and Macroscopic Changes

Microscopic changes, generally associated with the destruction and degradation of the middle lamella and wood cell wall, as well as the enlargement of pits apertures, often accompany the macroscopic changes in wood during weathering (Evans et al. 2005). Later, micro checks occur because of shrinkage in cell walls. Throughout the weathering process, the leaching and plasticising effects of water can accelerate the enlargement of the micro checks (Evans et al. 2005, Rowell 2005). Destruction of the lignin-rich middle lamella and cell wall is associated with degradation of lignin and leaching of water-soluble lignin degradation products due to weathering (Kuo and Hu 1991, Evans et al. 1992).

Changes in physical properties

Wood exposed outdoors promptly undergoes discolouration caused by the weathering process, indicating a chemical change. Light coloured woods, which includes most coniferous species, initially darken and then become yellowish or brownish due to accumulation of the photodegradation products of lignin and extractives on wood surfaces. However, after long periods of exposure the wood surface becomes grey due to leaching out of extractives and photodegraded lignin fragments from the surface leaving behind surface layers that become enriched with cellulose (Feist 1989, Evans et al. 2005, Rowell 2005). Finally, the surface becomes dark grey with a patchy appearance due to the presence of fungal spores, hyphae, and pigments (Evans et al. 2005, Rowell 2005). The glossiness and roughness of wood also change during weathering. These phenomena are related to the abrasion of wood surfaces and the associated erosion process. Consequently, the scatter of light on the surface increases, which changes the glossiness (Hon and Shiraishi 2000). In addition, cyclic changes of moisture content in wood generate stresses which lead to repeated swelling and shrinkage of wood, resulting in the formation of micro cracks and checks on the wood surface. Enlargement of micro checks can later cause the development of visible checks and cracks on the wood surface,

eventually increasing surface roughness (Chang et al. 1982, Evans et al. 2005, Rowell 2005, Evans 2015). Another consequence of weathering is the increase in surface wettability of wood. This change is primarily attributed to the loss of extractives and lignin from the exposed surfaces (Kalnins and Feist 1993, Rowell 2005). Discolouration and macroscopic changes of Scots pine due to natural weathering are shown in Figure 3.

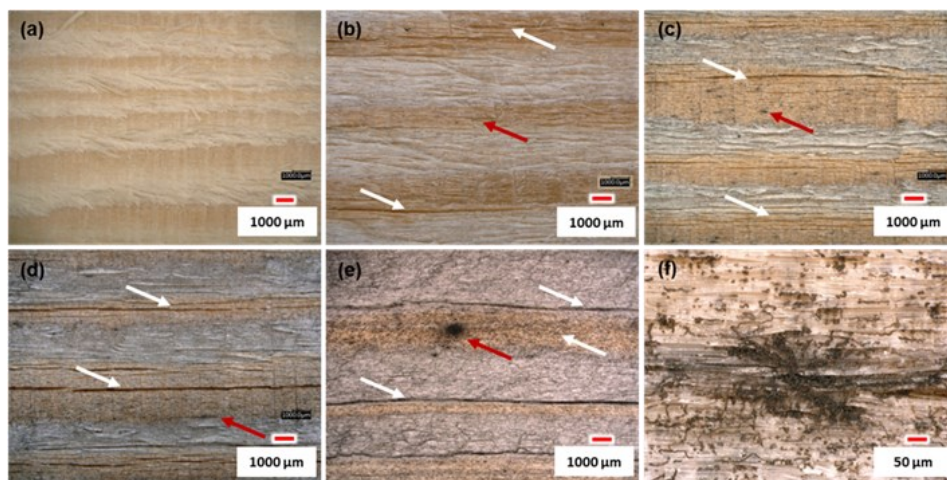


Figure 3. Discolouration and macroscopic changes of Scots pine wood exposed to natural weathering: (a) unexposed wood; (b) visible micro/miniature cracks and checks with darkening of the wood surface after 3 months of natural weathering; (c) enlargement of cracks with raised fibres and darkening of the wood surface caused by colonisation of fungi, visually detected after 6 months of exposure; (d) large cracks and checks, dark grey, blotchy appearance visible after 9 months of exposure; (e) large cracks and checks, dark grey surface with heavy colonisation of fungi following 12 months of exposure; (f) colonisation of fungi on the wood surface after 12 months of exposure. White arrows: cracks and checks; red arrows: fungal colonisation.

2.1.3 Protection

The weathering of wood can cause substantial economic loss by deteriorating and reducing the lifespan of wooden structures. It's important to protect the wood from these effects to maintain its functionality and aesthetic appeal. To tackle this challenge, researchers and industry professionals have developed different wood treatment solutions to mitigate weathering-induced damage. These treatments are designed to preserve the integrity and appearance of wood, thereby extending its service life and reducing maintenance costs.

Among the various wood protection methods, application of coatings emerges as one of the most prevalent and versatile solutions due to its cost-effectiveness and simplicity (Cogulet, Blanchet, and Landry 2018). In this context, a coating is described as a physical barrier that shields the bulk of the wood from environ-

mental factors and microorganisms. Typically, coating solutions can be categorised into two main types: film-forming and various impregnation approaches (Feist 1989, Rowell 2005). Film-forming finishes encompass a range of products, including various paints, varnishes, solid-colour stains, and overlays that bond to the surface and protect the wood from solar radiation. These finishes, which form a protective barrier over the wood surface, not only screen against the effects of solar radiation but also act as a deterrent to surface wetting and erosion (Feist 1989, Evans et al. 2005). Conversely, impregnation or penetration involve saturating the finished subsurface with chemical mixtures that contain hydrophobic substances capable of penetrating the wood and undergoing curing. It includes oils, water repellents, stains, preservatives, and surface treatments (Feist 1989). In contrast to paints, which establish a physical barrier, water repellents operate by creating a hydrophobic coating that plays a key role in minimising moisture absorption, thereby improving the dimensional stability of wood (Rowell 2005). Additionally, they decrease the leaching of photodegraded fibres from wood surfaces. Despite their prevalence, coatings are susceptible to weathering over time. Common issues include the photofading of dyed and pigmented polymers in the coating (Pintus, Wei, and Schreiner 2016, Cogulet, Blanchet, and Landry 2018), crack formation and peeling (Rowell 2005), a change of gloss and surface roughness (Christensen et al. 1999), the yellowing of clear coating (Singh, Tomer, and Bhadraiah 2001, Rosu, Rosu, and Cascaval 2009), and aesthetical problems caused by microorganisms (Horvath, Brent, and Cropper 1976, Gobakken and Westin 2008). The vulnerability of coatings to weathering highlights the need for continued research into more durable and effective wood protection solutions.

Applying preservatives extends the lifespan of wood structures, reducing replacement costs and improving the utilisation of forest resources. The effectiveness of protection depends on the choice of preservative with proper chemical penetration and retention. Moreover different wood types requiring specific approaches for optimal protection against weathering and decay (Ross 2010). Wood preservatives are classified into two broad categories: i) oil-type preservatives which include substances like creosote and petroleum solutions of pentachlorophenol. While effective, they can affect cleanliness of the wood, odour, colour, paintability, and fire performance. Heavy oils like creosote provide strong weather protection but may alter the wood's appearance and other properties, ii) waterborne preservatives which include copper-based compounds like Acid Copper Chromate (ACC), and Alkaline Copper Quat (ACQ), as well as borate compounds like inorganic boron (Borax-Boric Acid) (Ross 2010).

Wood modification is defined as *“the action of a chemical, biological or physical agent upon the material, resulting in a desired property enhancement during the service life of the modified wood. The modified wood should itself be nontoxic under service conditions and, furthermore, there should be no release of any toxic*

substances during service, or at end-of-life following disposal or recycling of the modified wood. If the modification is intended for improved resistance to biological attack, then the mode of action should be nonbiocidal” (Hill 2006). Wood modification aims to improve decay resistance, dimensional stability, water sorption, weathering performance, and other attributes (Hill 2006, Sandberg, Kutnar, and Mantanis 2017). It can be categorised into four main groups: i) Chemical modification, which involves chemical reactions with wood polymers, forming covalent bonds. For example, acetylation, which enhance resistance to swelling and decay, and improves dimensional stability, ii) Thermal modification, involves applying heat to wood in a low-oxygen environment, improving dimensional stability, hygroscopicity, and microbiological resistance. However, it may decrease certain mechanical properties, iii) Surface modification alters wood surface properties through physical (e.g. plasma discharge, microwave treatments) or chemical methods (e.g. grafting, sol-gel methods). It can improve stability, hydrophobicity, and compatibility with coatings, and iv) Impregnation modification involves filling wood with inert materials like monomers, polymers, resins, or waxes. This passive modification increases density, prevents water penetration, and enhances weathering resistance (Hill 2006, Sandberg et al. 2017).

Wood preservatives like Chromated Copper Arsenate (CCA) have proven to be highly effective; however, growing environmental concerns have led to increased scrutiny of their use. The toxicity of copper, chromium, and arsenic to aquatic organisms is well-documented (Flemming and Trevors 1989). Consequently, many countries have either banned CCA or restricted its use to specific applications (Wong and Chang 1991, Hill 2006). Similarly, modern coating formulations face challenges due to their composition. Many surface finishing products contain toxic chemicals that can negatively impact the environment and pose a risk to living organisms, including humans (Gesthuizen 2020). The growing awareness of environmental protection has led to increasingly restrictive international regulations on the use of previously allowed toxic substances. This shift in regulatory focus emphasises the importance of considering the long-term environmental impact and potential health risks associated with certain wood preservatives. It also encourages the development and adoption of alternative, more environmentally friendly preservative technologies in the wood treatment industry (Hill 2006).

2.2 *Aureobasidium pullulans*

Aureobasidium pullulans (DE BARY) ARNAUD (synonym: *Pullularia pullulans* (DE BARY) BERKHOU) is a polymorphic, oligotrophic and ubiquitous black yeast-like fungus. It is widely distributed across diverse habitats including forest soil, fresh and seawater, plants and animal tissues, building materials, etc. The name

“Black Yeast” was given due to its ability to produce a black melanin pigment (Deshpande, Rale, and Lynch 1992, de Hoog 1993). *A. pullulans* has been studied for over 100 years with different names from a wide range of habitats (Cooke 1959). The fungus belongs to the genus *Aureobasidium*, the phylum Ascomycota, and the order Dothideales (Schoch et al. 2006, Thambugala et al. 2014).

A. pullulans strains are classified as polyextremotolerant organisms (Gostinčar et al. 2010, Gostinčar, Grube, and Gunde-Cimerman 2011). These microorganisms can tolerate a wide range of harsh environmental conditions, such as cold environments (Andrews et al. 1994, Zalar et al. 2008, D’Elia et al. 2009, Branda et al. 2010, Vaz et al. 2011), environments with fluctuating levels of water activity (Zalar et al. 2008), hypersaline waters in salterns (Gunde-Cimerman et al. 2006, Gostinčar et al. 2010), polluted water (Vadkertiova and Slavikova 1995), bathrooms, food and feeds (Samson, Hoekstra, and Frisvad 2004), rocks and monuments (Urzi et al. 1999), and acidic and basic conditions (Ranta 1990, Shiomi et al. 2004). *A. pullulans* can grow in temperatures ranging from 4°C to 30°C with optimal temperature at 25°C (Zalar et al. 2008), in addition, it can withstand temperatures of 80 °C (Evans et al. 2005, Rowell 2005).

2.2.1 Morphology and life cycles

Aureobasidium pullulans exhibits polymorphism (Ramos and García Acha 1975, Kocková-Kratochvílová et al. 1980). The vegetative morphology of *A. pullulans* exhibits significant diversity; depending on environmental conditions, it can grow as budding yeast or as mycelia and form dark coloured chlamydospores or resistant spores (Ramos and García Acha 1975, de Hoog and Yurlova 1994, Slepecky and Starmer 2009, Gaur et al. 2010), nitrogen sources (Ramos and García Acha 1975), and carbon sources (Yurlova and De Hoog 1997).

The young colonies are initially smooth and later covered with a slimy mass of spores. The colour of young colonies is yellow, cream, light pink, or light brown, but eventually, often turn brown due to the development of dark-pigmented hyphae or conidia (Cooke 1959, Kocková-Kratochvílová, Černáková, and Sláviková 1980, Deshpande et al. 1992, Zalar et al. 2008). As the colonies mature, they usually turn black except at the margins. In some cases, blackened areas develop irregularly within the colony, forming patterns like radiating lines or sectors, appearing along the edges, or creating concentric rings that may cover parts or the whole colony (Cooke 1959), see Figure 4a.

Young hyphae are hyaline with smooth thin-walled, and transversely septate (Cooke 1959, Zalar et al. 2008). Dark-brown hyphae are occasionally formed in older cultures. As described by Zalar et al. (2008), conidiogenous cells in *A. pullulans* are undifferentiated and can be located intercalary or terminally on hyaline hyphae. Conidia are produced synchronously in dense groups from indistinct scars

or small denticles (Zalar et al. 2008). Colour of the conidia vary from hyaline to dark brown; hyaline conidia one-celled are smooth wall, ellipsoidal, and variable in shape and size ($7.5\text{--}16 \times 3.5\text{--}7 \mu\text{m}$) often with an indistinct hilum (Figure 4c). Dark brown conidia one - celled have a dimension of $10\text{--}17 \times 5\text{--}7 \mu\text{m}$; two - celled conidia, slightly constricted at the septum have a dimension of $14\text{--}25 \times 5\text{--}11 \mu\text{m}$ (Figure 4b). Budding of hyaline and dark brown conidia is commonly observed, where the secondary conidia are smaller compared to the primary ones (Figure 4d) (Zalar et al. 2008).

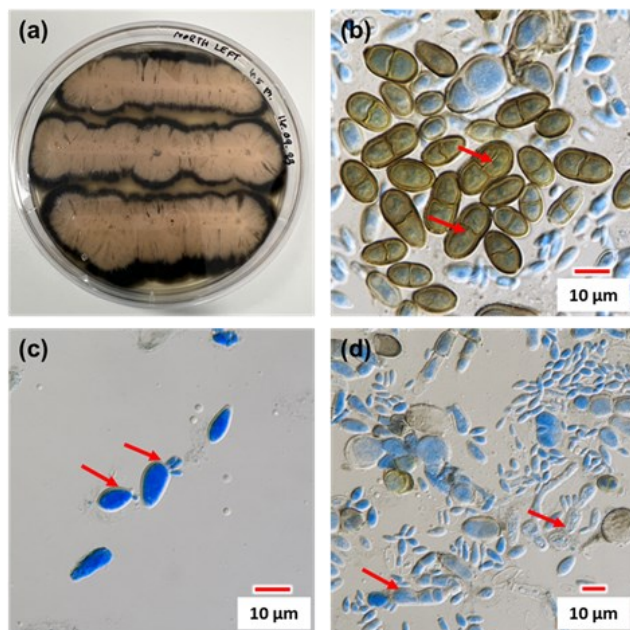


Figure 4. Macromorphology of *A. pullulans* incubated for 14 days at 25 °C in the dark on MEA (a) dark brown conidia with thick cell wall (b); liberated conidium transforming to budding cells (c); short hyphae synchronously producing conidia (d).

2.2.2 Bioproducts from *A. pullulans* and their potential applications

A. pullulans is biotechnologically important due to its ability to produce a wide range of natural products such as pullulans, siderophore, single-cell protein, and various types of enzymes including amylases, cellulase, proteases, esterases, pectinases, xylanases, and mannanases which are considered safe for biotechnological and environmental applications (Chan et al. 2011, Bozoudi and Tsaltas 2018). Some strains of *A. pullulans* have been widely used as a biocontrol agent for plant protection due to their antagonistic activity against plant pathogens (Chi et al. 2009, Chan et al. 2011, Prasongsuk et al. 2018).

Pullulan

A. pullulans synthesise pullulan - a unique biopolymer with economic importance. It is one of the biopolymers that has attracted great attention over recent years due to its peculiar characteristics and structural properties. Pullulan is a dry, white, odourless, and tasteless powder that is edible, nontoxic, non-mutagenic, non-hygroscopic, non-reducing, and biodegradable. It is highly soluble in hot/cold water and organic solvents, producing a clear and viscous solution with high adhesion and film-forming abilities (Leathers 2003, LeDuy et al. 2014). The thin film made up of pullulan is clear, highly oxygen-impermeable, thermally stable, non-hygroscopic under normal atmospheric conditions, and biodegradable (Rekha and Sharma 2007, LeDuy et al. 2014). These properties enhance its appeal for diverse industrial applications. In the food sector, pullulan serves as a low-calorie bulking agent and stabiliser, creating edible films for food preservation and enhancing the texture and shelf-life of various products. Due to its non-toxic, non-immunogenic, non-mutagenic, non-carcinogenic, and biodegradable nature, pullulan is currently intensively studied and widely used in various biomedical applications (Rekha and Sharma 2007). Furthermore, the lack of odour and taste, non-toxicity, non-irritability, high water solubility, tackiness, moisturising, and film-forming abilities make it suitable for cosmetic applications, particularly in skincare and antiageing products, shampoos, hair lacquers, and toothpaste (Joe Dailin et al. 2019, Ahmed and Soundararajan 2020).

Enzymes from A. pullulans

Many strains of *A. pullulans*, in different environments can produce different enzymes that show great potential in a wide range of applications. Amylases are enzymes that catalyse the hydrolysis of starch into diverse products including dextrin and progressively smaller polymers composed of glucose sugar units (Windish and Mhatre 1965, Gurung et al. 2013). Amylases are among the most important enzymes and are widely used in biotechnology, ranging from the bread and baking industry, starch liquefaction and saccharification, textile desizing, paper industry, detergent applications, medical and clinical chemistry to food, brewery, and pharmaceutical industries (Gupta et al. 2003, Aehle 2007, Gurung et al. 2013). Lipases are enzymes that catalyse the hydrolysis of long-chain acylglycerols to glycerol, free fatty acids, and mono- and diglycerides (Aehle 2007). Generally, lipases are used in the textile industry, detergent industry, food industry, biolipolysis in the processing of meat and fish, medical and cosmetic industries. Additionally, they are used as biosensors and in biodegradation across various industries, including palm oil, dairy, slaughterhouses, soap, and wastewater (Gurung et al. 2013). *A. pullulans* produces extracellular proteases PL5 that play a role in biocontrol activities against some postharvest pathogens of apple and peach

(Zhang et al. 2012) and in the production of bioactive peptides from shrimp muscle and spirulina powder (Chi et al. 2009, Zheng et al. 2022).

A. pullulans also produces xylanase, an enzyme that degrades the linear polysaccharide xylan, a major component of hemicellulose found in plant cell walls, into xylose (Beg et al. 2001). Xylanase is of special interest for use in various industrial applications such as the pulp and paper industry, fermentation and food and feed industries, and wastewater management (Beg et al. 2001, Chi et al. 2009). Laccases are multi-copper-containing enzymes that are well-known as lignin-degrading enzymes due to their ability to catalyse the oxidation of aromatic compounds, particularly phenols and anilines (Thurston 1994, Gianfreda, Xu, and Bollag 1999). Laccases play an important role in fungal enzyme systems for lignin degradation (Rich et al. 2013).

2.2.3 *Aureobasidium pullulans* - natural presence on weathered wood and its protective potential

A. pullulans is recognised as one of the most prevalent fungal species on weathered wood in various climates, both temperate and tropical (Evans et al. 2005, Rowell 2005). This prevalence is attributed to several key factors such as:

- 1) Extracellular enzyme production: this fungus can produce a range of extracellular enzymes e.g. laccase, amylase, protease, lipase, cellulase, and xylanase (Chan et al. 2011, Bozoudi and Tsaltas 2018). These enzymes can potentially degrade wood chemical components, such as cellulose, hemicellulose, lignin, and extractives (Rowell 2005).
- 2) Temperature resilience: the fungus can withstand high temperatures of up to 80°C, making it highly resilient to environmental extremes (Evans et al. 2005).
- 3) pH tolerance: *A. pullulans* can grow over a wide pH range (1.9–10.1), demonstrating its adaptability to various acidic and alkaline conditions (Evans et al. 2005).
- 4) Desiccation resistance: *A. pullulans* can survive for long periods without moisture (Evans et al. 2005)
- 5) Metabolism of lignin breakdown products: the fungus has the ability to metabolise lignin breakdown products allowing *A. pullulans* to thrive on weathered wood, where such substances are abundant (Schoeman and Dickinson 1996, Schoeman and Dickinson 1997).

Despite *A. pullulans* being capable of producing a wide range of extracellular enzymes, its ability to degrade cellulose and hemicelluloses in wood cell walls is generally limited (Rowell 2005). The fungus primarily targets sugars, carbohydrates, starch, proteins, fats, and extractives, which are easily accessible nutrients found in the ray parenchyma cells of the wood (Schmidt 2006). This selective

targeting limits its impact on the structural components of wood. Consequently, while *A. pullulans* may cause discolouration and aesthetic issues that can affect the appearance and quality of wood products, it does not lead to significant degradation of wood (Schmidt 2006, Zabel and Morrell 2020). The limited degradative impact of *A. pullulans* on wood, combined with its ability to colonise various wood species and treatments, has generated interest in its potential beneficial applications as a protective coating in construction.

2.3 Bioinspiration for wood protection

The world today faces critical challenges stemming from the rapid depletion of non-renewable petrochemical resources and severe global pollution, largely driven by population growth and industrialisation. As awareness of sustainability issues grow, there's an increasing public demand for green manufacturing processes in energy production and materials development. Nature's solutions, honed over billions of years by the relentless forces of natural selection, exhibit remarkable efficiency, sustainability, and optimisation. This efficiency, elegance, and adaptability that has emerged in the natural world serve as an abundant source of inspiration across various fields, including architecture, engineering, and materials science. Through meticulous examination and analysis of biological systems, mechanisms, and strategies found in nature, researchers can replicate, mimic, and modify these ingenious natural approaches. This process adopts creativity and enables the development of innovative materials, cutting-edge technologies, and biomimetic designs that address pressing human needs while drawing inspiration from the time-tested blueprints perfected through eons of evolution. Several terms are employed to convey the concept of “learning from nature” including bioinspiration, biomimicry, biomimetics, bionics, or biologically inspired design (BID) (Fayemi et al. 2017). Bioinspiration is defined as “*using phenomena in biology to stimulate research in non-biological research and technology*” (Whitesides 2015). Despite the established international standard on this topic, ISO 18458:2015(E) that describes the subtle differences, these terms are currently used synonymously in the literature (Fayemi et al. 2017, Katiyar et al. 2021). The subject matter covered in this thesis is a combination of the terms mentioned above.

2.3.1 Biological Systems and Bioinspiration

Nature, through the process of evolution, has created biological materials of remarkable performance from macro to nanoscales for the modification of their environment and survival. Biological materials exhibit a high degree of organisation, spanning from the molecular level through the nanoscale and microscale, up to the macroscale. This organisation is often hierarchical, featuring intricate

nanoarchitectures that collectively form a diverse array of functional elements (Sandak and Butina Ogorelec 2023). The complexity and efficiency of these natural structures offer a wealth of inspiration for technological innovation. Three intellectual vectors—function, simplicity, and dissipation (Whitesides 2015)—have been identified as particularly interesting and useful for selecting characteristics of biological systems. Generally, the concept of drawing inspiration from biological role models or natural phenomena can be categorised into three levels: organism, behaviour, and ecosystem (Benyus 1997, Chayaamor-Heil 2023; Zari 2007).

The organism level involves drawing inspiration from specific features, structures, or functions observed in living organisms (Chayaamor-Heil 2023). Researchers and designers study the forms and adaptations of various organisms, examining how these structures and features function to help the organism survive. This exploration serves as a basis for developing innovative solutions or technologies that incorporate nature-inspired designs. For instance, superhydrophobic and self-cleaning surfaces inspired by Lotus leaves (Nun, Oles, and Schleich 2002, Bhushan, Nosonovsky, and Jung 2008, Spaeth and Barthlott 2008, Eadie and Ghosh 2011), adhesives inspired by geckos' feet (Autumn et al. 2000, Geim et al. 2003, Hu, Xia, and Dai 2013), and structural colour inspired by animals, invertebrates, insects, and plants (Parker 1998, McPhedran et al. 2003, Vukusic and Sambles 2003, Parker 2004, Sun, Glover and Whitney 2010, Bhushan, and Tong 2013).

The behaviour level in biomimicry involves studying and emulating the process and performance exhibited by organisms in their natural environments (Chayaamor-Heil 2023). Instead of directly imitating the physical characteristics of the organism, the emphasis is on understanding and replicating the functional aspects of their behaviour. This may include observing how organisms interact with their surroundings, adapt to changing conditions, and achieve specific tasks or functions. By drawing inspiration from the behavioural strategies of organisms, designers and researchers aim to develop innovative solutions that address complex challenges and enhance efficiency in various fields. For instance, bionic adaptive camouflage material that can dynamically change its surface colour in response to optical environments (Drozdowski and Gupta 2009, Eid et al. 2021, Qiao et al. 2021) was inspired by animals that can rapidly change colour and pattern in response to various external stimuli such as chameleons, octopuses, squids, cuttlefishes, and other cephalopods. Bioinspired self-repairing materials are inspired by self-repair mechanisms in living beings; for example, self-healing concrete (Jonkers 2007, Tittelboom and Belie 2013, Seifan, Samani, and Berenjian 2016, Belie et al. 2018), self-healing polymers (Bollinger et al. 2001), self-repairing wood coating (Sailer, van Nieuwenhuijzen, and Knol 2010, van Nieuwenhuijzen et al. 2016), self-healing and superhydrophobic coatings (Speck and Speck 2019).

The ecosystem level in biomimicry draws inspiration from complex interactions, symbiotic relationships, and waste utilisation observed in natural ecosystems. These aim to create systems that mimic the resilience and sustainability found in nature. Architects working at this level require a broader understanding of biology, including how organisms interact with each other and their environments, expertise in ecological domains such as biodiversity, niche construction, food webs, complex networks, and social ecology (Chayaamor-Heil 2023). Notable examples of architectural projects at this level include the Sahara Forest Project (2012) and the Regen Village (2016) (Chayaamor-Heil 2023), the Lloyd Crossing Project proposed for Portland, Oregon, by a design team that includes Mithūn Architects and GreenWorks Landscape Architecture Consultants. John and Nancy Todd's Living or Eco Machines mimic the process of wastewater treatment in ecosystems by integrating it with plants (Todd and Josephson 1996, Todd 2003), the Australian-developed Biolytix® system mimics soil-based decomposition for the treatment of grey and black water. It integrates actual worms and soil microbes into the process (Zari 2007).

2.3.2 Bioinspiration for wood protection with a living coating system

Coating application is a common, economical, and convenient method for wood protection. However, weathering can lead to various forms of coating degradation as described previously. These degradation processes necessitate frequent maintenance, resulting in significant economic losses. Repeated application of coatings for maintenance purposes contributes to increased environmental impact through the consumption of additional resources and the potential release of harmful substances during the reapplication process. Additionally, many of these coatings contain hazardous chemicals that have the potential to negatively impact the environment and living organisms, including humans. Growing environmental awareness has led to increasing restrictions on the use of these toxic substances through international regulations (European Commission 2020).

Biofinish technology exemplifies a bioinspired and environmentally friendly solution with protective, decorative functionalities, and self-repairing properties for wood treatment. This approach inspired by the natural growth of microorganisms on wood leverages the functional biofilm produced by the black, yeast-like fungus *Aureobasidium pullulans* for wood protection (Sailer et al. 2010). The concept generally includes impregnation of the wood substrate with vegetable oil, specifically linseed oil. Subsequently, the wood's surface is coated with a proprietary Biofinish emulsion, which contains the necessary ingredients for *A. pullulans* to develop a long-lasting, self-sufficient living biofilm (Sailer 2013). The oil enhances the hydrophobicity of the wood, which improves the dimensional stability of the

wood substrate and serves as an additional nutritional source for *A. pullulans*, the living and active ingredient of the coating (Sailer et al. 2010). The presence of *A. pullulans* living cells plays a crucial role in protecting wood against colonisation of other wood-infestation fungi. The melanin pigment produced by the fungus contributes to an aesthetically appealing dark surface and simultaneously protects against UV radiation (Sailer et al. 2010). Moreover, the attractive aspect of using *A. pullulans* as a living coating system is its potential self-healing ability. The concept idea of Biofinish is shown in Figure 5. Biofinish is composed entirely of natural substances, offering a completely sustainable wood treatment solution with low environmental impact and low maintenance requirements (Sailer 2013). Biofinish coated timbers are currently produced at an industrial scale and Biofinish coating is available on the market (<https://www.xyhlo.com/en/>).

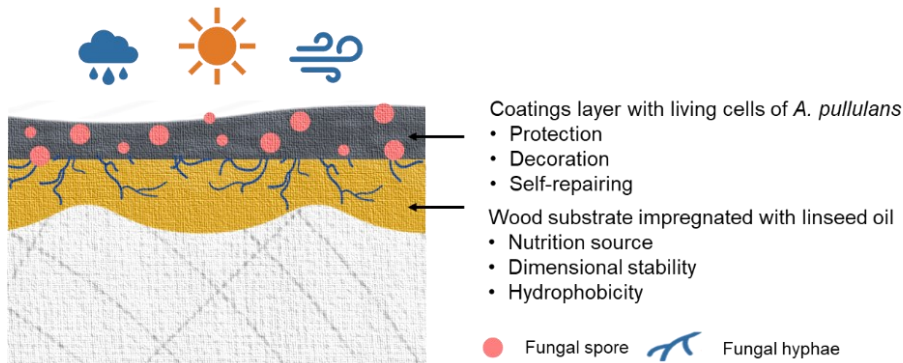


Figure 5. The concept idea of Biofinish coating.

3 Materials and Methods

3.1 Wood and natural weathering of wood

3.1.1 Sample preparation

The wood material used in **Paper 1** and **Paper 3** consisted of Scots pine sapwood blocks each with dimensions of 150 mm (length) × 75 mm (width) × 20 mm (thickness). In **Paper 3**, half of the sample set was coated with Biofinish (Biofinish, Xyhlo B.V. Deventer, The Netherlands) following the standard industrial application procedures. The wood samples were conditioned under controlled climatic conditions at a temperature 20°C and 65% RH before the tests. Note that the samples were not sterilised prior to the experiment.

In **Paper 2**, the wood samples included a variety of treatments, natural wood, thermal, chemical, and surface treatments, as well as impregnated wood, biobased composites, and hybrid modifications combining more than one of these categories. All information available about the samples can be found in **Paper 2**.

For **Paper 4**, the study was conducted on the external surface of the InnoRenew CoE building located in Izola, Slovenia. The façade is made of European larch wood (*Larix decidua* Mill.), was impregnated with linseed oil and coated with 3 layers of Biofinish (Xyhlo B.V. Deventer, The Netherlands), following the standard industrial application procedures; further details can be found in **Paper 4**.

3.1.2 Natural weathering of wood

In **Paper 1**, natural weathering of wood was conducted in two distinct European climate zones: Izola (Slovenia, 45°32'12.98"N, 13°39'42.98"E), representing warm temperate climates (Cfa) with hot, fully humid summers, and cool to mild winters; and Skellefteå (Sweden, 64°45'2.41"N, 20°57'10.04"E), representing subarctic or boreal climates (Dfc) with very long, cold freezing winters, and short, cool summers (Kottek et al. 2006). Locations were selected to represent diverse scenarios of deterioration mechanisms and corresponding changes to the aesthetical performance of wood exposed to natural weathering. Samples were mounted on a vertical stand, arranged such that the grain direction of the samples was perpendicular to the stand base, mimicking vertical façade siding configuration.

The wood samples were simultaneously exposed to natural weathering at both locations for 12 weeks, starting from July 1st until September 30th, 2021. Hourly local weather conditions were recorded during the exposure period (<https://www.meteoblue.com>).

In **Paper 2**, natural weathering of the wood materials was conducted at the weathering test station located on the roof of the InnoRenew CoE building in Izola, Slovenia (45°31'49.1"N 13°39'28.0"E). The wood samples were placed on vertical stands corresponding to a typical building façade configuration and exposed to the four cardinal directions. The wood grain direction of samples was parallel to the stand base, mimicking horizontal façade siding configuration. Meteorological data were obtained from the Historical Weather API (<https://open-meteo.com/>).

In **Paper 3**, natural weathering tests were performed in San Michele, Italy (46°11'15"N, 11°08'00" E), for a total period of 12 months, starting in March 2017 to March 2018. Samples were mounted on a vertical south-facing stand. The wood grain direction of samples was perpendicular to the stand base, mimicking vertical façade siding configuration.

Paper 4, the study was conducted on the external surface of the InnoRenew CoE building located in Izola, Slovenia (45°31'49.1'' N, 13°39'28.0'' E). The meteorological data were obtained from the Historical Weather API (<https://open-meteo.com/>).

3.2 Evaluation of microbial growth on wood surface

3.2.1 Sampling, cultivation, and molecular identification of fungi

The sampling and cultivation procedure in **Paper 1** and **Paper 4** involved using a wet sterile cotton swab to mechanically swab the surface of wood samples, which was then streaked out on Malt Extract Agar (MEA, Biolife, Milan, Italy). The plates were incubated in a growth chamber at 25 °C for 7 days. The representative colony of the prevalent morphology was isolated to a pure culture on MEA plates.

In **Paper 2**, the collection of surface samplings from wood samples was carried out using wet, sterile nylon swabs (Citotest, Haimen, Jiangsu, China). Thereafter, the swabbed material was directly spread on Dichloran glycerol agar plates (DG-18, NutriSelect® Plus, Merck Millipore, Merck KGaA, Darmstadt, Germany). The representative colony of the prevalent morphology was isolated to a pure culture on potato dextrose agar (PDA, Biolife, Milan, Italy) plates. Methodological overview of sampling, cultivation, and identification of fungi employed in **Paper 1**, **Paper 2**, and **Paper 4** is shown in Figure 6.

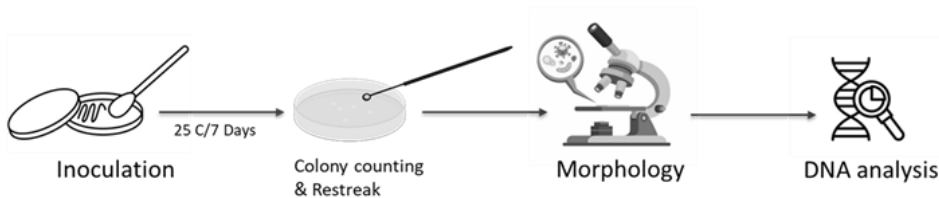


Figure 6. Methodological overview: fungal sampling, cultivation, and identification of fungi employed in **Papers 1, 2, and 4.**

In **Paper 1**, **Paper 2**, and **Paper 4**, the genomic DNA of the isolates were extracted after mechanical lysis in CTAB buffer according to the protocol described by Gerrits van den Ende and de Hoog (Gerrits Van Den Ende and De Hoog 1999). Identification was based on PCR amplification and Sanger sequencing of certain DNA regions/genes. The fungi were identified by comparing the sequences of the most similar type strains to other closely related strains stored in the non-redundant GenBank nucleotide database with the blast algorithm (Altschul et al. 1990). Further details regarding molecular identification of fungi can be found in **Paper 1**, **Paper 2**, and **Paper 4**.

3.3 Assessment of surface performance

3.3.1 Surface topography

In **Paper 4**, the 3D surface topography of wood samples was assessed using a Keyence VHX-6000 digital microscope (Keyence, Osaka, Japan). An area of 20 mm x 20 mm was examined by implementing a stitching algorithm for acquired 3D images. The proprietary software (VHX-6000v.3.2.0.121) of the Keyence microscope was used for post-processing the surface topography.

3.3.2 Gloss and colour measurement

In **Paper 4**, a portable MicroFlash 200D spectrophotometer (DataColour Int, Lawrenceville, NJ, USA) was used to assess colour changes in the wood samples. The selected illuminant was D65, with a viewer angle of 10°. The colour measurements were taken at ten randomly spots across the surface of the investigated wood samples. The mode of light reflection from the evaluated surfaces was measured using a REFO 60 (Dr Lange, Düsseldorf, Germany) portable gloss meter with incidence and reflectance angles of 60°.

3.3.3 Wettability

In **Paper 4**, the optical tensiometer Attension Theta Flex Auto 4 (Biolin Scientific, Gothenburg, Sweden) was used to determine the dynamic contact angle with distilled water. Five measurements were performed on each specimen, using the sessile drop method, with a nominal volume of each drop of 4 μL .

3.3.4 Visual appearance and microscopic observation

In **Paper 4**, high-quality images of the wood samples were obtained using a Konica Minolta Bizhub C258 office scanner with a resolution of 600 dpi. Micro-scopic observations were carried out using the Keyence VHX-6000 digital microscope (Keyence, Osaka, Japan).

4 Results and discussion

4.1 Colonisation of *A. pullulans* on wood surfaces

Microbial colonisation of wood is a complex process influenced by different factors which can be broadly categorised into direct or indirect effects (Brischke, Bayerbach, and Rapp 2006). Whereas climate conditions are indirect factors, direct factors can be further classified into endogenous factors which relate to the inherent properties of the wood itself, and exogenous factors; these encompass various physical and biological factors and environmental influences (Brischke et al. 2006, Schmidt 2006, Zabel and Morrell 2020). Physical and chemical factors include nutrients, water, air, temperature, pH value, light exposure, and gravitational force (Schmidt 2006). Biological influences arise from interactions between different organisms, such as antagonism, synergism, and symbiosis (Schmidt 2006). However, fungal species exhibit varying adaptability to environmental conditions, including climate factors, nutrient availability, pH levels, and other hazards. While some fungi thrive in specific environmental niches, others demonstrate resilience to diverse types of fluctuations. The broad adaptability of certain fungal species allows them to thrive in diverse environmental conditions, enabling them to occupy a wide range of ecological niches and effectively respond to environmental changes. This adaptability is crucial for their survival and persistence in natural ecosystems, as well as on wood substrates.

Exploring the resilience of *Aureobasidium pullulans* across different climate conditions, wood species, and treatment types is essential to maximize the effectiveness of bioinspired living coatings. *A. pullulans* demonstrates remarkable adaptability, yet understanding how it responds to specific climate variables, such as temperature extremes, humidity fluctuations, and UV exposure, will be crucial in tailoring its application for diverse environments. Additionally, the interaction of *A. pullulans* with various wood species and treatments can influence its colonisation ability, biofilm stability, and overall protective efficacy. By investigating these factors, we can refine the selection and application of *A. pullulans* to develop robust, adaptable coatings that perform consistently across different ecological settings, wood types, and environmental stressors. This comprehensive understanding will enhance the resilience and durability of fungal-based coatings, supporting their potential as sustainable wood protection solutions.

4.1.1 Colonisation of *A. pullulans* in Response to Weather Conditions

Paper 1 revealed fungal species from the genera *Aureobasidium*, specifically *A. pullulans* and *A. melanogenum*, as the predominant fungal species found on the weathered wood surfaces exposed to natural weathering under the period of 12 weeks in two distinct climate zones (see Table 1). The first zone, Izola, is characterised by a humid subtropical climate (Cfa) with hot, humid summers. The second zone, Skellefteå, is classified as a subarctic or boreal climate (Dfc) with short, cool summers. Notably, *Aureobasidium* colonisation was detected on the surface of weathered wood in both locations after just two weeks of exposure, indicating its rapid establishment. *A. pullulans* successfully colonised weathered wood during September, despite moderately low temperatures characteristic of the subarctic climate in Skellefteå, Sweden. Conversely, in Izola, Slovenia, the same species thrived during high summer temperatures in the humid subtropical climate. Paper 2 further corroborated these findings, showing *Aureobasidium* sp. as the predominant genus on most test materials after one month of exposure to natural weathering from October to November with moderately low temperatures in Izola, Slovenia (Figure 7). These consistent results across diverse climatic conditions highlight *Aureobasidium*'s remarkable adaptability and its capacity to promptly colonise weathered wood surfaces regardless of geographic and climatic zones.

Table 1. Culturable fungal species identified in Skellefteå, Sweden (■) and Izola, Slovenia (□) over a 12-week natural weathering period, **Paper 1**.

Fungal species	Time of exposure (weeks)					
	2	4	6	8	10	12
<i>Aureobasidium melanogenum</i>	□■	□■	■			
<i>Aureobasidium pullulans</i>		■	□■	□■	□■	□■
<i>Cladosporium allicinum</i>	□					
<i>Cladosporium crousii</i>	□	□				
<i>Cladosporium pseudocladosporioides</i>	□	□				
<i>Cladosporium</i> sp.		□				
<i>Cladosporium westerdijkiae</i>			■			
<i>Endoconidioma carpetanum</i>				■		
<i>Lithohypha guttulata</i>					□	
<i>Phoma herbarum</i>	□	□			□	
<i>Pseudotaeniolina globosa</i>				□	□	
<i>Stachybotrys</i> sp.	□					
<i>Sydowia polyspora</i>						■

4.1.2 Colonisation of *A. pullulans* on different untreated wood species

Wood properties such as the ratio of sapwood and heartwood, surface quality, extractives content, and permeability influence its susceptibility to colonisation by microorganisms, including mould fungi (Theander, Bjurman, and Boutelje 1993, Viitanen 1994, Terziev and Boutelje 1998, Johansson, Mjörnell, and Arfvidsson 2017). The susceptibility of wood to colonisation of fungi may vary among tree species, among individual trees, and among different parts within individual trees. However, the results from **Paper 2** highlight the ability of the mould fungus *A. pullulans* to colonise different untreated wood species, regardless of whether they are hardwoods or softwoods. Specifically, *A. pullulans* was observed to colonise kiln-dried Scots pine heartwood, kiln-dried fir, and oak (corresponding to samples 3, 23, and 29 in Figure 7.). Notably, kiln-dried Scots pine heartwood was less susceptible to mould growth compared to other untreated wood. This can be attributed to the presence of pinosylvin, a phenolic substance extractive, in Scots pine heartwood which plays an important role in the natural durability of Scots pine (Ek, Henriksson, and Lennholm 2009).

The findings indicate that *A. pullulans* is exceptionally adaptable to different wood substrates, despite the inherent differences in chemical and physical properties. This adaptability makes *A. pullulans* a promising candidate for developing broadly applicable bioinspired living coatings across a wide range of wood substrates.

4.1.3 Colonisation of *A. pullulans* on different treatments

In **Paper 2**, colonisation of *Aureobasidium* sp was detected on different wood treatments, as shown in Figure 7. These treatments encompass various modification techniques and surface applications, demonstrating the fungus's adaptability to different wood substrates, such as

- thermally modified spruce
- thermally modified spruce with the addition of FeSO₄
- furfurylated Scots pine
- Scots pine impregnated with TiO₂ nanoparticles
- Scots pine impregnated with TiO₂ nanoparticles and linseed oil
- thermally modified fir
- Scots pine coated with industrial transparent coating thermally
- thermally modified oak
- thermally modified spruce

These treatments correspond to samples numbered 1, 2, 7, 8, 9, 24, 25, 30, and 33 in Figure 7, respectively.

This finding supports earlier research demonstrating *A. pullulans*' capacity to colonise diverse surfaces, including painted wood (Horvath et al. 1976, Bardage

1998, Bardage and Bjurman 1998, Schmidt 2006), and wood with semi-clear coatings (Cogulet, Blanchet, Landry, et al. 2018). *A. pullulans* has also been identified as a predominant fungal species on coated thermally modified and furfurylated wood (Gobakken and Westin 2008). Nevertheless, these modifications of wood have been recognised as effective methods to enhance its resistance to biological attack (Hill 2006), and numerous studies have consistently demonstrated the improved decay resistance of thermally modified wood (Kamdern, Pizzi, and Jermannaud 2002, Weiland and Guyonnet 2003, Boonstra et al. 2007, Yilgör and Nami Kartal 2010). This is particularly significant as it demonstrates the fungus's ability to colonise not only untreated wood but also modified wood specifically designed to resist fungal growth.

4.1.4 Colonisation of *A. pullulans* across cardinal directions

Considering fungal composition in response to cardinal directions, in **Paper 2**, fungi from the genus *Aureobasidium* were consistently observed on samples exposed to all cardinal directions, see Figure 7. This suggests the broad adaptability and prevalence of *Aureobasidium* across varying environmental conditions, regardless of cardinal direction. The doses of UV, wind, and wind-driven rain depend significantly on the exposure direction, influencing weathering of material. This, in turn, may directly and indirectly affect fungal growth.

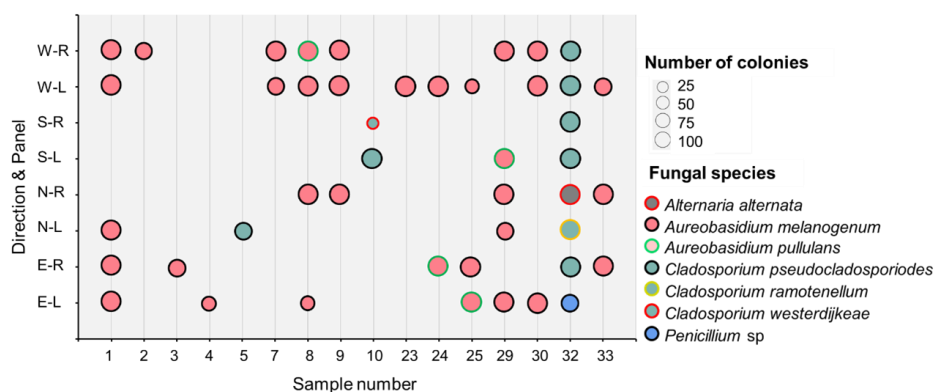


Figure 7. Culturable fungal colonies and fungal species identified on different wood specimens after one month of exposure to natural weathering (**Paper 2**).

Based on the results obtained from **Paper 1** and **Paper 2**, *A. pullulans* exhibited prolific colonisation, consistently being the most frequently observed species on wood surfaces regardless of climate conditions, cardinal directions, wood species, and treatments. This success can be attributed to its adaptability to a broad range of environmental conditions, minimal nutrient requirements, ability to

metabolise lignin degradation products, resilience against desiccation and high temperatures (Kühne et al. 1970, Feist and Hon 1984, Sharpe and Dickinson 1992, Garg and Dhawan 2006, Schoeman and Dickinson 1997, Rowell 2005), and antagonistic action against a diverse spectrum of fungi, including wood-decaying fungi (Schena et al. 1999, Castoria et al. 2001, Sugawara and Nikaido 2014). One of the key characteristics enabling *A. pullulans* to survive in a wide range of ecological niches and tolerate diverse environmental challenges is its capacity for phenotypic plasticity. This phenomenon involves *A. pullulans*' ability to respond to environmental cues by changing their morphology, physiological state, or behaviour (Slepecky and Starmer 2009, Han et al. 2019). In response to fluctuating environmental conditions, this fungus can adjust its morphologies, such as colony texture, colour, size, and varying degrees of melanin accumulation (Slepecky and Starmer 2009). It can also modulate its physiological condition by regulating metabolic pathways, enzyme production, and energy utilisation to optimise growth and survival (Zajc et al. 2020). Additionally, *A. pullulans* may adapt its behaviour by forming biofilms or exhibiting antagonistic activity against competing microorganisms to enhance its adaptability to various ecological niches even during periods of strong wind and heavy rain (Andrews et al. 1994, Slepecky and Starmer 2009, Zajc et al. 2020) as the evidence shows in **Paper 1** and **Paper 2**.

In conclusion, the findings from **Paper 1** and **Paper 2** highlight the exceptional suitability of *A. pullulans* as the active ingredient for bioinspired living coatings for wood protection. Its resilience and adaptability to diverse environmental conditions, along with its strong antagonistic activity against other wood-decaying fungi, make it a promising candidate for bioinspired living coatings. These properties will establish the fungal-based coating inspired by nature as an alternative, sustainable, and robust solution for long-term wood protection. It is expected that such coatings can effectively protect wood surfaces across a wide range of climate conditions, offering versatility and reliability in wood preservation efforts.

4.2 Performance of bioinspired living coating

Wood exposed outdoors undergoes degradation due to the natural weathering process which is the combined action of environmental factors and biological attacks. Applying a coating is one of the most common methods to prevent wood alteration and extend the service life of the material (Cogulet, Blanchet, and Landry 2018). Durability or "service-life performance" is a critical value criterion for wood coatings, which must exhibit longevity and maintain their protective and aesthetic qualities over time to ensure the preservation of wooden surfaces. Effective wood coatings must protect against UV radiation, water ingress, and microbial attack. Despite the application of coatings that are widely used for wood protection,

degradation due to weathering can occur through various deterioration processes (Horvath et al. 1976, Allen 1994, Singh et al. 2001, Rowell 2005, Bulcke, Acker, and Stevens 2008, Gobakken and Westin 2008, Rosu et al. 2009, Cogulet, Pintus et al. 2016, Blanchet, and Landry 2018). These changes can occur not only on the surface of the coating but also within the interior of the coating and the underlying wood substrate (Bulcke et al. 2008). High-performance wood coatings should therefore adhere well to the wood substrate, remain flexible, and resist cracking, peeling, and fading.

This section evaluates the performance of wood coated with the commercial bioinspired living coating (Biofinish) after one year of exposure to natural weathering. It focuses on changes in surface colour, wettability, gloss, and surface topography - critical indicators of the coating's durability and effectiveness in protecting wood surfaces outdoors. Understanding these changes over an extended period assesses the coating's ability to maintain its protective and aesthetic qualities. This knowledge can guide the development of enhanced bioinspired living coating systems with optimised performance capabilities, enabling more robust and long-lasting protection for wood surfaces in various outdoor applications.

4.2.1 Surface Colour

Colour fading of wood coatings is a common issue caused by the degradation of pigments due to exposure to sunlight, particularly UV radiation. Exposure to UV radiation can initiate complex photochemical processes within the coating matrix, leading to the degradation of pigments, binders, and other additives (Cogulet, Blanchet, and Landry 2018). These chemical changes can manifest as visible alterations in the coating's appearance, such as fading, discolouration, chalking, or increased brittleness (Christensen et al. 1999, Cogulet, Blanchet, and Landry 2018). The degradation of the coating's components can compromise its protective properties, making the underlying substrate more susceptible to further deterioration.

The colour stability of Biofinish coating is significant, demonstrating its efficacy in protecting the wood surfaces over time. Comparative studies, such as those outlined in **Paper 3**, reveal that surfaces treated with Biofinish exhibit superior resistance to colour degradation compared to uncoated wood. This is evidenced by negligible variations of the $CIE L^*a^*b^*$ colour and significantly lower values of ΔE , indicating minimal colour change, even after one year of exposure to environmental factors (Figure 8). Such exceptional colour stability highlights the effectiveness of Biofinish in maintaining the aesthetic appeal and visual integrity of wood surfaces, making it a preferred choice for outdoor applications. This enhanced colour stability of Biofinish can be attributed to the presence of melanin pigment produced by *A. pullulans*. Melanin, produced by *A. pullulans*, plays a pivotal role in

Biofinish coatings, contributing to both colour stability and protective functions (Sailer et al. 2010). As a pigment, melanin determines the colour of the coating, ensuring that the wood surface maintains its original hue over time. Beyond its aesthetic role, melanin acts as a robust protective barrier, shielding the wood against environmental stressors such as UV radiation.

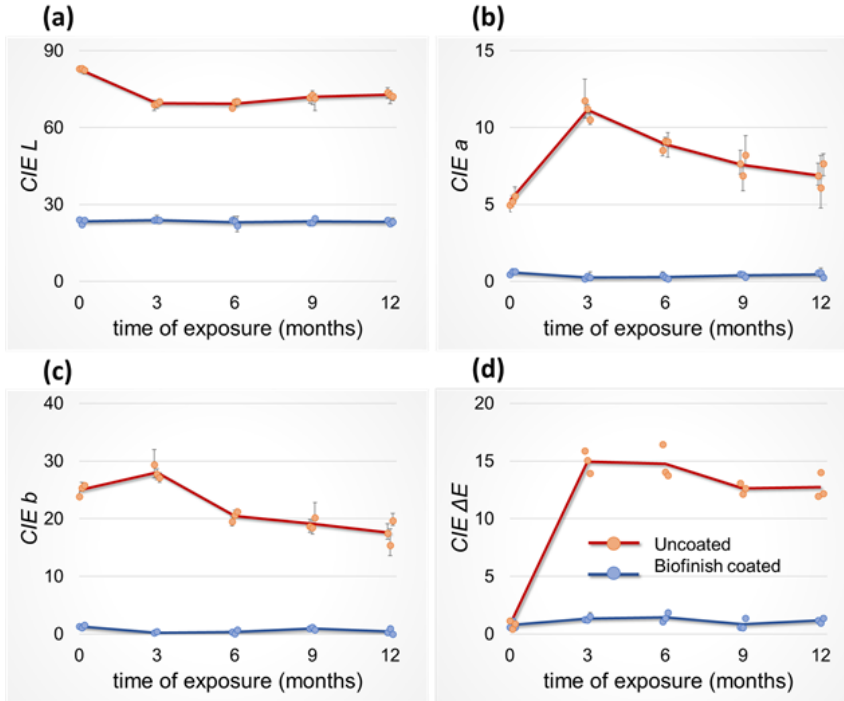


Figure 8. $CIE L^*a^*b^*$ colour coordinates of uncoated and Biofinish – coated Scots pine wood as a function of exposure time. Error bars correspond to the range (minimum–maximum) of results observed: (a.) $CIE L^*$, (b.) $CIE a^*$, (c.) $CIE b^*$ and, (d.) $CIE \Delta E$ (**Paper 3**).

4.2.2 Gloss evaluation

Glossiness is one of the key parameters that indicates the aesthetic performance of coatings, reflecting both physical and chemical changes within the coating material. In certain cases, gloss loss is even used for predicting the service life of coatings (Guseva et al. 2003). As coatings degrade due to environmental factors such as UV radiation, moisture, and temperature fluctuations, their glossiness can diminish. This parameter is often a visible sign of underlying deterioration processes, including surface roughening, pigment fading, and binder breakdown (Bulcke et al. 2008). Maintaining high glossiness is essential for preserving the visual appeal and protective properties of coatings, making it a crucial aspect of coating performance

evaluation. The change in gloss of weathered wood is generally attributed to surface mechanical abrasion and chemical erosion (Rowell 2005).

Paper 3 demonstrated the exceptional performance of the Biofinish coating in maintaining gloss for 1 year of exposure to natural weathering. The gloss measurements, conducted both parallel and perpendicular to the wood fibres on Biofinish-coated Scots pine specimens, revealed that the coating's gloss levels remained consistent throughout the entire weathering process (Figure 9a and b). This gloss stability is an indication of the coating's ability to resist the degradative effects of environmental factors, such as ultraviolet radiation, moisture, and temperature fluctuations, which typically cause a loss of gloss in conventional wood coatings. The gloss retention capabilities of the Biofinish coating can be attributed to its unique composition and the self-healing properties of *A. pullulans*. As the coating ages or experiences minor surface damage, *A. pullulans* can continuously regenerate and replenish the protective layer, preserving the desired gloss levels. This outstanding gloss performance not only enhances the visual appeal of the coated wood but also has practical implications for maintaining the coating's protective properties.

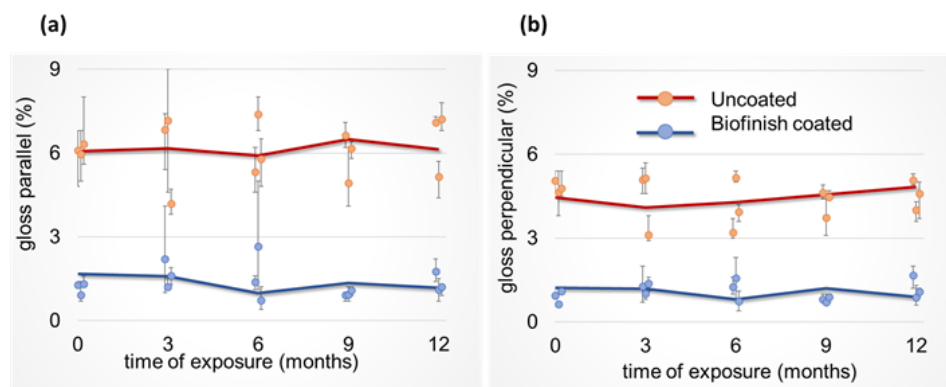


Figure 9. Gloss changes parallel (a) and perpendicular (b) to grain of uncoated and Biofinish – coated Scots pine wood as a function of exposure time. Note: Error bars correspond to the range (min to max) of results observed (**Paper 3**).

4.2.3 Wettability

Wettability of a solid surface by a liquid is typically quantified by measuring the contact angle formed between the solid and the liquid interface (Yuan and Lee 2013). A smaller contact angle indicates higher wettability, while a larger contact angle signifies lower wettability (Gast and Adamson 1997, Nejad and Cooper 2017). Contact angles lesser than 90° correspond to high wettability, but conversely, contact angles greater than 90° signify low wettability (Yuan and Lee 2013). Wood's natural hygroscopicity, due to its porous structure and chemical composition,

allows it to readily absorb water, which can lead to swelling, warping, and deterioration over time. Weathering significantly increases the wettability of wood caused by the depletion of extractives and lignin from exposed surfaces (Kalnins and Feist 1993, Rowell 2005). The increased wettability of weathered wood surfaces contributes to further subsurface degradation, resulting in more pronounced surface erosion and corrosion. Additionally, high levels of surface wetting can raise moisture content, leading to the loosening of cellulose fibers. The removal or washing away of these fibers by wind-driven rain initiates the formation of micro-cracks, leading to an increase in micro-roughness (Gonzalezde et al. 2016). Modifying the wettability of wood through coatings, sealants, modification, and chemical treatments is crucial for enhancing its water-repellence and weathering resistance (Hill 2006). As a result, it extends the service life and durability of wood, reducing maintenance costs and environmental impact in both construction and decorative applications. The contact angle on wood is also an important parameter in assessing the performance of wood coatings (Petric and Oven 2015).

Biofinish coating demonstrated the ability to significantly enhance the hydrophobicity of wood, as evidenced in **Paper 3**. The initial contact angle of the Biofinish-coated wood was approximately 120°, indicating a high degree of hydrophobicity and water-repellent behaviour. This contact angle was notably higher than the contact angle measured on uncoated Scots pine surfaces, which was around 60° (Figure 10). The substantial improvement in hydrophobicity achieved by the Biofinish coating can be attributed to the pre-treatment of the wood with linseed oil before applying the coating. Linseed oil, a natural vegetable oil, is known for its ability to penetrate the wood substrate and impart water-repellent properties (Terziev and Panov 2010, Arminger et al. 2020, Liu et al. 2021). During the initial three months of exposure to natural weathering, the uncoated Scots pine samples experienced a decrease in their respective contact angles. Previous studies have revealed similar results on Scots pine (Oberhofnerová and Pánek 2016), radiata pine, (Sandak et al. 2021), western redcedar (Kalnins and Feist 1993), and other softwood and hardwood species (Oberhofnerová and Pánek 2016). The drops were more pronounced for the uncoated wood compared to the wood coated with Biofinish, with the contact angle plummeting to less than 20°, a value that remained consistent until the end of the exposure period. Such a small contact angle corresponds to instantaneous surface wetting and the immediate spread or spill-out of the water droplet upon contact with the measured wood surface. In contrast, the Biofinish coating substantially mitigated the decline in wood hydrophobicity as a decrease in the contact angle on the Biofinish-coated wood surface was considerably observed after 6 months of exposure. After that point, the contact angle steadily decreased throughout the duration of the natural weathering test. This suggests that the Biofinish coating was able to preserve the wood's

hydrophobic properties for a longer duration, even though the hydrophobicity eventually decreased with prolonged exposure to weathering.

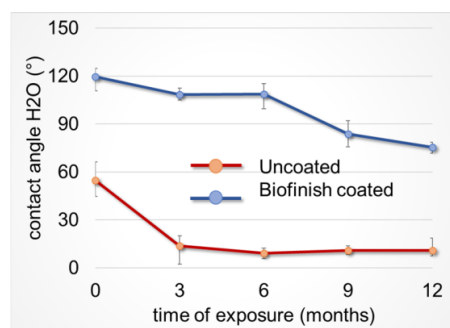


Figure 10. Changes in the contact angle of uncoated and Biofinish - coated Scots pine wood as a function of exposure time (**Paper 3**). Note: Error bars correspond to the range (minimum to maximum) of results observed.

4.2.4 Surface Topography

Wood weathering leads to the degradation and removal of lignin and extractive components, resulting in increased surface roughness and wettability. As the surface becomes rougher, it becomes more prone to moisture absorption, accelerating further degradation and erosion (Hon and Chang 1984, Rowell 2005, Cogulet, Blanchet, and Landry 2018). In **Paper 3**, uncoated Scots pine wood specimens exhibited a progressive increase in surface roughness where all examined surface topography descriptors: Sa (Arithmetical mean height), S_{kt} (Kurtosis), and S_{sk} (Skewness) changed. In contrast to uncoated wood samples, Scots pine wood treated with the Biofinish coating exhibited an opposite trend in surface roughness alteration. As weathering progressed, the Sa value of the coated samples decreased, indicating a progressive smoothing of the surface. (Figure 11). The reduction in surface roughness for the Biofinish-coated samples can be attributed to two factors. First, the gradual secretion of linseed oil, which was initially impregnated into the wood substrate prior to coating application. Second, the self-healing capability of the living *A. pullulans* fungal cells present in the coating formulation. Self-healing properties refer to the ability of a material to partially or completely heal or repair itself to its original properties or functionalities (Hager et al. 2010). This phenomenon occurs as the fungal growth gradually fills surface micro-voids and other irregularities present on the wood surface, resulting in a smoother surface texture. This self-healing ability is visually evident in the surface topography profiles, where the excessive initial waviness and roughness observed in the uncoated wood samples seem to diminish on the Biofinish-coated profiles. Unlike the uncoated wood surfaces that exhibit roughening due to weathering, the

Biofinish coating maintains a smoother surface owing to the self-regenerative properties of the living fungal cells and their ability to continually fill in surface irregularities. This ability to heal itself helps maintain the integrity and aesthetics of the wood, even in harsh environmental conditions. Compared to traditional wood treatments that may require frequent reapplication or maintenance, Biofinish offers a sustainable solution that reduces the need for costly repairs and extends the lifespan of wood products.

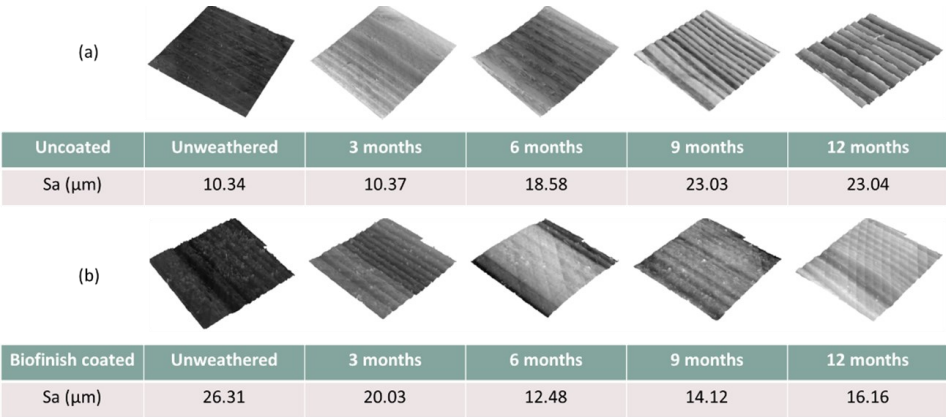


Figure 11. 3D surface topography images and surface roughness of uncoated Scots pine (a) and Biofinish - coated Scots pine (b), **Paper 3**.

4.3 Survival of *A. pullulans* during in-service period

Performance and protective functionality of the Biofinish coating primarily rely on the presence of the fungus *A. pullulans*. Therefore, the ability of *A. pullulans* to survive within the coating throughout its service period are crucial factors determining the effectiveness of the Biofinish coating. This aspect not only highlights important insights into the coating's protective abilities against other wood-infesting fungi but also elucidates the dynamic interactions between *A. pullulans* in the coating matrix and environmental conditions. Understanding these dynamics is essential for comprehending the long-term efficacy of the bioinspired living coating in preserving wood integrity.

Paper 4 revealed the survival of *A. pullulans* and effective protection of Biofinish coating against wood-infesting fungi during the 9 months in-service under fluctuating climate conditions and seasonal changes. As shown in Figure 12, fungi from the genus *Aureobasidium*, specifically *A. pullulans*, were predominant and consistently observed on façades coated with Biofinish, irrespective of cardinal directions and heights. This highlights its survival and resilience against

environmental fluctuations, ranging from low temperatures with high humidity of winter to contrasting conditions of high temperatures with low humidity, and intense direct solar radiation typical of Izola's summer climate. This adaptability can be attributed to its phenotypic plasticity. Furthermore, the prevalence of *A. pullulans* on the façade can be attributed to its antagonistic activity against various fungal species, including *Aspergillus niger*, *Alternaria alternata*, *Penicillium expansum*, and *Rhizopus stolonifera* (Schena et al. 1999, Castoria et al. 2001, Bozoudi and Tsaltas 2018, Di Francesco et al. 2020, Don et al. 2021). These fungi are recognised as typical colonisers commonly found on weathered wood surfaces (Rowell 2005). *A. pullulans*' antagonistic activity serves as a natural defence mechanism, inhibiting the growth of competing fungal species and facilitating its establishment and proliferation on the substrate surface. This enhances its effectiveness as a biocontrol agent in architectural coatings.

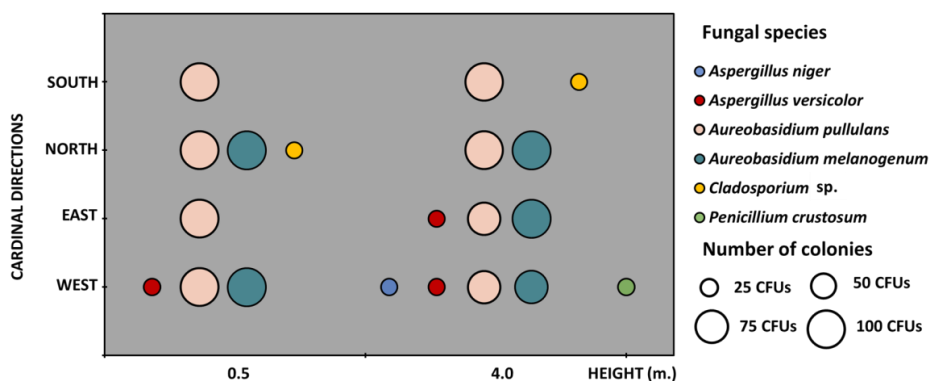


Figure 12. Distribution of fungal species on the Biofinish-coated façade of the InnoRenew CoE building in Izola, Slovenia after a 9-month exposure period, sampled from heights of 0.5 and 4 m across all four cardinal directions (north, south, east, west), **Paper 4**.

5 Conclusions and future perspectives

This thesis was conducted to develop a comprehensive understanding of fungal colonisation on wood materials in the context of environmental factors and to identify fungal strains that could potentially be utilised as protective layers in building materials. Fungi that are perceived as negative under certain circumstances might be beneficial for some applications. The bioinspiration in this case was to benefit from phenomena that is already occurring in nature. By gaining a deeper understanding of these processes, we can effectively regulate fungal growth on surfaces of materials, allowing us to strategically select fungal strains that do not contribute to the degradation of materials. This knowledge will facilitate the control and optimisation of fungal biofilm and contribute to the development of bioinspired living coatings for wood protection.

Certain species, such as *Aureobasidium pullulans*, was predominantly identified as primary colonisers on weathered wood surfaces, irrespective of geographical location, cardinal direction, and surface treatment. Their adaptability and capacity to thrive in a relatively broad range of ecological conditions makes this fungal strain suitable for use as a protective layer for building materials.

The predominance of *A. pullulans*, the living and active ingredient in the Biofinish coating on façades, after 9 months in-service, under fluctuating climate conditions and seasonal changes, demonstrates this fungus's ability to survive and adapt to diverse climate conditions. Additionally, it highlights the protective functionalities of the Biofinish coating against other wood-decaying fungi.

The performance of Scots pine (*Pinus sylvestris* L.) wood coated with Biofinish was compared with uncoated reference wood after a 12-month natural weathering trial. Through a multi-sensor approach focusing on aesthetic deterioration aspects, the wood treated with Biofinish demonstrated superior performance in all examined aspects compared to the uncoated reference. An intriguing observation was the dual self-healing mechanism, evidenced by a decrease in surface roughness throughout weathering, contrary to the typical deterioration observed in many materials. The entirely bio-based composition of the Biofinish coating enhances its sustainability and compatibility with natural environments, making it an attractive alternative to contemporary wood surface protection solutions.

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This doctoral thesis explores an innovative, sustainable solution for wood protection through a bioinspired living coating system. The research focuses on the fungal species *Aureobasidium pullulans*, examining its ability to form biofilms, resist environmental stresses, and protect wood from decay. Natural weathering trials demonstrated *A. pullulans*' adaptability and its effectiveness as a protective coating, outperforming untreated wood in durability and resistance.

The study highlights the potential of this entirely bio-based coating to enhance sustainability and ecological compatibility in wood protection. By advancing the understanding of fungal biofilms, this work contributes to the development of environmentally friendly solutions that harmonize with natural ecosystems.

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