



Fungal Deterioration of Cellulosic Textiles: a Review

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ABSTRACT

Microbiological agents, such as bacteria, actinomycetes, and fungi, degrade cellulose. The most severe deterioration in indoor environments is primarily caused by cellulolytic fungi, resulting in loss of fiber strength and actual material failure. Other microbial spoilage can occur as a result of the permanent staining from pigmentation and mycelial penetration by both cellulolytic and non-cellulolytic fungi, the latter using food sources ranging from starch sizing to surface dirt, and oils. Much has been written concerning biocidal testing against these microfungal agents from an industrial point of view; less from a conservation standpoint. This paper submits a limited review of current literature (c. 25 years) focusing on the information and its relevance for the conservator. The following topics related to cellulosic deterioration by fungal microbes are covered: 1. current theories about the mechanism of

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cellulolytic attack; 2. biocides which may be employed in fungal control; and 3. choice of environmental conditions as a means of static control of fungal biodegradative agents. An annotated reference list is provided.

INTRODUCTION

Connections between dampness and moldy growth on textiles were made as early as biblical times. The study of microbial growth as a direct consequence of spores in the air was begun in the late 19th century and only within the last 40 years has the mechanism of cellulolytic attack on fibers begun to be understood. Beckwith *et al.* (1940), lists some of the early references to studied in the field of fungal deterioration of textiles.

The literature is extensive on microbial vectors found on textiles exposed outdoors (Siu, 1951, is probably the most comprehensive early work on the subject). It has been shown that the outdoor microbial population in the air is similar to the communities of organisms found in soil (Siu, 1951). Hence, one of the most frequently used test procedures on treated fabrics is the soil burial test (Darby & Kempton, 1962; Turner, 1972; Walton & Allsopp, 1977; Kaplan, 1978; Kowalik, 1980; Raschle, 1983, 1989), (cf. other commonly used test methods: Hueck, 1965; Hueck *et al.*, 1966; Hueck, 1969; Park, 1976; Kowalik, 1980; Vigo, 1983; Sager, 1987; Raschle, 1989.) Although these types of tests are not directly appropriate to routine conservation investigations, they have proven of interest in the peripheral information which has evolved from them, for example, how the growth of fungal colonies can be affected by various environments.

Other biological agents besides fungi work alone or synergistically with fungi to destroy textiles in the soil. The high water content (60%) found in the most effectively destructive soils (Raschle, 1983) and the high RH found outdoors make it also possible for bacteria and actinomycetes to accomplish cellulolytic decomposition (*Bacteria references*: Hueck, 1965; Hueck *et al.*, 1966b; Adema *et al.*, 1967; Quesnel, 1968; Lashen, 1971; van der Kerk, 1972; Vigo, 1980; Vigo & Benjaminson, 1981; Vigo, 1983. *Actinomycetes references*: Waksman, 1967; Betrabet *et al.*, 1968; Mandels, 1975; Hagerdal *et al.*, 1979; Fermor & Eggins, 1981; Williams, 1985). It has been noted that the amount of water in and around a textile is of limited biological importance; the water that is physically bound to the fibers themselves seems to be a controlling factor in microbiological growth (Block, 1953; Hueck, 1972). [References for the derivation of the 'water activity' $a(w)$ formula are Sagar (1987) and especially Rose (1981)]. Both bacteria and actinomyceti require a water

activity reading $a(w)$ of at least 0.90 (water has $a(w) = 1.0$), making them unlikely attackers in indoor environments (except for emergency wet disaster conditions). They will not be considered here beyond the references given.

Interest in rot-proofing textiles increased during World War II when the US Army had needs for textiles primarily used in wet tropical and subtropical regions, and it continued into the 1950s and 1960s (Vigo, 1983). Vigo lists Siu (1951) as an excellent review of cellulosic deterioration up to 1950, Howard & McCord (1960) for the next decade, and Mahomed (1971) for the 1960s. His own work (Vigo, 1983) competently takes the subject through the 1970s. A brief look at the Allsopp & Allsopp (1983) survey of commercial biocides (already obsolete) gives an indication of the continuing appropriateness of such research. Unfortunately for the conservator, most of the tested commercial biocides are designed to be applied at the time of manufacture. For the most part, those that can be used effectively later have been proven either chemically or physically inappropriate for use on museum textile objects, or too dangerous to humans.

PART 1: MECHANISM OF ATTACK

Cellulose is a linear polysaccharide made up of β -D-glucopyranose molecules linked together through a glycosidic bond to an OH group at the carbon 1 and 4 positions (Allsopp & Seal, 1986). The basic unit cell of cellulose is an attached pair of glucopyranose units, called cellobiose. The degree of polymerization (DP) of native cellulose (cotton) is about 10 000 (other plant species of cellulose-containing fibers vary in their DP). The individual cellulose chains align by means of hydrogen bonding, creating 'elementary fibrils', containing crystalline and amorphous regions in at least a 70:30% ratio, but probably even more crystalline (Selby, 1968; Allsopp & Seal, 1986). These elementary fibrils bond together in bundles of about 2000, called 'microfibrils'. Four hundred microfibrils group together to make a single 'fibril'. The fibrils organize themselves in parallel, random, or spiral fashion around the central hollow lumen, giving a primary and a secondary wall (Selby, 1968).

If this complex organization of cellulose is thought of in three dimensions, it is possible to imagine the existence of a matrix surrounding these members that is filled with non-cellulosic materials, and microcapillaries. The nature of the chemical and structural makeup of these matrix materials (pectin, hemicellulose, and lignin) can affect its susceptibility to microbiological deterioration (Kowalik, 1980; Vigo,

1983). Pectins act as a food source and promote microbiological attack (Vigo, 1983), while lignin (an amorphous, cross-linked polymer which bonds to carbohydrate materials) and naturally occurring waxes are deterrents (Kowalik, 1980; Vigo, 1983; Millett *et al.*, 1979). Natural plant sources contain varying amounts of cellulose: cotton, 94%; hemp, 77%; flax, 75%; sisal, 75%; ramie, 73%; jute, 63%; wood, reed, straw, and bamboo all are less than 50% cellulose (*Encyclopedia of Polymer Science and Engineering*, 1987).

Studies underline the complexity of fungal enzyme attack on cellulosic textiles (Howard & McCord, 1960; Selby, 1968; Gong *et al.*, 1979; Gritzali & Brown, 1979; Wood & McCrae, 1979; Sagar, 1985). The enzymes are produced by the organisms for the purpose of changing insoluble cellulose into soluble sugars, to be metabolized as food. Production of these enzymes can be induced by the introduction of cellulose, and inhibited in the presence of cellulose derivatives (simpler saccharides) which do not require the evolution of the full enzyme complex for digestion (Bravery, 1968; Hueck & Hazeu, 1969; LaBrijn & Kauffman, 1972; Gong *et al.*, 1979; Vigo, 1983; Allsopp & Seal, 1986). Natural hydrolysis of cellulose, i.e. cleavage of the 1,4-glycosidic bond in the presence of water, is a normally slow process. However, it is catalyzed by enzymes, and together they can completely degrade a textile in only a few days under the right conditions (Hueck, 1972; Hamlyn, 1983). The enzymes themselves can still continue degrading the fibers, even after the organism which produced them has been destroyed (Allsopp & Seal, 1986).

Cellulose passes through intermediate forms (oligomers, cellobiose, glucose) as it is broken down by a system of several enzymes referred to as the *cellulase complex*. There is a general consensus in the literature as to the existence of three main enzyme component types: certain exoglucanases (commonly referred to as C1), certain endoglucanases (Cx), and enzymes with β -glucosidase capabilities. The β -glucosidases play an important role by cleaving one of the intermediate products, cellobiose, into its two glucose components. The presence of cellobiose, similar to the presence of other alternate carbon sources, acts as one of the inhibitors to the production of cellulolytic enzymes.

It is still unclear how the more crystalline areas of cellulose are solubilized. Selby (1968) gives an interesting list of early research references and the methods they employed in attempting to determine the breakdown mechanism. It was thought that only the C1 enzyme had the capability to attack the crystalline regions of the cellulose chain (according to early research done by Reese *et al.*, 1950, among others). C1 would supposedly open the cellulose chain and the Cx enzymes

(numbering up to 20, Millett *et al.*, 1979) would then hydrolyze the more soluble cellulose derivatives. Wood and McCrae (1979) report that it is not really as simple as previously thought. Fractionation studies isolating C1, Cx and β -glucosidase from various cellulolytic fungi have shown varied synergistic activity between the enzyme components, seemingly variable with regard to the strain of organism, environmental conditions, differences in crystallinity of the substrate, presence of alternate food sources, and the state of swelling and decomposition of the fibers (Wood & McCrae, 1979; Gong *et al.*, 1979; Vigo, 1983; Allsopp & Seal, 1986). Wood and McCrae conclude that although it seems that C1 does have an affinity for crystalline cellulose, Cx seems to be the first to cleave the glycosidic linkage, almost immediately followed by C1 which is already in place.

The role of C1, according to Sagar (1987), is still controversial. It appears to be able to catalyze the hydrolysis of the 1,4 bonds between D-glucose units, where the spatial placement of hydroxyl groups available for hydrogen bonding with the enzyme molecule is of a particular configuration (Sagar, 1985). As a result of the testing of cell-free enzyme component isolates, it appears that some variations exist in the capabilities of these components of different cellulolytic fungi equally to degrade cellulose. This may account for some inconsistencies in laboratory tests conducted on biocides using different species of fungi. Other factors which have contributed to some confusions in testing are listed by Gong *et al.* (1979). Table 1 gives a list of 10 commonly tested fungi found on textiles and the reference number of the studies which selected them.

PART II CHEMICAL PROTECTANTS

It seems at present that textile conservators generally do not attempt to identify specific strains of fungus found on textiles in their collections. Articles devoted to the screening and detection of fungal activity describe methods which require chemical tests done to the surface of the textile, immersion of the textile into deleterious chemical baths, or sample taking of too large a size (van der Toorn *et al.*, 1965; Allsopp *et al.*, 1970; Mills *et al.*, 1972; Lashen, 1971; McCarthy, 1983, 1984, 1987). Carter (1984) describes simpler methods, more appropriate for museum textiles, using microscopical identification, UV fluorescence, and variations in pH.

The textile laboratories of several museums in New York City have indicated a general consensus that no chemical treatments have been proven safe enough (private communications). Instead, a combination

TABLE 1

<i>Fungi</i>	<i>Cellulolytic activity</i>	<i>References</i>
<i>Trichoderma viride</i>	+	1, 28, 29, 30, 36, 37, 39, 40, 43, 52, 56, 63, 64, 73.
<i>Aspergillus niger</i>	-	1, 25, 28, 29, 30, 39, 43, 46, 49, 52, 55, 56, 69, 73.
<i>Chaetomium globosum</i>	+	1, 5, 28, 29, 30, 39, 40, 43, 52, 55, 56, 69.
<i>Myrothecium verrucaria</i>	+	1, 28, 29, 30, 39, 40, 56, 58.
<i>Penicillium funiculosum</i>	-	39, 40, 43, 52, 63.
<i>Memnoniella echinata</i>	+	28, 39, 40, 69.
<i>Stachybotrys atra</i>	?	40, 52, 63.
<i>Aspergillus flavus</i>	-	39, 40, 52.
<i>Fusarium</i> sp.	+	43, 56, 63.
<i>Rhizopus nigrican</i>	?	43, 56, 63.

+ Or - indicates cellulolytic activity.

of mechanical methods: brushing, vacuuming under a binocular microscope, dry-cleaning, and better environmental control are used. Older literature recommends various washing and mechanical techniques: Glover (1973) recommends that textiles be dried in hot air, brushed outdoors, washed, rinsed and dried. Rice (1973) recommends mild bleach when possible, and drying in sunlight. Flury-Lemberg (1988) also mentions both a soda-alkaline wash, and a bleaching method for 'mildew'. All authors correctly stress control of indoor humidity. However, there are collections in parts of the world which are not able to be controlled by air-conditioning, where fungal infection is common. In such cases, where there is a need for emergency treatment of active growth, as well as preventive use on objects at risk, chemical biocides could be considered.

The decision to use a chemical biocide on museum textiles is not easy. It has been recommended that the identification of species of fungi be determined before any biocidal decisions are made. Fungi respond differently to toxic chemicals, and proper prescriptions can only be made

knowing the species (Nair, 1974). However, as has been shown by others, the slightest variations in pH, colony age, RH, and alternate food sources enormously change the parameters for fungal growth, and thus change the biocidal requirements for the individual colony. Almost every publication which discusses the testing of biocides begins with the disclaimer that no panacea exists for chemical control of microbes on textiles. Several references list the minimum requirements of a biocide useful for conservation (Kaplan, 1968; Baynes-Cope, 1972; Yeager, 1977; Hamlyn, 1983; Sagar, 1987). The industry-related testing reports rarely discuss conservation issues like changes in hand, flexibility, degree of lightening or darkening, or effect on colorants.

Burgess and Binnie (1990), in their report on research approaches to cellulosic textile testing, address the types of analytical monitoring they feel are necessary during biocide testing and which would give the conservator quantifiable data for biocidal decision-making: acidity/alkalinity, degree of polymerization, presence of carbonyl functional groups, color by reflectance measurements, tensile strength, and various methods of functional group or chemical residue determination. Even without sophisticated instrumentation, some simple test methods can be devised to ascertain if the fungicide is oxidizing or acidifying the textile (Jedrzejska, 1967).

Chemical protection of cellulose from fungal (cellulolytic) attack is best accomplished by using impregnation methods due to the microscopic size of the fungi spores (Hueck, 1972; Vigo, 1980, 1983). Recent fumigation tests have proven ineffective. Gustafson *et al.* (1990), tested eight commonly used fumigants, and concluded that none of the eight exhibited acceptable fungicidal activity when used in standard thymol cabinets. Haines and Kohler (1986) agree according to the chemicals they tested in similar setups. Impregnation methods include:

1. Insolubilizing biocidal toxicants onto the surface. This is the normal method of biocidal deposition onto the textile (Vigo, 1980; Hamlyn, 1983; Vigo, 1983). Care should be taken to test for the presence of sizing or other chemical treatments as there may be an undesirable interaction between the fungicide and the surface (Bomar, 1966; Burgess & Binnie, 1990). Consideration must be given also as to the effect of the vehicle delivering the active biocide, whether it is a solvent or an emulsifying agent (Hueck, 1972). A study of the history of biocides used in museums might be valuable. According to Yeager (1977), before 1926 zinc chloride was the principal fungicide used for textiles, along with salicylic acid, sodium fluoride, sodium silicofluoride, phenol, cresylic acid, and formaldehyde.

2. Altering the cellulose chain structure so as to make it indigestible. Acetylation and cyanoethylation were studied in the 1960s (Howard & McCord, 1960; Hueck, 1965; Mahomed, 1971; Yeager, 1977; Vigo, 1980, 1983). Such methods, along with polymerization of monomers onto the surface fibers, are not easy processes and have not proven fully safe for fabrics (Hueck, 1972).
3. Creating a barrier-type film coating on the fibers. Early work by Ruperti *et al.* (1964) discusses the 'Arigal' method of resin incorporation (Hueck, 1969). It is not yet clear whether protection is due to covalent bonding of these varied nitrogenous resins to the cellulose at the molecular level or that they simply penetrate the fibers and create a physical barrier (Kaplan *et al.*, 1972; Isquith *et al.*, 1972; Yeager, 1977; Vigo, 1980, 1983). The combination of a barrier resin with an active toxicant is accomplished by using organosilicon polymers containing pendant quaternary ammonium groups (Vigo, 1983).

One of the controlling factors of the effectiveness of a biocide is the amount of structural degradation already present in the fiber (lowered degree of polymerization, decreased crystallinity, etc.). A higher degree of structural degradation will increase susceptibility to microorganism attack (Hueck, 1965; Millett *et al.*, 1979; Vigo, 1983), and also make chemical substitutions on active sites unpredictable. An interesting unexplained result is reported on cellulosic textiles that were weathered outdoors: they were shown to be less susceptible to fungal attack (Kaplan *et al.*, 1970).

Another factor in controlling the effectiveness of the biocide is an understanding of the chemical status of the textile surface in its relation to the toxic agent. For example, variations in pH of the biocide, fungal colony, or the textile can drastically change its effectiveness (Wessels & Adema, 1968; Allsopp & Barr, 1976; Kaplan, 1978; Vigo, 1980). Other factors involved are the technique itself, the moisture content $a(w)$ of the fibers, the effect of light on the treated fabric, the maturity of the organisms, and the presence of alternate food sources.

These variable factors, and others, were shown to change test results drastically: even those carefully attempting to duplicate test procedures. A study done by the International Biodegradation Research Group showed significant differences in the results from identical experiments performed at 30 different testing laboratories in nine countries (LaBrijn & Kauffman, 1972).

Industrial testing reports sometimes contain useful information for the conservator, such as the ineffectiveness of a biocide against specific

agents, resultant yellowing, reaction to UV light, and loss of tensile strength. Those parameters alone can help to rule out certain chemical treatments. The improved sensitivity in toxicity and carcinogenic tests has also limited the number of possible biocides available to the conservator. Many chemicals used in the past have since been banned for health reasons or they were found to be harmful to museum textiles. The conservator must carefully review the prospective environmental conditions before making a decision.

Table 2 lists some of the commonly tested biocides, applied by *impregnation*, which in the past have been thought to have possible fungicidal applications for conservation. Each is followed by a brief description and references. Caution should be taken of the formulations of commercial fungicides. Kaplan (1968) discusses the role of the formulator in his search for more effective products and the problems of constantly changing formulas. The delicate interaction of additives in a biocide is an important issue, introducing the possibility of side-effects for artefacts (see: van der Kerk, 1971; Block, 1979; Rose, 1981).

PART 3: ENVIRONMENTAL CONTROL

The most common and least intrusive method for the prevention of fungal growth is the control of the environment. Factors that affect the growth of fungi specifically on textiles are described by Siu (1951), Hueck (1972), Vigo (1980), and Raschle (1989). A guide to the inhibition of microbes in general, relating many methods of environmental control, is found in Skinner & Hugo (1976). As is constantly stated, humidity and temperature exert the most influence on colony growth, but there are subtle interrelations among other factors such as pH, $a(w)$, oxygen, and light which determine the overall activity of a microorganism (Vigo, 1983).

The range of temperatures which supports fungal growth is large. Dormant conditions for spores, waiting for the proper environment to re-emerge, can range from 0 to 70°C (Hueck, 1972). Most cellulolytic fungi are mesophilic (25–45°C) (Vigo, 1983), and thus it is suggested that a satisfactory temperature for long-term storage be about 10°C (Hueck, 1972). Nutrient status, $a(w)$, and pH have a direct relationship on the temperature an organism can bear (Ingram & Mackey, 1976). For example, the effect of reducing $a(w)$ to below the minimum tolerance for continued existence of the spores would not necessarily cause their demise if the other related parameters were well above the basic support measurements. However, the effect of reducing the $a(w)$ would be to raise

TABLE 2
Commonly Tested Biocides

<i>Biocide</i>	<i>Merck Index No.</i>	<i>Comments</i>	<i>Refs</i>
1. <i>O</i> -Phenylphenol	7 110	Toxicity similar to phenol (OPP) Potential carcinogen (NIOSH)	6, 20, 39, 52, 54, 63
2. Sodium <i>O</i> -Phenylphenol (NaOPP)	7 110	Hazards similar to OPP	52
3. Salicylanilide (SAL)	8 090	Skin & mucous membrane irritant	15, 25, 52, 68, 39
4. Tributyl tin oxide (TBTO)	—		15, 49, 52, 70, 23, 63
5. <i>P</i> -Chloro- <i>M</i> -cresol (CMC)	2 108	No toxicity found	52
6. Pentachlorophenol (PCP)	6 901	LD ₅₀ (oral rats) 146-175 mg/kg Prohibited in Switzerland 1989	1, 56, 52, 28, 6, 76, 39, 70, 63

7. Sodium pentachlorophenol (NaPCP)	6 901	—	—	1, 49, 15, 52
8. Pentachlorophenol esters (LPCP)	—	—	—	1, 29, 49, 68 69, 76, 23
9. Quaternary ammonium compounds (QAC)	—	—	—	23, 25, 30, 41, 49 76, 57, 69, 71
10. Fatty nitrogen compounds	—	—	—	30
11. β -Propiolactone (BPL)	7 610	LD ₅₀ (i.v. mice) 345 mg/kg	—	25
12. Trimethylmelamine	9 386	No toxicity found	—	69, 36
13. Dichlorophene	3 047	LD ₅₀ (oral rats) 2.69 g/kg	—	76, 69, 39
14. Cadmium salts	—	—	—	55

the minimum temperature requirement for growth. Loss of viability may occur upon freezing at that reduced $a(w)$ point due to the phenomenon of 'cold shock' (Ingram & Mackey, 1976).

Fungi grow most rapidly at RH levels above 80%. Relative humidity for a biologically safe museum environment has been suggested to be held at about 50% (Hueck, 1972). Constancy is the important factor. Fluctuating RH values leave opportunities for microbial growth. Micro-environments, caused by the limited ability of some areas of storage, exhibition space, or packaging to release trapped excess moisture during times of fluctuation, cause problem areas which can increase the already existing RH by up to 20%. Block (1953) had suggested 70% RH but studies have proven that under these conditions micro-environments can push up the RH into the unacceptable optimum growth range.

There is, however, a *minimum* RH value below which it is detrimental for the cellulosic textile. A severe lack of moisture could cause even the bound water within the fiber to escape (equilibrate with its environments), thus shrinking the fiber and effecting the hydrogen bonding between the microfibrils. This would embrittle the textile. The $a(w)$ of fibers are in equilibrium with the RH (Hueck, 1972; Dallyn & Fox, 1980; Rose, 1981). Few species of fungus can grow below $a(w)$ of 0.8 (Raschle, 1989), but a severely low RH which would lower the $a(w)$ to below 0.4 would be dangerous to the textile.

Most cellulolytic fungi produce cellulases within a pH range of 4.0–6.5 (Vigo, 1983) (Wessels & Adema, 1968 had previously reported findings of optimum cellulase activity to be within a pH range of 6.4–8.2). Simply washing out acidic oxidative by-products (along with surface dirt food sources) from textiles would seem to be an aid in fungal control. Reducing sources of photooxidation and acidic pollutants would seem to be within the control of the conservator.

REFERENCES

Key to annotation coding system:

- * Exceptional bibliography
- C Conservational interest
- G General interest
- B Specific biocides or biocidal treatments
- M Mechanism of deterioration or cellulosic structure
- F Specific fungal species
- T Specific test methods
- D Detection methods

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