A Critical Review of the Physiological, Ecological, Physical and Chemical Factors Influencing the Microbial Degradation of Concrete by Fungi

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Highlights

- Fungi survive and grow in concrete by modifying the local environment.
- Fungal influenced degradation of concrete is driven primarily by organic acid chemical attack.
- Fungi secrete keratinase and amino acids, accelerating concrete cracking.
- Fungi may attack concrete mechanically due to hyphal penetration.

Abstract

Concrete is the most extensively used material in construction and is generally relatively resistant, but under certain environmental conditions it is susceptible to microbially influenced degradation (MID) by bacteria, algae and fungi. Filamentous fungi, including *Fusarium oxysporum, Aspergillus niger* and *Cladosporium sphaerospermum*, are widely detected on corroded concrete surfaces. However, in contrast to bacteria, the extent of, and factors influencing, fungal influenced degradation (FID) of concrete are poorly understood. The extensive presence and survival ability of fungi in concrete may be explained by their remarkable environmental adaptability and capacity to modify potentially extreme environments, including alkaline pH conditions found in concrete, facilitating its exploitation by, and growth of, the organism. Furthermore, fungi produce dormant, resistant spores that remain viable

and survive for long periods of time, cellular autolysis conserves resources to maintain viability and growth in low nutrient conditions, and the mycelial network facilitates the transport of nutrients, substrates, water and oxygen (O_2) within fungal colonies. The concrete environment is rich in calcium (Ca), which is essential for hyphal growth, and the requirement for this important nutrient may explain why fungi grow in and exploit concrete as a resource. The identified mechanisms responsible for the FID of concrete, include: (1) the formation and leaching of soluble Ca salts from the reaction of organic acids secreted by fungal cells with Ca in concrete; (2) expansion due to formation of insoluble Ca salts, such as Ca citrate, from the reaction with fungal organic acids; (3) crack development by ettringite formation from the secretion of the enzyme, keratinase, and amino acids; and (4) potential mechanical attack by fungal hyphal growth and extension into solubilisation zones and cracks. The mechanisms of FID operate simultaneously and potentially have important, yet currently underestimated, consequences for the aesthetic, functional and structural properties of concrete structures. However, direct evidence of internal decay induced by fungal hyphae is lacking, and physical and chemical research is required to demonstrate the potential extent and significance of internal FID of concrete.

Keywords

Concrete; Biodeterioration; Fungi; Microbially Influenced Degradation (MID); Fungal Influenced Degradation (FID)

1. Introduction

Globally, concrete is the most widely produced material used in construction with an annual production exceeding 10 billion t [1]. Concrete has a low unit price, but repairing or replacing damaged material is technically challenging and expensive. Concrete structures may be damaged by a variety of physical, chemical, as well as biological mechanisms. Indeed, concrete is readily colonised by microorganisms, including bacteria, algae and fungi, and their metabolic activities, such as the secretion of enzymes, amino acids or excretion of metabolic by-products, for instance, acid forming waste materials, can lead to significant concrete biodeterioration. Biodeterioration can result in aesthetic, functional and structural problems in concrete structures. For example, *Cladosporium* was the predominant fungal genus associated with the aesthetic degradation observed on different bridge structures (Figure 1) [2]. In another example, the presence of sulphur-oxidising bacteria (SOB) in anoxic conditions within a French sewer network was responsible for the reduction in pH value and loss of material from the internal walls, and the reduction in strength and density of concrete pipes [3]. Concrete biodeterioration also has the potential to endanger human health due to bioaerosol emissions of fungal spores which can cause respiratory illness. This phenomenon is known as the ‘sick building syndrome’ (SBS) [4].

Figure 1. Surfaces of concrete structures covered and affected by biofilm where several fungal species, including *Cladosporium* and *Aspergillus*, have been isolated [2]

The kingdom, *Fungi*, represents the most widely distributed and diverse group of organisms on Earth, with approximately 144,000 known species [5,6]. Most taxa in the kingdom *Fungi* have a filamentous growth habit and have the unique property of growing by hyphal tip extension. The cell wall of filamentous fungi is strong and flexible, which, together with the growth extension from hyphae structures, enables the organism to penetrate into dense materials to seek and acquire nutrient resources. Indeed, when sufficient nutrients, energy and organic carbon sources are available, in favourable environments of temperature and moisture, filamentous fungi can colonise and grow in concrete structures, often with detrimental effects on the physical and chemical properties of concrete [7]. The principal species of filamentous fungi that demonstrate the ability to grow in, colonise, and cause the biodeterioration of concrete materials, include: *Fusarium oxysporum*, *Aspergillus niger* and *Cladosporium sphaerospermum* [2,8–10]. In contrast, other specific species of filamentous fungi, for instance, *Trichoderma reesei*, have the potential to act as self-healing agents when

applied to cracked or damaged concrete structures [11,12].

This review critically examines the negative effects of filamentous fungi on the physical and chemical properties of concrete and explores how these mechanisms influence the structural characteristics and behaviour of concrete. The specific objectives were to:

1) Understand why concrete provides a suitable matrix to support the growth of certain filamentous fungi.

2) Determine the growth requirements of filamentous fungi that can colonise concrete, including nutrient and environmental factors relevant to the biodeterioration of concrete.

3) Determine the changes in the physical and chemical properties of concrete that occur following colonisation by filamentous fungi.

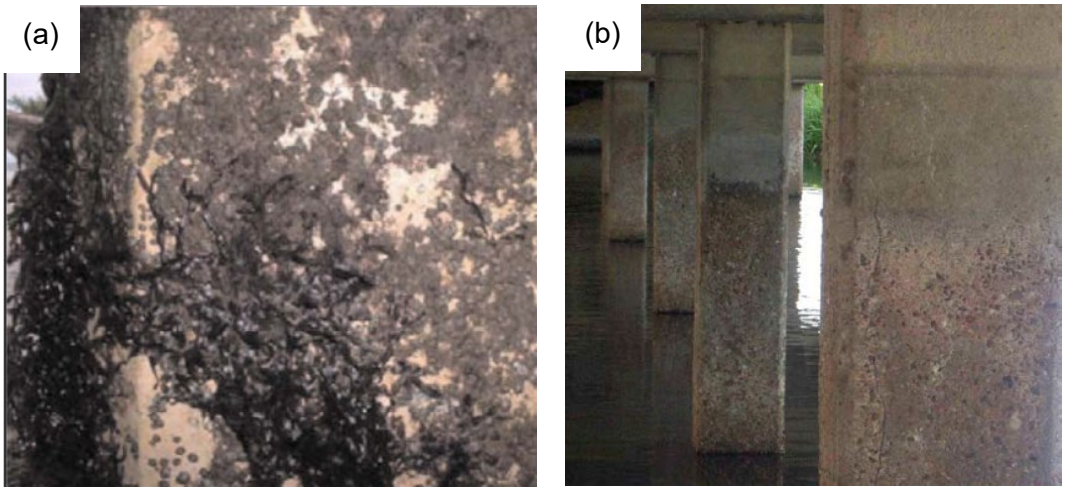
2. Concrete environment and fungal colonisation

2.1 Introduction to concrete biodeterioration

Biodeterioration of concrete, also described as microbially influenced degradation (MID) of concrete or microbially influenced concrete corrosion (MICC), is the physical or chemical process involving microorganisms (e.g. bacteria, fungi and algae) that

destroys the structure and chemical integrity of concrete [7] (Figure2). Parker (1945) first reported the link between microbial attack and concrete corrosion and isolated the sulphuric acid producing, SOB, *Thiobacillus thiooxidans*, from corroded concrete in sewage pipelines [13], which remains a significant problem for the integrity of concrete sewer networks [3]. Nitrifying bacteria have also been shown to degrade building masonry through the microbial production of nitric acid [14]. The mechanisms of concrete corrosion by bacterial mineral acids are relatively well understood, however, few studies have investigated fungal influenced degradation (FID). Indeed, early attempts, for example, by Milde et al. (1983), to measure organic acids, which are associated with FID, on Hamburg sewer pipes were unsuccessful [15], but *Thiobacilli* bacteria were isolated from the corroded concrete walls and it was therefore concluded that mineral acid produced by the bacteria was responsible for the MID of the structure. However, Gu et al. (1998) suggested that the possible role of fungal organic acids in concrete degradation could be overlooked [16]. This is because organic acids released into the concrete environment rapidly react with concrete minerals and are therefore short-lived and, consequently, may be difficult to detect [16]. Indeed, Gu et al. (1998) compared the effects of *Thiobacillus* sp. (bacteria) and *Fusarium* sp. (fungi) on concrete degradation and found the samples exposed to the fungus suffered greater weight loss and more serious degradation [16]. Microscopical examination of the concrete surface suggested that the fungus was present and growing in fractures of the damaged concrete (Figure 3), however, it is not clear to what extent or depth the

fungus had penetrated into the concrete [17]. Nica et al. (2000) also studied the microbiology of corroding concrete sewage pipes in Houston, USA and found fungal colonies in 60% of the samples, although the fungal species was not identified [18]. In another example, Geweely (2011) isolated fungi from corroded concrete bridges over the River Nile and concluded that *F. oxysporum* was the dominant fungal species [19], which is linked to the FID of concrete [6-9]. Thus, although the amount of quantitative investigation is currently relatively limited, the available evidence indicates that filamentous fungi potentially have a central role in and contribute to MID of concrete. Fungal influenced degradation involves specific chemical and mechanical mechanisms (see Section 3.1 and 3.2) and the consequences include significant changes and impacts on the aesthetic, physicochemical and structural properties of concrete (see Section 3.3).



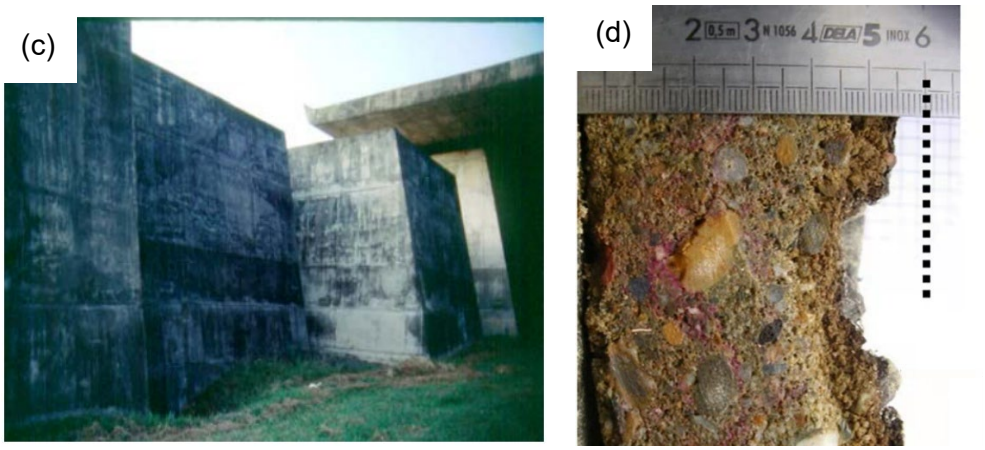


Figure 2. Cases of concrete biodeterioration (a) algae and lichens causing erosion of the concrete surface in a marine environment [20], (b) biodeterioration of concrete bridge columns in Texas [21], (c) aesthetic biodeterioration of concrete structures in Brazil [9], and (d) matter loss from the internal wall of concrete pipes in a sewer environment [3]

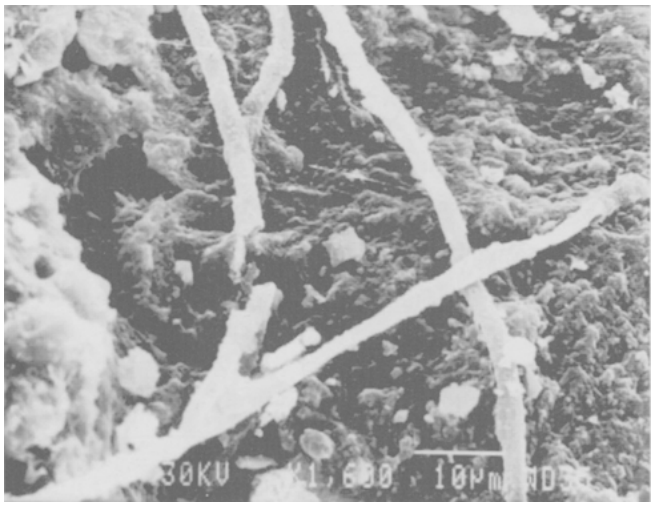


Figure 3. Fungal hyphae observed on the degraded concrete surface of a bridge pier immersed in a polluted river [17]

2.2 Fungal inoculation of concrete

The kingdom *Fungi* comprises of ten major phyla: *Cryptomycota*, *Microsporidia, Chytridiomycota, Monoblepharidomycota, Blastocladiomycota, Neocallimastigomycota, Zoopagomycota, Mucromycota, Ascomycota,* and *Basidiomycota*, based on molecular characteristics, which, by and large, have supported more traditional phylogenies relating to reproduction methods, hyphal structures and growth optima. *Ascomycota* (sac fungi) is the largest phylum and contains predominantly filamentous species, including those commonly isolated from concrete structures: *A. niger, F. oxysporum, C. sphaerospermum* and *T. reesei*. Except for yeasts, ascomycetes undergo both sexual reproduction by asci formation, and faster and more common asexual reproduction by conidia formation [22,23]. For example, in asexual reproduction of *Aspergillus* (Figure 4a), fungal growth begins with the germination of spores, which occurs in two stages where the dormant spore swells followed by the initial hypha growing out [24]. The nutrients stored in the spore allow the apical growth of the hypha; branching is apparent near hyphal tips and septation occurs between cells. Hyphal extension and branching continue to seek out, exploit and transport nutrients, resulting in the formation of a complex fungal mycelium structure [25]. Asexual development is established with spore dispersal, and the fungal lifecycle restarts [26]. Sexual reproduction is a more complex process, and produces ascospores following fertilization between the ‘male’ antheridium and ‘female’ ascogonium, mitosis and cell division [23]. These spores are commonly associated

with environmental stress and are relatively resistant, enabling survival under harsh conditions (for example moisture or nutrient depletion), and sexual recombination from different parents facilitates adaptive development of the organism [27]. Both types of spores, and particularly asexual spores, are produced in vast numbers, and are disseminated in air and water films, enabling widespread distribution and colonisation of favourable environments [28].

Concrete is a porous material with macropores, gel pores (<10nm) and capillary pores (10 to 10000nm) [29–31]. Also, concrete structures typically develop cracks classified as narrow (<0.5mm), medium (0.5 to 1.5mm) and wide (>1.5mm) [32,33]. Fungal spores, on the other hand, are considerably smaller (for example, the diameter of *A. niger* spores is typically in the range of 4-5μm) and can be readily transported into deeper regions of the concrete matrix through cracks and pores by air or water, increasing the distribution of fungi and the potential for FID [34]. The diameter of fungal hyphae is similarly typically in the range: 4-6μm [35], and hyphae of *A. niger* have an average diameter of 3.5μm (approximate range: 2.5-4.5 μm) [36], also permitting penetration into the concrete matrix directly through cracks and larger pores. In addition, concrete pores allow water and nutrients to enter from the external environment, potentially supporting fungal growth within the matrix. Consequently, concrete porosity has a significant influence on the development of microbial biofilms and MID of concrete [2].

Figure 4b shows the detailed structure of the hyphal tip of *Aspergillus*, one of the filamentous fungi most frequently detected in concrete structures, and the close relationship between extension and cell wall formation and organization. The hyphal cell wall is made up of chitin and complex carbohydrates rendering it strong and flexible, allowing the hyphal tip to penetrate into dense structural materials, such as concrete [7,37]. Thus, the potential growth and extension of fungal hyphae within concrete materials may be a key mechanism contributing to FID of concrete.

The inner layer of the hyphal cell, adjacent to the cell wall, is the plasma membrane, and is interspersed with globular proteins that govern the transport of nutrients into and of metabolites out of the cell [25]. Fungi have four modes of nutrient transportation through the plasma membrane: free diffusion, facilitated diffusion, diffusion channels, and active transport. The active mechanism in filamentous fungi is responsible for transportation of more complex substrates and nutrients, including sugars, amino acids, and nitrate (NO_3^-), ammonium (NH_4^+), sulphate (SO_4^{2-}), and phosphate (PO_4^{3-}) ions [25]. Hyphae contain vesicles and vacuoles, which are organelles that are responsible for storing and transporting materials within the organism. Endocytic vesicles form in the plasma membrane and transfer extracellular substances into the cell, while secretory vesicles carry proteins and enzymes and deliver them externally along the direction of hyphal growth, to support cell wall extension and tip growth. The region that is surrounded by secretory vesicles near the hyphal tip is called the Spitzenkörper [25,26]. Sac fungi have hyphae compartmentalized by perforated septa allowing

cytoplasm or the entire protoplasm to pass. Woronin bodies sometimes form to block the septa pores to seal off stressed or damaged hyphae to protect undamaged cells [25,26]. Consequently, the structural and growth features of filamentous fungi are well adapted to enable them to penetrate and exploit dense media, such as concrete environments, to acquire resources. However, as with all microorganisms, the microbiological and metabolic activities of filamentous Ascomycetes are strongly dependent on nutrient availability and environmental conditions including moisture, pH and O_2 status, which are discussed in Section 2.4.

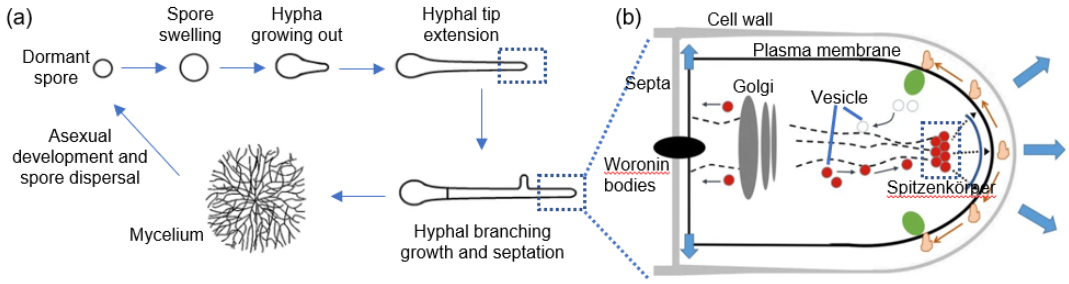


Figure 4. Lifecycle, growth and development of *Aspergillus* (a) asexual reproduction process and lifecycle and (b) intracellular and structural features of the hyphal tip [25,26]

2.3 Nutrient requirements

Fungi are heterotrophic organisms and require organic carbon substrates to grow and synthesise new bio-compounds in cells and to supply metabolic energy through oxidation of carbon compounds [38,39]. Nutrients enter hyphae through the plasma membrane, however, only small, soluble nutrient molecules in the growth environment,

such as sugars and amino acids, can be absorbed directly by the cell membrane and transported to the hyphal tips. Larger, complex and insoluble molecules, such as polysaccharides, lipids and proteins, are degraded by hydrolytic, exocellular enzymes (exoenzymes) secreted by fungi, into simple monomers for absorption, a form of heterotrophic nutrition described as saprophytic [40].

Exposed surface materials that lack a source of organic carbon may be initially colonised by phototrophic organisms which use sunlight and fix inorganic carbon dioxide (CO_2) as sources of energy and carbon, respectively. The excretion of carbohydrates, growth factors and antibiotics, and dead cellular biomass originating from the phototrophic organisms provide an organic matter source to support the growth of heterotrophic microflora, including fungi [41]. In addition, traces of organic matter may be present in construction materials, including: concrete admixtures (e.g. superplasticizers), rock matrices, gravel substrates and especially in fine aggregates used to produce concrete, due to the presence of decaying vegetation, humus or organic and industrial wastes [42]. Organic matter retards the setting of cement and negatively affects concrete or mortar strength, and the content is therefore controlled in construction materials. A simple test for organic matter is based on the colour change of sodium hydroxide solution, and a darker coloration compared to a standard colour index is indicative of excessive organic matter content [43]. Nevertheless, only traces of organic substrates in the concrete matrix may be necessary to support fungal growth and FID since organic resources are transported to actively growing regions of the

organism through the hyphal network, and autolysis of fungal cells occurs when localised nutrient and organic carbon resources are in limited supply (discussed in Section 2.5).

Other sources of organic carbon that can support the growth of heterotrophic microorganisms include anthropogenic and biogenic gaseous hydrocarbons, such as aliphatic compounds, acids and alcohols. These can be deposited onto concrete surfaces from the atmosphere or in rain [42], and gaseous hydrocarbons may enter the concrete matrix with moisture vapour by 'aspiration' [44–47]. Hydrocarbon compounds present in paint or coating formulations deliberately applied to concrete surfaces also provide a source of organic matter for microbial growth [48,49].

Once absorbed, the cellular structure of filamentous fungi enables the transport of organic resources to the hyphal tip to support: (a) the release of organic acids to modify the physical and chemical properties of the concrete (see Section 3.1.1) and (b) the growth and potential extension of the fungus into the concrete matrix. This behaviour is unique to filamentous fungi and allows them to exploit and grow in environments that would be unsuitable for colonisation by unicellular organisms, such as bacteria.

Nitrogen (N) is an essential nutrient required by all organisms for the synthesis of key biomolecules including: proteins, nucleic acids and chitin. Ammonium and L-glutamate are generally considered to be the preferred N sources for fungi [24]. For example, colonies of *A. niger* and *C. sphaerospermum* grew more rapidly with ammonium

sulphate ($(NH_4)_2SO_4$) as the N source, compared to ammonium nitrate (NH_4NO_3), sodium nitrate ($NaNO_3$) or urea [50,51]. Potassium nitrate (KNO_3) has been experimentally confirmed as the optimal N source for *F. oxysporum* f. sp. *ciceris*, followed by peptone, $NaNO_3$ and magnesium nitrate ($Mg(NO_3)_2$) [52]. In addition, fungi are also able to utilize NO_3^- and intercellularly convert the anion, via nitrite (NO_2^-), into NH_4^+ [24]. As is the case with organic matter, N compounds may directly or indirectly deposit onto concrete surfaces in soil, water and from the atmosphere [53]. Within concrete materials, NH_4^+ may be supplied in shale, which is a common raw feedstock used in cement production [54]. Another possible source of N is from the mineralisation of organic N compounds. Thus, biodegradation of organic N releases NH_4^+-N by microbial mineralization processes, including by fungi, which may be subsequently transformed to NO_3-N via bacterial nitrification [55,56]. According to Jang et al. (2016), aggregates used to produce mortar with increasing organic matter fractions have elevated cation exchange capacities and retain and maintain larger NH_4^+ contents compared to aggregates of lower organic matter status [56], which is favourable to fungal growth.

The addition of alternative aggregates to produce concrete from secondary waste resources is increasing, for instance to conserve natural habitats used for aggregate extraction [57]. For example, fly ash from waste incineration is one of the most common mineral additives used in concrete production which also improves concrete durability [57]. However, fly ash adsorbs $(NH_4)_2SO_4$ compounds and therefore provides a source

of N nutrition for fungal growth [58]. Indeed, Jang et al. (2020) showed that fly ash addition to cement increased the growth rate of *Fusarium spp*., *Nectria mauritiicola*, and *A. niger* on mortar discs [58]. Thus, there are multiple direct and indirect sources of N potentially available to support the colonization and growth of fungi on concrete materials.

Phosphorus (P) is also an essential, major nutrient, present in high concentration in fungal cells, and is necessary for biosynthesis of nucleic acids, phospholipids, adenosine triphosphate (ATP), glycophosphates, and polyphosphates. Filamentous fungi have developed strategies, such as medium acidification and organic acid production, to solubilize fixed P sources in soil, to mobilize and capture P, which is often poorly available in natural environments [25,59,60]. Concrete represents a high pH environment and contains large concentrations of Ca with smaller amounts of aluminium (Al) and iron (Fe). All of these ions are effective at binding and retaining soluble P, for example, from fertilisers in run-off or industrial and domestic chemicals in wastewater, that may come into contact with the concrete surface, thus representing a potential mechanism and source of P retention for fungal growth [61].

Calcium ions (Ca^{2+}) are essential for fungal apical growth and are required in high concentrations at the hyphal tip, but in low concentration in other hyphal regions. When such a gradient exists, hyphal extension is encouraged, hyphal branching is inhibited and a normal hyphal morphology develops [62]. To maintain the gradient in Ca

concentration, fungi are able to control the Ca^{2+} concentration status by both active and passive transportation between the extracellular environment and cytoplasm [63]. Concrete is a high Ca environment offering a rich source of Ca^{2+} for fungal growth. Indeed, Ca^{2+} plays a prominent role in organic acid attack of concrete during FID, changing the physico-chemical properties of, and promoting fungal extension into, the concrete matrix (see Section 3.1.1).

Other essential resources required in small amounts for fungal growth and reproduction include trace elements (eg. zinc (Zn), copper (Cu)) and vitamins (eg. thiamine, biotin) [39]. Zinc and Cu are present in trace amounts in concrete and are derived mainly from the raw feedstock materials and fuel used in cement production [64]. Microbial metabolites containing low-molecular-weight organic acids (LMWOAs), such as citric and tartaric acids, influence the desorption of metals and their bioavailability in concrete. In addition, the synthesis of essential vitamins in concrete may occur within biofilms by bacteria and fungi [65]. For instance, *A. niger* can biosynthesize thiamine from sugar and minerals such as NO_3^- [66].

2.4 Environmental requirements

Moisture, in liquid or vapor form, is essential for fungi to inoculate, grow and colonise a substrate material [39]. Bathroom environments, for instance, are typically dark with high humidity and moderately high temperatures, and provide an ideal habitat for fungal growth. For example, *Cladosporium* was the most frequently isolated genus

from building surfaces and air in Japanese bathrooms [67].

The amount of water available to microorganisms can be described in terms of water activity (Aw), which represents the equilibrium relative humidity (ERH)/100 [68]. The minimum Aw limit for fungal growth depends on the species and environmental conditions. For example, at a temperature between 20 and 25°C, *Aspergillus* and *Penicillium* species inoculated on malt extract agar required an Aw in the range 0.76-0.79, which decreased with increasing temperature and nutritional status of the substrate [69]. Water resistant coatings are often applied onto the surfaces of building materials to reduce the Aw and prevent biodegradation. These products are designed to allow water vapour to evaporate from building materials, but prevent water entry from the environment [70]. However, high moisture contents, on the other hand, can block porous void spaces with free water, reducing the supply of O_2, potentially inhibiting the growth of obligate aerobic microorganisms, such as fungi [39].

Temperature has a strong influence on fungal growth, spore germination and reproduction. Generally, maximum growth rates of most fungi, including *F. oxysporum*, *C. sphaerospermum, T. reesei* occur at a temperature of approximately 25°C, depending on other environmental factors, such as Aw and pH value [39,71–73]. Optimum temperatures also differ between species, for example, *C. sphaerospermum* prefers 25°C and does not grow at 37°C, whereas the optimum temperature for growth of *A. niger* is usually above 30°C, but can be as high as 41°C [74,75]. Indoor and

outdoor ambient temperatures used in building design in the UK are typically assumed to be in the ranges: 23°C to 28°C and 1°C to 30°C, respectively, which are ideal for the growth of fungi, and particularly those species linked to FID of concrete [76,77]. However, localized extreme temperature conditions on building surfaces may be inhibitory to fungal growth. For instance, the maximum temperature of a vertical wall with a dark colour exposed to direct sunlight increased to 88°C [78].

Most microorganisms grow in environments with pH in the range 2-9, and the optimum pH value is species dependent. In general, fungi are more active in acid environments compared to bacteria, but growth is inhibited below pH 3 [79]. By contrast, environments with high alkalinity can prevent fungal colonisation, for instance, the establishment and growth of *C. sphaerospermum* on the surface of concrete was inhibited at 25°C and 100% relatively humidity (both of which are favourable to fungi) at pH values above 10 [80]. Indeed, the pH of uncarbonated concrete is typically greater than 12.5 and prevents fungal growth [81]. Carbonation reactions in concrete between CO_2, usually from ambient air, and Ca, form calcite ($CaCO_3$) and reduce the pH value of concrete over time to approximately 8.5 [81]. The carbonation reaction usually proceeds from the concrete surface, and is amplified in the presence of microbial biofilms due to the localized metabolic production of CO_2, which reacts with moisture in the biofilm to form carbonic acid (H_2CO_3), further reducing the pH value of the concrete [7]. Hydrogen sulphide (H_2S) in air also assists in reducing the initial alkalinity of concrete through direct contact and the formation of thiosulphuric and

polythionic acid from abiotic oxidation [7].

Environmental O_2 and CO_2 concentrations are also critical factors influencing fungal growth. Most filamentous fungi are obligate aerobes and require O_2 to grow, although the demand is limited. In the presence of an O_2 concentration gradient, O_2-transporting aquaporin, a protein responsible for the metabolic transport of water and other small neutral molecules, can transport O_2 absorbed by fungal hyphae throughout the entire mycelium, including to areas of low O_2 status [82]. Consequently, within concrete structures, where O_2 deficiency may prevail due to restricted diffusion from the surface, fungi can access and transport O_2 from aerobic zones, to deficient areas, to maintain growth under localised anoxic/anaerobic conditions (discussed in Section 2.5). Nevertheless, fungi are sensitive to the O_2 status of the environment and fungal activity and biodegradation increase in the presence of greater O_2 supply [38,39]. Interestingly, however, certain fungal species in the phylum *Ascomycota* are facultative anaerobes, and are capable of either aerobic or anaerobic respiration, and examples include *F. oxysporum* and *A. niger*, both of which are involved in FID of concrete [83,84].

Fungi are sensitive to environmental CO_2 accumulation which is inhibitory to the growth of all fungal species. However, species vary in CO_2 tolerance [38,39,85]. For instance, under normal atmospheric O_2 concentrations (21%), growth of *Cladosporium* decreased with increasing CO_2 and 50% inhibition occurred at CO_2 concentrations of 20-23% [86,87]. *Fusarium*, on the other hand, has a higher tolerance of CO_2 and the

growth of this fungus can increase with rising CO_2 concentrations up to 20%; however, 50% growth inhibition was observed at a CO_2 concentration of 45% [86,87]. In addition, fungal secretions are negatively affected by increasing CO_2 levels, for example, citrate production by *A. niger* was reduced in the presence of elevated CO_2 [88]. Tolerance to CO_2 is influenced by other environmental factors, for instance, increasing Aw promoted colony radius development of *A. niger* under equivalent CO_2 levels [85].

Generally, fungal growth is inhibited by exposure to light, depending on the illumination intensity, exposure period and wavelength. Indeed, blue and ultra-violet light are widely used for potable water disinfection treatment and are inhibitory to the propagation of most fungi, including *Fusarium* [38,89].

2.5 Environmental adaptation by fungi

Aspergillus niger, F. oxysporum and *C. sphaerospermum* are the principal species of fungi linked to the FID of concrete media. *Fusarium oxysporum* is a common vascular wilt pathogen of plants, whereas *A. niger and C. sphaerospermum* tend to flourish under a wider range of growth conditions, as well as causing plant infections (although usually as non-specialised necrotrophes). As plant pathogens, the organisms penetrate host tissues through stomata, or wounds and cracks on the plant, or they can invade directly through local mechanical pressure and by releasing enzymes. Fungi have the ability to adapt to the external nutrient environment and, in the pathogenic mode of nutrition, are able to exploit nutrient resources from the host

organism, a behaviour that enables these species of fungi to also exploit the low nutrient status conditions in concrete. Interestingly, fungi are considered to be in a starvation condition during spore germination and in the penetration phase of the plant host [90]. The spore swelling stage does not require external resources, however, they are necessary for the growing out phase of the hyphae [24]. Starvation conditions may therefore hinder subsequent fungal growth and development.

However, fungi are remarkably tolerant of low carbon environments and fungal autolysis enables survival and hyphal growth under nutrient stress conditions by reusing cellular components. During this process, vacuoles degrade cytosolic materials in old hyphae by micro-autophagy and transport them to young hyphae to support tip extension into new host regions enabling the organism to explore new plant tissues for potential nutrients [91]. For instance, within filamentous fungi, the nuclei in mature hyphae may be degraded to produce N and P that can be recycled and reused in metabolic pathways [92]. Nitsche et al. (2012) studied the hyphal morphology of *A. niger* under carbon starvation by cultivating the organism in controlled conditions [93]. After 16 hours of carbon depletion, empty hyphal compartments in mature regions appeared and new hyphae extended in a non-branching manner with significantly reduced diameter from 3μm to 2μm. During a 140 hour period of carbon starvation, the number of empty hyphal compartments increased, although the cell wall exoskeleton remained intact [93]. Similar autophagic processes are observed in *F. oxysporum* and *T. reesei* enabling the organisms to adapt to and survive starvation conditions [94,95].

Indeed, Bindschedler et al. (2016) observed that most fungal hyphal networks in soil environments were empty [63]. Thus, cellular autophagy may permit the extension of young hyphae to seek nutrient resources and support the survival and growth of filamentous fungi within concrete environments where nutrient availability is low.

As well as adapting to their immediate microenvironment, filamentous fungi can transport nutrients and modify the local external environment to improve growth conditions. Thus, water and nutrients acquired from the external environment can be transported by the mycelium network to support growth at the hyphal tips [25]. For example, organic acids can be transported within the mycelium by free diffusion based on the concentration gradient, whereas the movement of sugars, amino acids and ions is by active transport mechanisms [25]. Therefore, nutrients, water and O_2 are transported to support fungal growth within deeper and otherwise inaccessible regions of the environmental matrix. This behaviour also permits colonisation and growth of other microorganisms, particularly bacteria, within the vicinity of the fungus. Thus, the development of SOB, such as *T. thiooxidans* and nitrifying bacteria, is also encouraged, allowing the simultaneous development of multiple corrosion mechanisms in concrete structures [14,96].

Overall, filamentous fungi are generally not particularly fastidious organisms and grow in a wide range of environments in humid conditions [8]. Furthermore, fungal spores remain dormant and can survive extreme environments, for instance, low moisture or

high temperatures. Growth is triggered when water availability increases and continues by forming a three-dimensional mycelial structure. Fungal growth depends on the number of hyphal tips and the availability of nutrients [39] and the mycelium expands into substrate materials to supply nutrient resources to support cell growth. Under starvation conditions, fungal autolysis can release nutrients stored in older fungal structures. The transport of nutrients, water and O_2 through the hyphae to the tip, supports apical extension to explore and access potential nutrient resources and colonise new regions of the substrate. These physiological mechanisms and the ability to tolerate relatively extreme environments explain why filamentous fungi are highly versatile and successful at media colonisation and exploitation. These ecological characteristics and the physiological behaviour of filamentous fungi, therefore, enable them to exploit concrete as a resource, causing changes in the physical and chemical properties, and ultimately the deterioration, of concrete.

3. Fungal influenced degradation of concrete

3.1 Chemical attack

3.1.1 Organic acids

Organic acids are metabolic end-products secreted by fungi to modify (acidify) and improve the growth conditions of their immediate external environment, which react with Ca and participate strongly in the chemical biodeterioration of concrete. Table 1 presents the major filamentous fungi and the commonly secreted organic acids

involved in concrete FID. The types and quantities of organic acids produced are influenced by environmental conditions and have potentially important consequences for the progression of FID [79]. For example, Gutarowska and Czyżowska (2009) observed that high initial pH values of mortar stimulated the production of organic acids, including oxalic, malic, succinic and fumaric acids [97]. Organic acid production is dependent on fungal species and strain. *A. niger*, for example, is well known for its ability to produce a large range of commercially important organic acids, such as citric acid. Indeed, Liaud et al. (2014) showed that the concentration of citric acid produced by *A. niger*, strain BRFM422, grown in a glucose medium, increased to 2.5 g/L, however, the production by other strains was not detectable under the same conditions [98].

Table 1. Organic acids secreted by filamentous fungi involved in FID

Fungal taxa	Organic acids	Reference
Fusarium	Fusaric	Bacon et al. (1996) [99]
	Acetic, citric, fumaric, glyoxylic and oxalic	Sterflinger (2000) [100]
Aspergillus	Oxalic	Dutton and Evans (1996) [101]
	Acetic, formic, fumaric, gluconic, glyoxylic, itaconic, oxalic	Sterflinger (2000) [100]
	Succinic	Vazquez et al. (2000) [59]
	Oxalic, citric, pyruvic, gluconic, lactic, formic, acetic, fumaric, propionic and butyric	Jestin et al. (2004) [102]
	Oxalic, citric, gluconic succinic	Rashid et al. (2004) [103]
	Oxalic, citric, gluconic, succinic	Fomina et al. (2007) [104]
	Gluconic, oxalic	Chuang et al. (2007) [105]

	Acetic, ascorbic, butyric, citric, fumaric, formic, oxalic, gluconic, itaconic, isobutyric, lactic, malic, propionic, succinic, and tartaric	Liaud et al. (2014) [98]
Cladosporium	Formic, fumaric, gluconic and lactic	Sterflinger (2000) [100]
Other fungi: *Trichoderma, Alternaria, Phoma, Penicillium, Epicoccum*	Other organic acids: Malonic, tenuazonic	Sterflinger (2000) [100]

Portland cement contains the major elements: silicon (Si), Al, Fe, Ca and a small amount of magnesium (Mg), which are typically present as oxides in cement clinker (Table 2), and are involved in the chemical reactions leading to FID [103]. The maximum content of magnesium oxide (MgO) should not exceed 5% by mass of cement materials [107]. Both organic acidic hydrolysis, with associated metal salts formation (examples for acetic acid are shown in *Equation 1* to *5*), and complexation reactions, are active in concrete FID, with the former being the main driving mechanism

[108]. Generally, complexation reactions only occur when metal ions have been released into solution after acidolysis [109]. The solubilities of Ca, Al, Mg and Fe salts of organic acids are listed in Table 3 and show that Al and Fe salts have relatively low solubility. Bertron et al. (2005) measured the concentrations of various elements after exposing cement produced with different binders to an acidic solution containing acetic, propionic, butyric, iso-butyric and lactic acids and found that the element mass of released Si, Al and Fe after 6 h was less than <1% of the initial mass of the elements in the cement specimens [106]. The stability of these elements in acid environments is an important parameter when assessing the chemical resistance of cement binders [103]. By contrast, Ca and Mg salts have much larger solubilities, ranking first and second respectively, in terms of dissolution rate, compared to the other major metal ions present in cement [106]. The reactions between Ca and most organic acids (except for tartaric, oxalic and citric acid) form soluble salts within the concrete, resulting in Ca leaching and concrete degradation. For example, *Equation 5* to *7* describe the process of acetic acid attack of concrete, which follows the order of: portlandite (calcium hydroxide, $Ca(OH)_2$), C-S-H (calcium-silicate-hydrate) and ettringite (hydrous calcium aluminium sulphate, $Ca_6Al_2(SO_4)_3(OH)_{12}\cdot 26H_2O$) (AFt) [108].

Table 2. Average concentrations of chemical components in typical Portland cement [110]

Component	Average concentration (%)
SiO_2	21.0
Fe_2O_3	2.9
Al_2O_3	5.0
CaO	64.2
MgO	1.7
SO_3	2.6
Na_2O	0.24
K_2O	0.70
Equivalent alkalis	0.68
Free lime	1.2

$$Al^{3+} + CH_3COO^- \rightarrow Al(CH_3COO)^{2+}$$ *Equation 1*

$$Fe^{2+} + CH_3COO^- \rightarrow Fe(CH_3COO)^{+}$$ *Equation 2*

$$Fe^{3+} + 3CH_3COO^- \rightarrow Fe(CH_3COO)_3$$ *Equation 3*

$$Mg^{2+} + 2CH_3COO^- \rightarrow Mg(CH_3COO)_2$$ *Equation 4*

$$Ca(OH)_2 + 2H - acetate \rightarrow Ca - (acetate)_2 + 2H_2O$$ *Equation 5*

$$C - S - H1.8 + 3.6H - acetate \rightarrow 1.8\ Ca - (acetate)_2 + H_4SiO_4 + 1.6H_2O$$ *Equation 6*

$$AFt + 6H - acetate \rightarrow 3Ca - (acetate)_2 + 2gibbsite + 3gypsum + 26H_2$$ *Equation 7*

Table 3. Solubility of Ca, Al, Mg and Fe organic acid salts (g/100ml) [111,112,116]

		Organic acid types							
		Acetic	Propionic	Butyric	Iso-butyric	Lactic	Citric	Tartaric	Oxalic
Ca salts	Formula	$Ca(C_2H_3O_2)_2$	$Ca(C_3H_5O_2)_2 \cdot H_2O$	$Ca(C_4H_7O_2)_2 \cdot H_2O$	$Ca(C_4H_7O_2)_2 \cdot 5H_2O$	$Ca(C_3H_5O_3)_2 \cdot 5H_2O$	$Ca_3(C_6H_5O_7)_2$	$CaC_4H_4O_6 \cdot 4H_2O$/ $CaC_4H_4O_6 \cdot 3H_2O$	CaC_2HO_4/ $CaC_2O \cdot H_2O$
	Sol. cold water g/100ml	37.4 (0°C)	4.9 (0°C)	5	20	3.1 (0°C)	0.085 (18°C)	sls/i (0°C)	0.00067/i (18°C)
	Sol. hot water g/100ml	29.7 (100°C)	55.8 (100°C)	sls	sls	7.9 (30°C)	0.095 (25°C)	sls/0.16 (100°C)	0.0014/i (95°C)
Al salts	Formula	$Al(C_2H_3O_2)_3$	$C_9H_{15}AlO_6$			$Al(C_3H_5O_3)_3$	$C_6H_5AlO_7$	$C_{12}H_{12}Al_2O_{18}$	$C_6Al_2O_{12}$

	Sol. cold water g/100ml	s; sls				vs			i
	Sol. hot water g/100ml	d					s	s	i
Mg salts	Formula	$Mg(C_2H_3O_2)_2$ or $Mg(C_2H_3O_2)_2 \cdot 4H_2O$				$Mg(C_3H_5O_3)_2 \cdot 3H_2O$	$C_6H_6MgO_7$	$C_4H_4MgO_6$	MgC_2O_4
	Sol. cold water g/100ml	vs or 120 (15°C)				3.3	20	0.008 (18°C)	
	Sol. hot water g/100ml	vs				16.7 (100°C)		0.0144 (90°C)	0.104 (20°C)

Fe salts	Formula	$FeOH(C_2H_3O_2)_2$				$Fe(C_3H_5O_3)_3$	$C_6H_5FeO_7$	$C_{12}H_{12}Fe_2O_{18}$	$FeC_2O_4 \cdot 2H_2O$
	Sol. cold water g/100ml	i				s	0.5		
	Sol. hot water g/100ml					vs	s		0.008 (20°C)

Sol: solubility; vs: very soluble; sls: slightly soluble; s: soluble; d: decomposes; i: insoluble

The component changes in Portland cement paste chemistry due to organic acid attack exposed to *A. niger* have been described by the reactive transport model, HYTEC, which divides cement paste into three zones: sound zone, intermediate degraded zone and outermost degraded zone (Figure 5) [108,111,112]. Experimental (Figure 5a) and modelled (Figure 5b) chemical element changes reported by De Windt and Devillers (2010) showed similar trends, however, several differences can be observed [108]. For example, model calculations predicted an intermediate degradation zone depth of approximately 1mm, but experimental measurements showed it was 2.5mm, which may be explained due to the heterogeneity of C-S-H decalcification processes in actual cement samples. Another major difference was observed in the distribution of Al gel, which covered the entire degraded zone in the experimental investigation, but the model predicted that this layer would be completely removed by leaching within the degraded layer due to the calculated low pH value and the presence of silicon dioxide (SiO_2) gel. Figure 5b and 5c show the simulated distribution of chemical minerals and elements and the changes in contents within the cement paste after 450 days of bioleaching. The processes represented in *Equation 5* and *6* occurred at the interface between the sound and intermediate degraded zones, resulting in the complete dissolution of portlandite and C-S-H near the interface. The AFt content subsequently declined rapidly, represented by *Equation 7*, promoting sulphur (S) leaching and gypsum (calcium sulphate hydrate, $CaSO_4 \cdot 2H_2O$) formation. Gibbsite (aluminium hydroxide, $Al(OH)_3$) and gypsum (*Equation 7*), or precipitated $CaCO_3$ from the reaction

of portlandite and CO_2 from microbial respiration, can increase the density of the intermediate degraded zone [108,111]. The leaching of Ca, Mg, S and alkali components (OH^-) from the cement material and, consequently, the increase in concentrations of SiO_2 and aluminium oxide (Al_2O_3) (Figure 5b) indicated the formation of very porous SiO_2 and Al gels in the outermost degraded zone [108,111,112]. The formed gel regions have weak mechanical resistance, but they reduce the alteration kinetics by forming a semipermeable zone that may enhance the durability of the material [106,113]. The outermost zone is usually considered as decalcified, however, a small quantity of Ca may be detected in the gel layers when the pH is in the range 4 to 7 [108,113].

The acid dissociation constant, referred to as pKa, describes the strength of acids governing their corrosion potential. For a given pH value, acids with a lower pKa are more dissociated and, therefore, more aggressively dissolve cement [106]. In addition, poly-acidity is apparently more effective at cement dissolution compared to single acids [114].

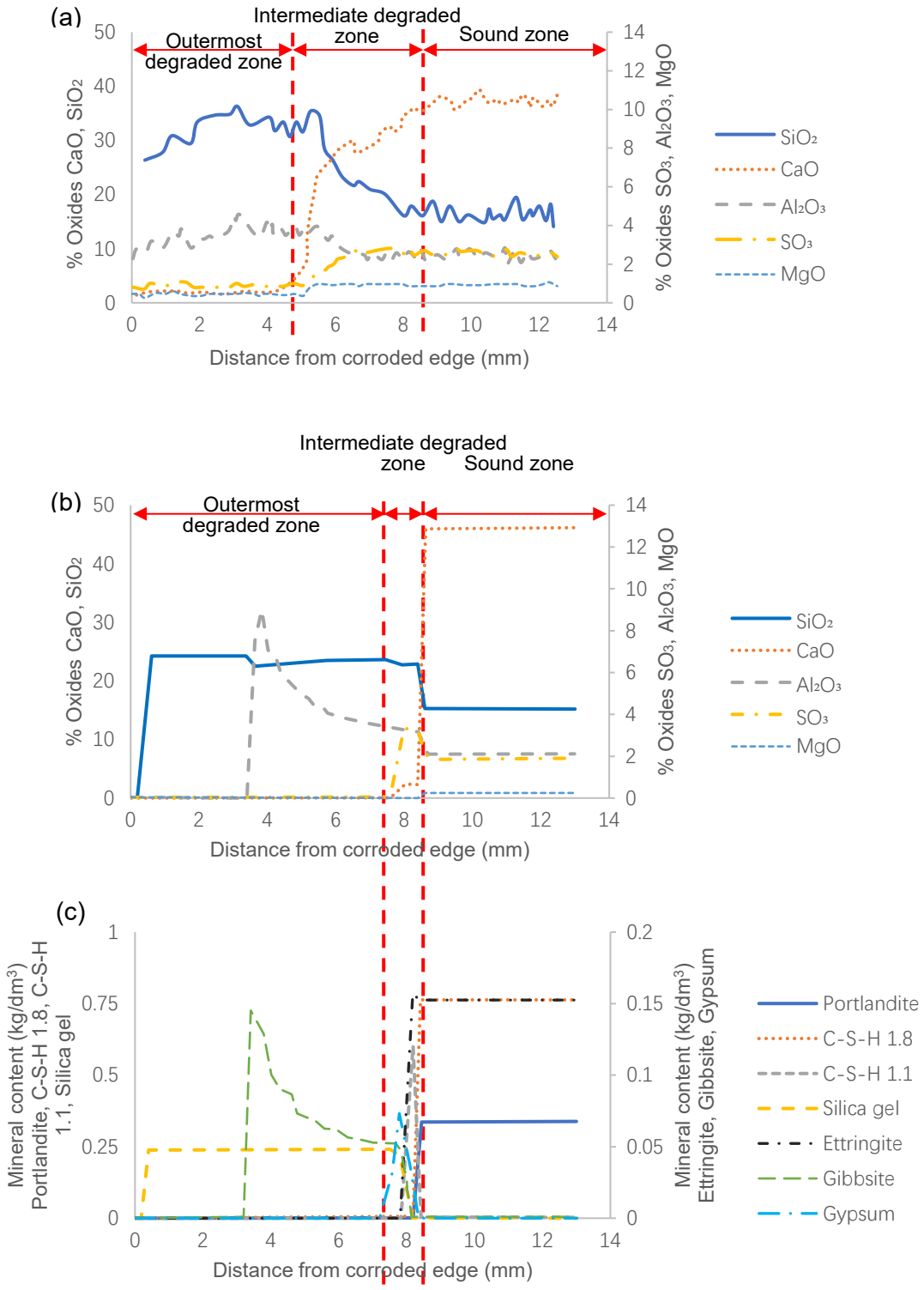


Figure 5. Changes in cement components following exposure to *A. niger* for 450 days (a) experimentally measured changes in chemical distribution, (b) predicted changes in chemical distribution, and (c) predicted changes in mineral content. The vertical

dotted lines divide the specimen into three zones: sound zone, intermediate degraded zone and outermost degraded zone (adapted from [108]).

The degree of concrete degradation is also affected by the properties of the Ca salts produced, and is particularly dependent upon their solubility. For example, calcium acetate ($Ca(C_2H_3O_2)_2$), calcium propionate ($Ca(C_3H_5O_2)_2$), calcium butyrate ($Ca(C_4H_7O_2)_2$), calcium iso-butyrate ($Ca(C_4H_7O_2)_2$) and calcium lactate ($Ca(C_3H_5O_3)_2$) are soluble in water (see Table 3), however, other Ca-organic acid salts, including: calcium citrate ($Ca_3(C_6H_5O_7)_2$), calcium malate ($C_4H_4CaO_5$) and calcium oxalate (CaC_2O_4), are sparingly soluble or relatively insoluble with solubility values equivalent to 85, 321 and 0.6 mg per 100ml H_2O (18°C), respectively [102,114]. At pH values <4, the solubility of Al salts is increased and should also be considered [111]. However, given the strongly alkaline nature of cement, acidification of the general matrix to this extent seems unlikely, nevertheless, it is possible to speculate that it could occur in localised zones, such as the point of organic acid secretion in close proximity to the fungal hypha. The formation of soluble salts accelerates the leaching of Ca, increasing the porosity of cement and promoting further degradation. By contrast, insoluble Ca salts produced by specific organic acids, such as, tartaric and oxalic acid, can protect the cement to a degree [114]. However, precipitated Ca salts do not always play a protective role. Indeed, Figure 6 shows the effects of different acids (citric, acetic, tartaric and oxalic at a concentration equivalent to 0.28 mol/L) on concrete and, although calcium citrate has low solubility (Table 3), citric acid attack is the most

aggressive. This can be explained by the expansive nature of citrate salts and also because they do not adhere to cement [114,115].

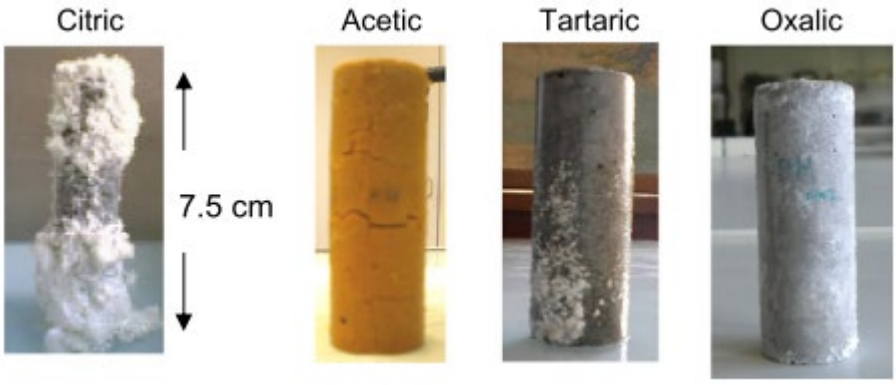


Figure 6. Effect of concrete immersion for 1 month in different organic acids [114].

Another factor influencing organic acid attack is the physicochemical characteristics of the cement matrix. In general, increasing the Ca content and porosity of the original matrix may result in more serious corrosion [116]. Aluminous cement contains less free lime and can, therefore, resist weak acid solutions (eg. tannic acid) more effectively than pure Portland cement. However, due to the rapid decomposition of ettringite, high alumina cement may be more susceptible to acetic acid than blast furnace slag cement or fly ash cement [117,118]. Moreover, incorporating acid resistant materials into concrete, such as quartz aggregate, can protect concrete from acid attack [119].

De Windt and Devillers (2010) showed that, with adequate nutrition and suitable environmental conditions for fungal growth, the biogenic acid concentration produced by *A. niger* on Portland cement pastes increased exponentially during the first 200 days to a maximum value of 300 mmol/L, when the production and consumption of

organic acids reached a steady-state equilibrium [108]. In parallel, the evolution over time of leached Ca closely followed the organic acid production profile, and increased from undetectable levels to 100 mmol/L in the first 100 days, which was consistent with the relationship between organic acid attack and cement bioleaching by fungi [108]. The degradation depth of cement increased in an approximately linear pattern with time to about 8.5 mm after 15 months. Interestingly, no hyphal growth was observed within the cement samples where organic acids were detected and cement degradation had occurred [108]. Thus, fungal organic acids diffused into the cement matrix along a concentration gradient between the external solution and the cement pore water resulting in organic acid degradation. Hence, the early stages of FID may be largely driven by chemical attack rather than physical mechanisms.

3.1.2 Enzymes

In addition to organic acids, fungi secrete small amounts of extracellular enzymes, which are also likely to participate in FID, although this mechanism has received little research [120]. Ilinskaya et al. (2018) detected hydrolytic enzymes, including proteases and lipases, secreted by *Aspergillus* strains isolated from aged concrete buildings [4]. Filamentous fungi, including *Aspergillus*, *Fusarium* and *Trichoderma*, can secrete keratinase which reacts with amino acids, also secreted by fungi, to produce thin needles of ettringite (Figure 7) [120,121]. In fresh concrete, the formation of ettringite plays a positive role by reducing the setting time and enhancing the early strength of the matrix. However, ettringite formation in hardened concrete may cause

cracks to develop and is therefore likely to be one of the mechanisms responsible for FID [122].

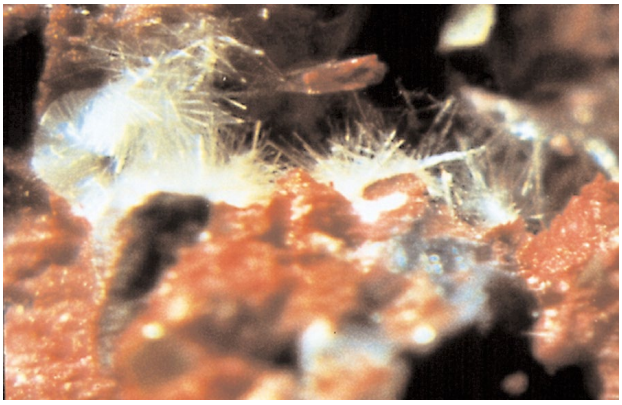


Figure 7. Ettringite formation in hardened concrete [123]

3.2 Mechanical attack

Under suitable environmental conditions and nutrient supply, fungal hyphae adhere to the surface of porous or fissured materials and may grow and extend into the inner material, sporulating and establishing new colonies that spread and sporulate further, increasing the distribution of the fungus within the matrix. In the case of impervious organic substrates, for instance, fungal enzymes weaken the material and assist hyphal entry and growth [37]. Turgor pressure within hyphae exerts mechanical force on the solid material being colonised by the organism, which ranges from 0.3 to 2.5 N/mm^2, depending on the fungal species and growth medium, facilitating the expansion of fungal structures such as hyphal tips and the process of fungal penetration [124]. Phytopathogenic fungi, including *Fusarium*

species, with specialised penetration structures, such as appressoria [125], are capable of generating considerably greater pressures in the range 5 to 8 N/mm^2 [126–128], although the possible role of these structures in concrete colonisation is yet unknown. Given our understanding of fungal behaviour and interactions with concrete, it is possible to hypothesise that similar, physical mechanisms may operate in, and contribute to FID of, concrete. Thus, initial chemical attack by acids and enzymes may facilitate fungal penetration and subsequent mechanical degradation, increasing the porosity and formation of cracks in concrete. For example, Jestin et al. (2004) observed crack formation in CEM I paste after three and six months of fungal degradation (Figure 8) [97]. These processes would allow entry of water, organic matter, and nutrients and further ingress of acids and enzymes, creating an environment within the concrete matrix that is suitable for fungal colonisation and growth. As hyphae penetrate into the concrete matrix, internal pressure increases potentially causing fragmentation and disintegration, leading to more crack formation and surface exposure and, consequently, further degradation [4,100]. A hypothetical framework of the chemical and physical mechanisms and interactions involved in the FID of concrete is therefore proposed and presented in Figure 9.

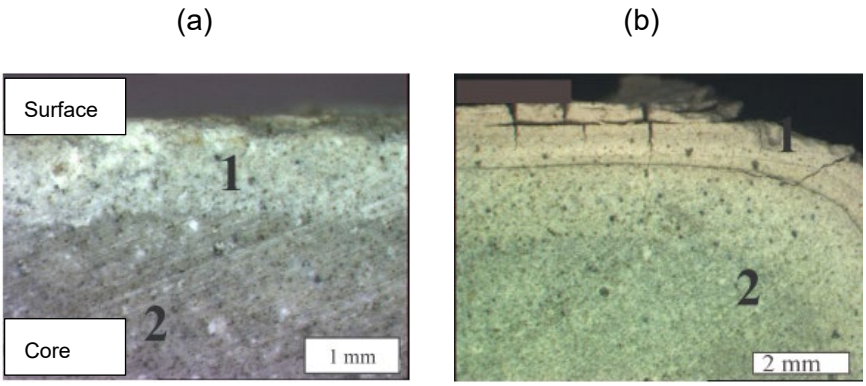


Figure 8. Crack formation on CEM I paste exposed to *A. niger* and *Trichoderma viride* (a) after three months and (b) after six months degradation [102]. Zone 1 and 2 represent the damaged and partially damaged layers, respectively.

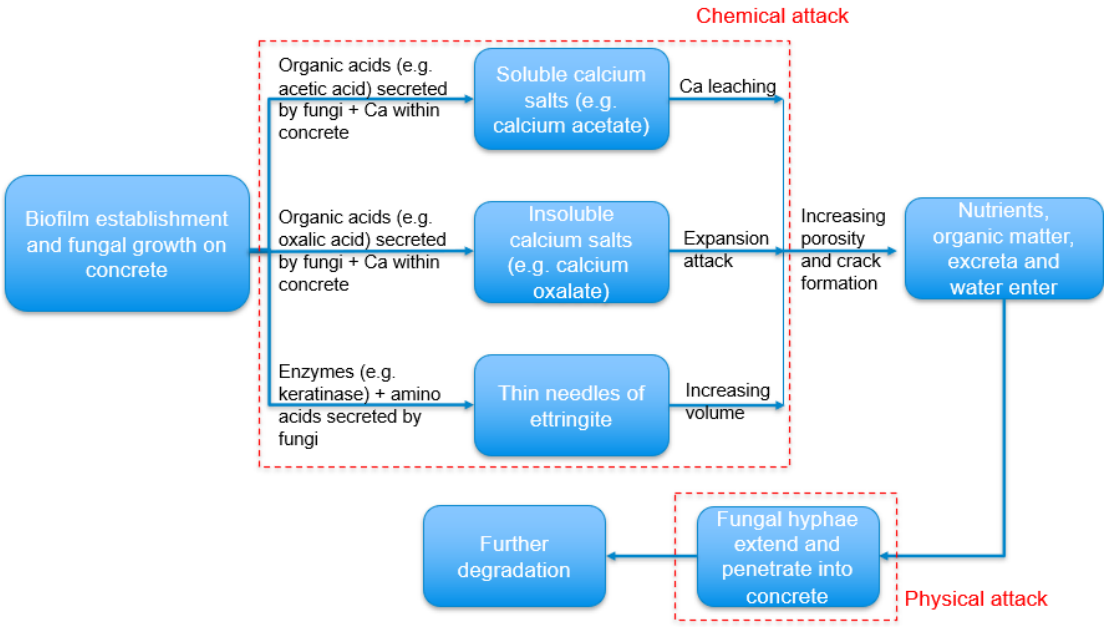


Figure 9. Hypothetical framework of the chemical and physical mechanisms involved in fungal influenced degradation of concrete [4]

Scanning electron microscope (SEM) images show the colonisation and penetration

of mortar and degraded concrete samples by filamentous fungi, respectively (Figure 10 and 11). However, images are only available showing fungal colonisation on the surface. Direct evidence is also required, using micro-structural, physical and chemical analysis techniques, to confirm that filamentous fungi can grow and cause degradation internally within the concrete matrix. Indeed, experiments simulating cracking of reinforced concrete indicated that failure pressures range from 2.25 to 7.43 N/mm^2 for uniform reinforcement corrosion and 10 to 70 N/mm^2 due to localised reinforcement corrosion, and that the pressures are related to the ratio of cover thickness and reinforcing bar diameter, and the orientation of reinforcing bar [129,130]. Thus, a fungal hypha growing and extending in parallel and near to the exposed concrete surface is more likely to cause cracking than a hypha at greater depth in perpendicular extension to the concrete surface. For example, for a failure pressure of 2.5 N/mm^2 [124] the depth/diameter would have to be <0.5. A single hypha with a diameter of 4μm may therefore only cause cracking if less than 2μm from the concrete surface [129].Multiple hyphae and successive rounds of delamination may produce cumulative damage, but, even so, this is still likely to be only possible at very shallow depths. This simplistic analysis suggests that failure pressures are generally larger than those exerted by fungal hyphae, further emphasising that mechanical attack by direct fungal penetration is most likely to be a secondary process following chemical degradation, once the concrete is at least partially microstructurally compromised.

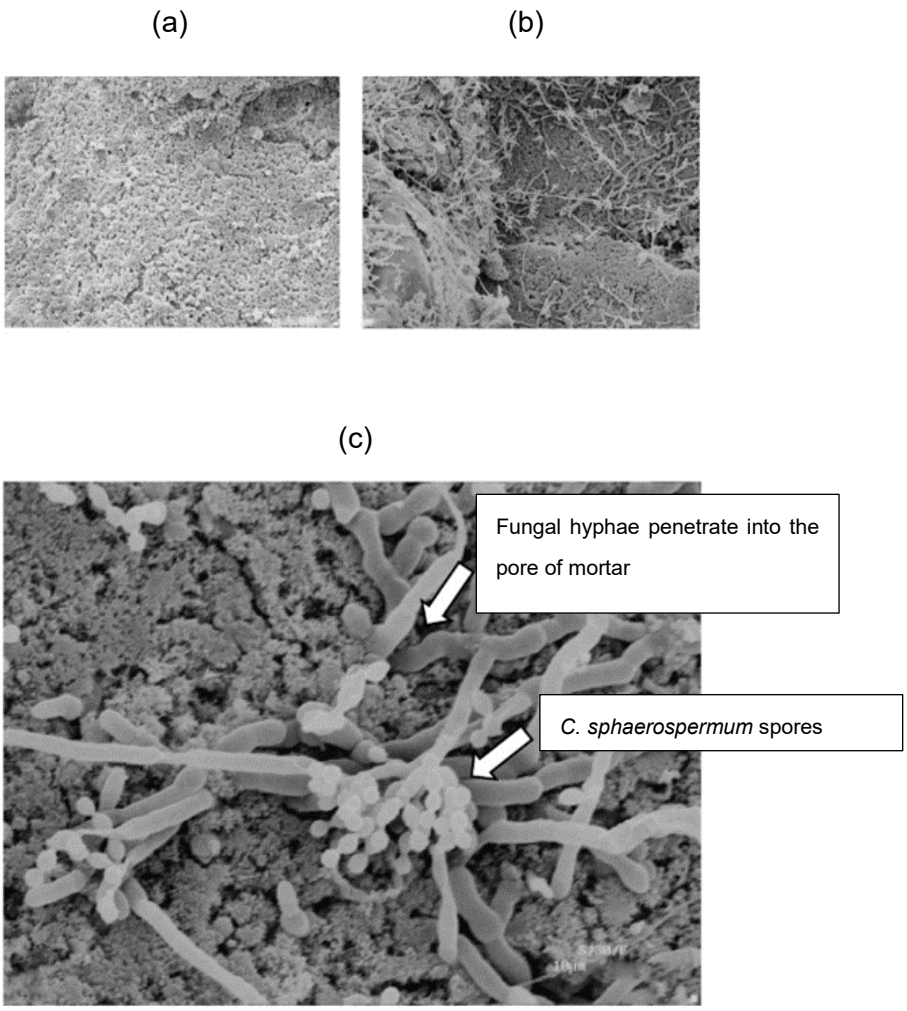


Figure 10. Electron-micrographs of mortar samples after 14 days of incubation at 25°C and 100% RH (a) without fungal inoculation, (b) showing the growth of *C. sphaerospermum*, and (c) fungal growth in (b) at higher magnification [80]

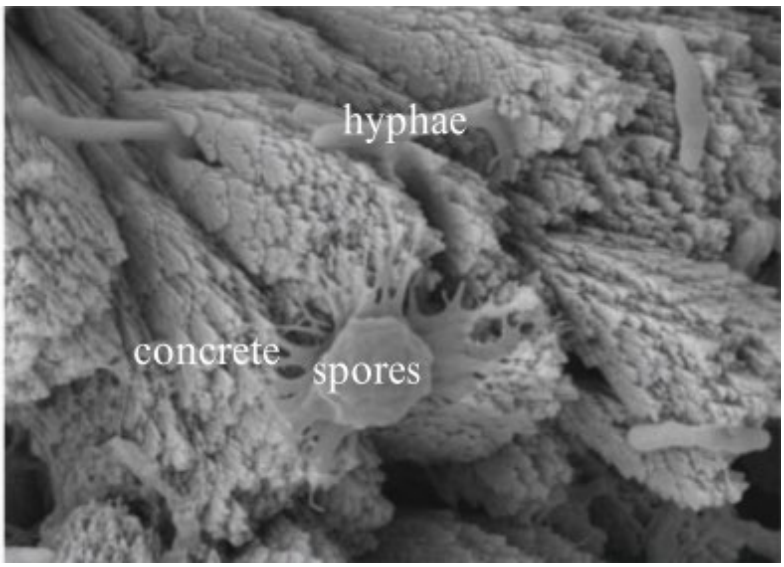


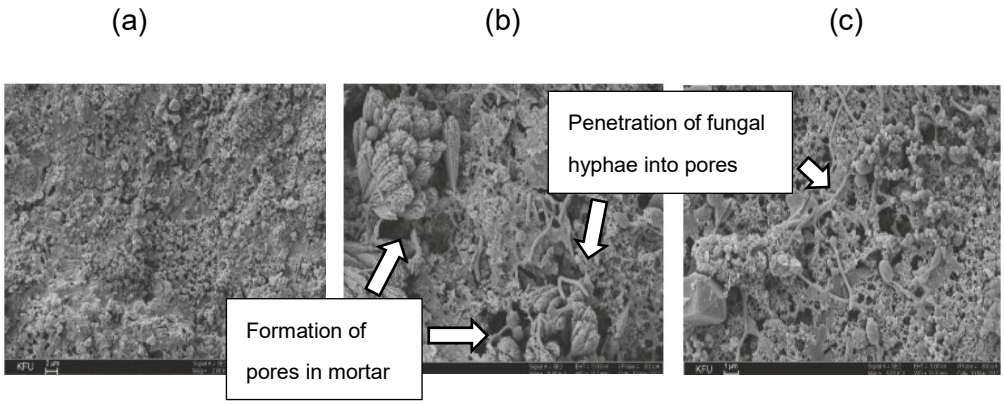
Figure 11. Fungal hyphae penetration of a concrete sample collected from former military hospital in Kazan, Russia, affected by *A. niger* [4]

3.3 Interaction between fungal growth and concrete properties

Several experiments report changes in concrete properties following fungal inoculation [2,108,111,112]. These include effects on aesthetic properties and mineral composition, and also an increase in the porosity of concrete. After 11 months exposure to *A. niger*, Perfettini et al. (1991) found the total porosity of cement samples (discs with thickness 4 mm and diameter 70 mm) increased to 31% compared to the uninoculated control, which had a porosity equivalent to approximately 20%. However, the amount of Ca leached was similar under both conditions, which may be explained due to the release of oxalic acid by *A. niger* and the formation of insoluble calcium oxalate [131]. Nevertheless, *A. niger* significantly reduced the bending strength by 78%, demonstrating the potential major impact of FID on the mechanical properties of concrete [131].

Yakovleva et al. (2018) conducted a similar experiment inoculating concrete beams (160*40*40 mm) with *Penicillium brevicompactum*, immersing the lower half in a culture medium and the upper surfaces were exposed to a humid atmosphere [10]. Both immersed and exposed surfaces showed evidence of fungal deterioration after a relatively short incubation period of 28 days (Figure 12). In this case, however, Ca leaching of immersed and exposed concrete inoculated with fungus increased by approximately 41% and 32%, respectively, compared to the uninoculated control [10]. The compressive and flexural strengths of inoculated concrete were also reduced, albeit not significantly compared to the control [10]. Interestingly, as may be expected, concrete samples with higher initial strengths were less susceptible to strength loss due to fungal attack [10]. Thus, *A. niger* reduced the compressive and flexural strengths of concrete with an initial compressive strength of 400 kg/cm^2 by 1.5% (from 6.60 to 6.50 MPa) and 0.46% (from 32.30 to 32.15 MPa), respectively, and, for concrete of higher initial compressive strength equivalent to 1000 kg/cm^2, they were reduced to a smaller extent by 0.41% (from 12.20 to 12.15 MPa) and 0.21% (95.2 to 95.0 MPa), respectively [10]. The reason why the apparent effects of FID on concrete properties described by Yakovleva et al. (2018) were far less pronounced may be explained by the shorter incubation period and larger concrete specimens used compared to Perfettini et al. (1991). The Young's modulus (E) is another mechanical property, describing the stiffness of solid materials, including concrete, and the value of the outer zone of concrete affected by FID can decrease to as low as 4.5% of sound

concrete [111].



(a) Surface of control mortar sample immersed in culture medium without fungal inoculation (magnification: 200k)
(b) Concrete specimen inoculated with *P. brevicompactum* immersed in the culture medium (magnification: 500k)
(c) Concrete specimen inoculated with *P. brevicompactum* and exposed to humid atmosphere

Figure 12. Exposed and immersed surfaces of concrete samples inoculated with *P. brevicompactum* after 28 days of growth on a nutrient medium [10]

As may be expected, concrete formulation and environmental conditions have an important influence on the extent of fungal colonisation and growth on concrete and the severity of degradation. For example, concrete with rough surfaces, low pH values and treated by accelerated carbonation tend to be more susceptible to fungal attack [9,80]. Mineral additions used in cement production may also significantly influence fungal colonisation and development. For example, Jang et al. (2020) showed the colonisation area of *A. niger* on the surface of Portland cement mortars increased with the rate of fly ash addition from zero, for the unamended control, to approximately 35 and 75% at incorporation rates of 10 and 20%, respectively [58]. The effect of fly ash on accelerated fungal growth may be explained by the reduced pH value and increased

rate of carbonation of amended concrete. For example, fly ash incorporation into Portland cement at a rate of 45% by mass achieved two to three times the full carbonation depth after a period of 14 days compared with the standard Portland cement control [132]. As discussed in Section 2.3, fly ash also provides a source of N increasing the growth of fungi in concrete environments [51].

4. Conclusions

The negative effects of bacteria on the physical and chemical properties of concrete are well established and have been extensively studied. The action of SOB and nitrifying bacteria on concrete occurs through a non-specific mechanism of mineral acid attack and low pH value, which increases the porosity and weight loss, and reduces the strength of concrete [133]. However, FID is potentially a more significant and wide-spread problem, due to the environmental tolerance and survival characteristics of fungi compared to bacteria. Filamentous fungi are ubiquitous in the environment and uniquely grow by hyphal tip extension, but, as with all microbes, require nutrients, sufficient moisture, and a suitable temperature and pH value. Nevertheless, they are less fastidious than bacteria and can self-supply nutrients and organic substrates through fungal autolysis, which is a strategy that enables the survival and growth of the organism in deficient environments, such as concrete. Therefore, hyphae can extend through low-nutrient areas to explore and exploit new

regions of the matrix for resources [91]. In addition, they are able to modify their local external environment through the transportation of nutrients, organic substrates and acids, water and O_2 within the continuous mycelial network, improving conditions for further growth of the fungus, as well as other microorganisms, such as bacteria [25]. In particular, fungi have a high Ca requirement for apical growth and this can be met by concrete materials, which may explain why they are extensively found in concrete environments [62].

The main types of filamentous fungi associated with concrete degradation include: *F. oxysporum*, *A. niger* and *C. sphaerospermum*, which damage the mechanical and chemical properties of concrete. The degradation mechanism is driven primarily by the reaction between organic acids, released by fungi, and Ca^{2+} in concrete forming soluble Ca salts, resulting in Ca leaching, and insoluble Ca salts, causing expansion attack [108]. Fungi also secrete keratinase, which reacts with amino acids producing thin needles of ettringite that develop cracks in concrete [120,121]. In addition, the growth and extension of fungal hyphae may degrade the concrete matrix mechanically due to expansion and increasing internal pressure, although this is likely to be a secondary process following initial chemical attack [4,100].

Overall, filamentous fungi can survive and grow in concrete structures due to the resistance of the organism to environmental stress, conservation and recycling of scarce nutrient resources by mycelial autolysis and transportation of nutrients,

substrates, water and O_2 within the continuous network of the fungal colony [25,91]. Fungal cell walls are strong and flexible and the unique three-dimensional structure of the colonies allow the organism to penetrate into dense matrices, including concrete [7,37]. Filamentous fungi, including, *F. oxysporum, A. niger and C. sphaerospermum* potentially cause chemical and mechanical damage of aged concrete due to several interactive processes that operate simultaneous: (1) Ca leaching due to the production of soluble Ca salts from the reaction of fungal organic acids and Ca^{2+} in concrete; (2) expansion by insoluble Ca salts, such as Ca citrate, also from the reaction between Ca^{2+} with fungal organic acids; (3) production of ettringite in concrete from the reaction between keratinase and amino acids released by fungi; and (4) direct mechanical damage from the increased internal pressure exerted by fungal hyphae [4,100,108,120,121]. Whilst the mechanisms of FID have been elucidated and critically discussed here, the main evidence shows the mode of attack is chemical and progresses from the concrete surface. Direct, quantitative evidence demonstrating: (a) fungal hyphae penetrating the concrete matrix, resulting in (b) internal chemical and mechanical degradation, has not yet been reported and is an area requiring further physical and chemical research to determine the potential extent and significance of internal FID of concrete.

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