

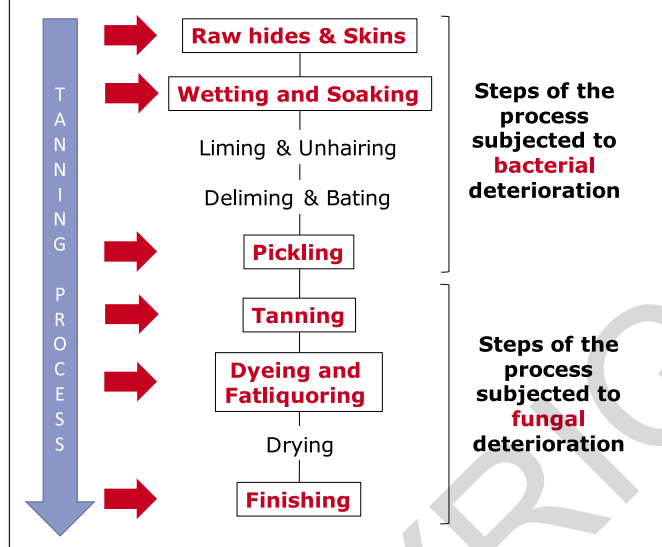
Management of microbial growth on hides and leather (part one)

Zulfe Biehl, managing director, ATC Brazil

Leather is a natural product susceptible to microbial growth owing to the presence of proteins and lipids. Tanneries provide an environment that lends itself to microbiological development since there are sources of nutrients and water throughout the manufacturing process as shown in Fig. 1 below. Pickled pelts, wet blue hides and vegetable-tanned moist leathers are especially prone to microbial attack, even when stored or shipped^{1,2}.

This paper is based on a presentation the author gave at the XXXV IULTCS Congress in Dresden in June 2019.

Fig. 1: Tanning process flow chart. In red, all steps in which it is possible to use biocides.



Chrome- and vegetable-tanned leathers contain several compounds, such as ammonium salts, phosphates, surfactants, fatliquoring agents and other organic agents that enable microbial (especially fungal) growth to cause damage to the material. For this reason, biocides have a role to play in the tannery.

Methods

This review was created by consulting the databases PUBMED (PubMed comprises more than 29 million citations for biomedical literature from MEDLINE, life science journals, and online books), Web of Science, (a platform with more than 9,200 journals) and ScienceDirect (a source for scientific, technical, and medical research), and other literature.

Environmental conditions for fungal proliferation in tanneries and fungal diversity in wet blue biodeterioration

Besides the fact that hides and leather are suitable substrates for microbial proliferation, other variables may directly influence the growth of fungi, as well as bacteria. In this sense, some steps of the process could be cited as major problems that favour fungal growth:

- Tanning processes with quality deviations, mainly related

to insufficient degreasing and the presence of reducer compounds;

- Longer storage time than the half-life of most of the fungicides;
- Poor storage conditions, with inappropriate cleaning and disinfection routines;
- Poorly controlled drying operations, where the humidity remains high or the drying process takes too long;
- Poor airflow;
- High temperatures inside drying rooms.

Environmental conditions also have an influence on fungal diversity in leather contamination. Temperature, for example, directly impacts fungal growth. As we can observe in Fig. 2 *Penicillium* species (sp.) and *Scopulariopsis* sp. hardly grow in temperatures above 28°C and most fungi are not able to grow appropriately in temperatures around 45°C². In this sense, it is important to highlight that at 45°C fungi are not necessarily dead, even though there is no colony growth. In this case (and in other stressful conditions) most fungi are able to generate spores, which are the most resistant form of fungi in nature. Then, when environmental conditions are favourable again, they can grow as usual.

Regarding the prejudices caused by fungal contamination diversity, the literature points always to the same genera: *Alternaria*, *Aspergillus*, *Mucor*, *Rhizopus*, *Paecilomyces*, *Penicillium* and *Trichoderma*. Among them, many distinct species may appear, as indicated by different authors in Table 1. On the other hand, no convergence is found on which are the most common fungi in leather contamination. Some authors point to *Trichoderma* spp. as the most frequent³ while others indicate *Aspergillus niger* as well as

Fig. 2: Temperature influence in fungal growth.

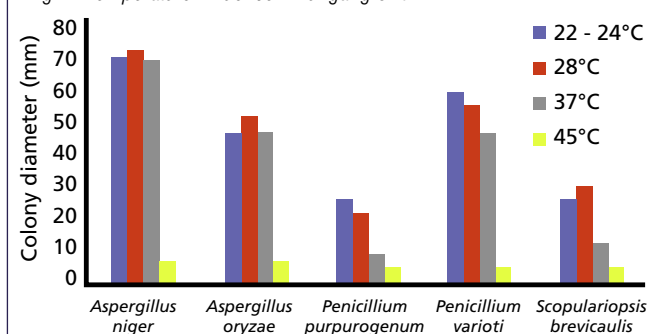


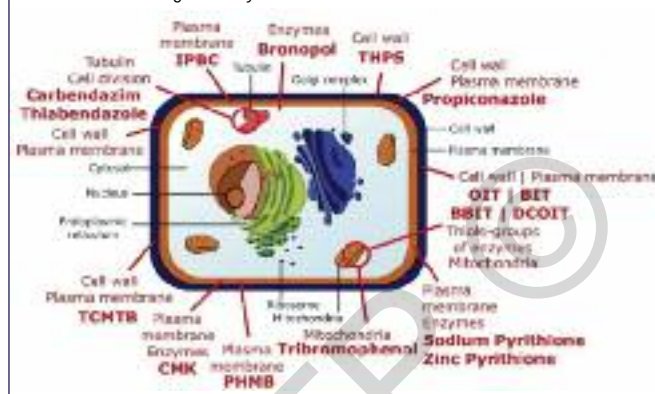


Table 1: List of fungi reported by distinct authors as sources of damage in wet blue leathers.

Most common fungi reported as agents of leather damage		Reference
Aspergillus sp.	Penicillium sp.	3
Mucor sp.	Rhizopus nigricans	
Paecilomyces variotii	Trichoderma viride	
Aspergillus niger	Penicillium sp.	7
Aspergillus terreus	Trichoderma atroviride	
Paecilomyces nivea	Trichoderma harzianum	
Alternaria alternata	Paecilomyces variotii	2
Aspergillus niger	Penicillium commune	
Aspergillus oryzae	Penicillium duclauxii	
Aspergillus wentii	Penicillium glabrum	
Aspergillus versicolor	Penicillium ochrochloron	
Aureobasidium pullulans	Penicillium purpurogenum	
Chaetomium globosum	Penicillium rubrum	
Cladosporium herbarum	Penicillium verrucosum	
Cladosporium sphaerospermum	Scopulariopsis brevicaulis	
Fusarium chlamydosporum	Trichoderma viride	
Myrothecium verrucaria	Verticillium tenerum	
Alternaria geophila	Curvularia luneta	4
Aspergillus niger	Drechslera papendorfii	
Aspergillus chevalieri	Fusarium sp.	
Aspergillus fumigatus	Helminthosporium sp.	
Aspergillus conicus	Mucor ambiguus	
Aspergillus flavus	Paecilomyces varioti	
Aspergillus terreus	Penicillium asperum	
Aspergillus repens	Penicillium camemberti	
Aspergillus sulphureus	Penicillium citrinum	
Aspergillus tamari	Penicillium funiculosum	
Aspergillus luchuensis	Penicillium oxalicum	
Aspergillus amstelodami	Penicillium purpurogenum	
Aspergillus sydowii	Penicillium stipitatum	
Botrytis cinerea	Rhizopus nigricans	
Cladosporium herbarum	Rhizopus oryzae	
Chaetomium globosum	Trichoderma lignorum	
Aspergillus niger	Paecilomyces sp.	8
Aureobasidium pullulans	Penicillium funiculosum	
Chaetomium globosum	Rhizopus stolonifer	
Cladosporium sp.	Trichoderma viride	
Fusarium sp. Mucor sp.		
Aspergillus sp.	Rhizopus nigricans	9
Mucor sp.	Trichoderma viride	
Paecilomyces variotii		
Penicillium sp.		

various species of *Penicillium*⁴. Other genera also are cited, such as *Cephalosporium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Scopulariopsis* and *Verticillium*, but they are not commonly found on leather samples^{1,5,6}.

Fig. 3: Summary of the mechanisms of action of the main fungicides used in the tanning industry.



Mechanisms of action of fungicides

The tanning industry applies biocides along its production process chain to avoid damage to leather from fungal contamination. In summary, *Fig. 3* schematically represents the mechanisms of action of the fungicides most commonly used in this industry sector.

In order to clarify the mechanisms of action of fungicides mentioned in *Figure 3*, we present below an overview of the known fungal growth inhibition pathways made responsible for the biological activity of these biocides.

Azole compounds – mechanism of action

The use of Azole compounds as fungicides appeared as an important alternative to the use of amphotericin B, in the 1980s. Azoles, as well as polyenes and allylamine/thiocarbamates, are the three major groups of antifungal agents in use today.

The most accepted mechanism of action of Azoles, (including TCMTB, propiconazole, carbendazim, OIT, BIT, BBIT, DCOIT and also thiabendazole) is based on the inhibition of ergosterol biosynthesis or on the direct interaction of azole compounds with it. Ergosterol is a key molecule in fungi, since it serves as a bioregulator of membrane fluidity and integrity (*Fig. 4*). Importantly, even though cell wall components might drastically vary among species, ergosterol is a molecule found on all fungi cell membranes, despite fungal genus (*Fig. 5*). That is why azole compounds usually have a broad spectrum of activity.

Fig. 4: Schematic representation of the mechanism of action of azole compounds. Red arrow indicates the target of azole compounds.

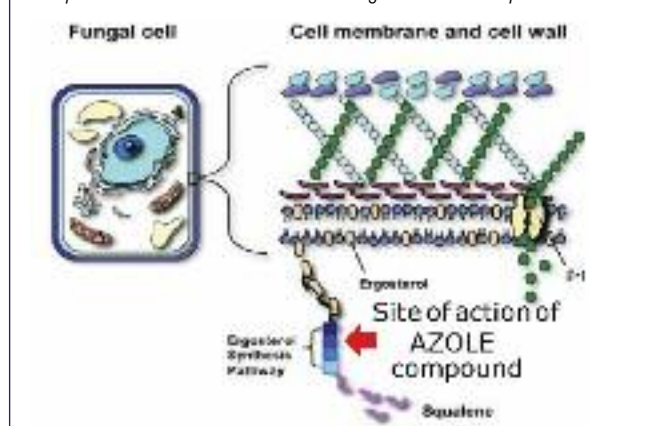
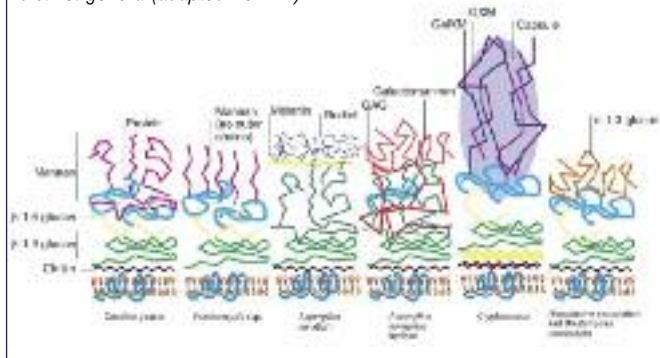




Fig. 5: Representation of the possible variation of the fungal cell wall in distinct genera (adapted from ¹¹).



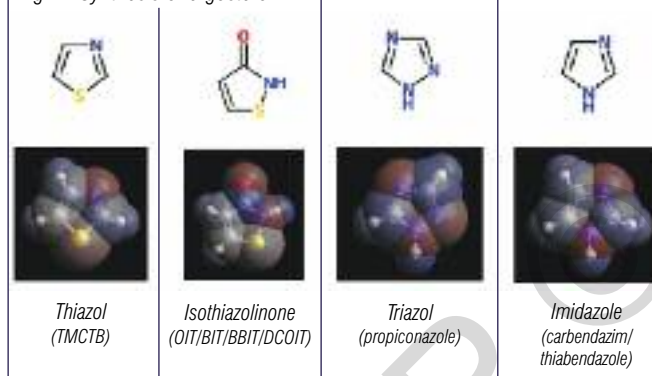
However, considering that the target is always the same, a question may arise: *how do the distinct azoles exert different activities upon fungal strains?*

The primary target of azoles is the heme protein, which cocatalyses cytochrome P-450-dependent 14 -demethylation of lanosterol (Fig. 6). Inhibition of 14 -demethylase leads to depletion of ergosterol and accumulation of sterol precursors, including 14a-methylated sterols (lanosterol, 4,14-dimethylzymosterol, and 24-methylenedihydrolanosterol), resulting in the formation of a membrane with altered structure and function.

Although contemporary azole antifungals are 14 -demethylase inhibitors, there is a heterogeneity of action among them. The main azole target, *cytochrome P450*, catalyses the oxidative removal of the 14 -methyl group of lanosterol or eburicol in fungi by a typical P450 mono-oxygenase activity. This protein contains an iron protoporphyrin moiety located at the active site, and the antifungal azoles bind to the iron atom via a nitrogen atom. Therefore, the azole molecule binds to the protein in a manner dependent on the individual azole's structure (Fig. 7).

The exact conformation of the active site differs between fungal species and among the many mammalian P450 mono-oxygenases. It is possible to observe the structural

Fig. 7: Synthesis of ergosterol.



differences between the two fungal P450 cytochrome protein (Fig. 8). These differences will influence the higher or lower interaction of the azole functional group and consequently its activity. In conclusion, the precise nature of the interaction between each azole molecule and each kind of P450 determines the extent of the azole's inhibitory effect in different fungal species.

Isotiazolinone compounds – mechanism of action

The isotiazolinone chemical group comprises OIT, BIT, BBIT, DCOIT and correlated compounds that exhibit the capacity to bind thiol-groups of fungal proteins, besides the previously described ability to inhibit ergosterol biosynthesis. Most of the scientific reports mention that the isotiazolinone chemical group acts on distinct proteins related to crucial Krebs cycle pathways. The Krebs cycle (or citric acid cycle) is a part of cellular respiration (Fig. 9). All these reactions occur inside mitochondria of all aerobic organisms.

Isotiazolinone biocides inhibit specifically sulfur-containing dehydrogenase enzymes related to crucial Krebs cycle pathways, including pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase, lactate dehydrogenase, and alcohol dehydrogenase. The disablement of these important enzymes can result in complete inhibition of critical metabolic functions concerned with energy generation and cell growth. Moreover, isotiazolinone biocides are known to react with nucleophilic materials and protein thiols, inactivating them. Thiol-active sites are common to dehydrogenase enzymes and other proteins¹³.

Carbendazim and thiabendazole exert their biological activity by binding to tubulin proteins. Tubulin is a major

Fig. 6: Synthesis of ergosterol. Red arrow indicates the step that azole compounds are able to inhibit.

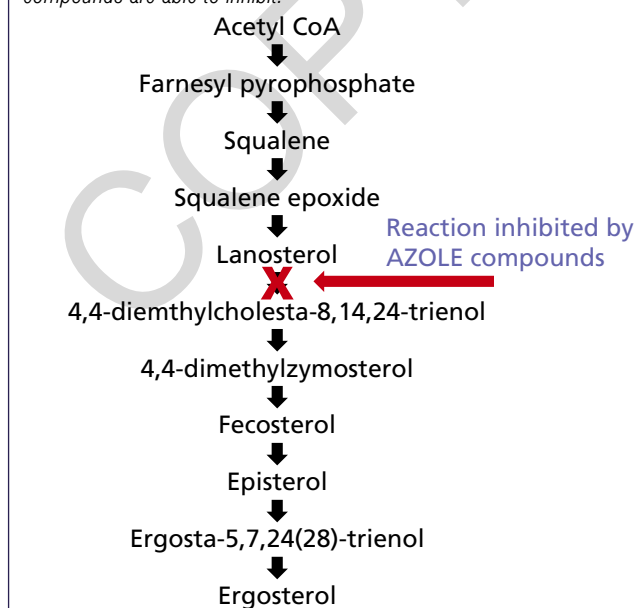


Fig. 8: Representation of the P450 cytochrome protein of two fungal species, displaying remarkable differences, which directly interferes in the fungicide activity.¹²

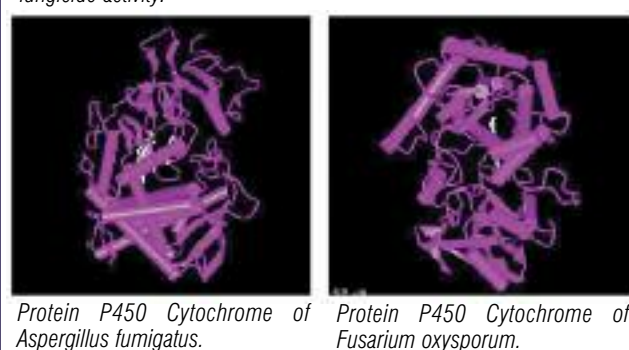




Fig. 9: Representative scheme of action of OIT upon Krebs cycle pathways.

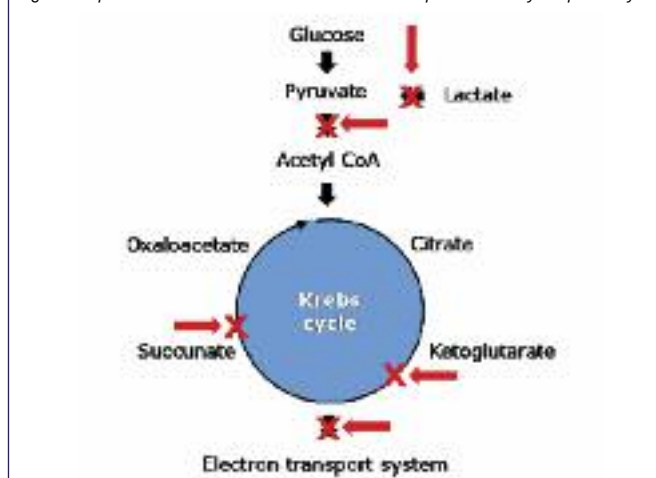
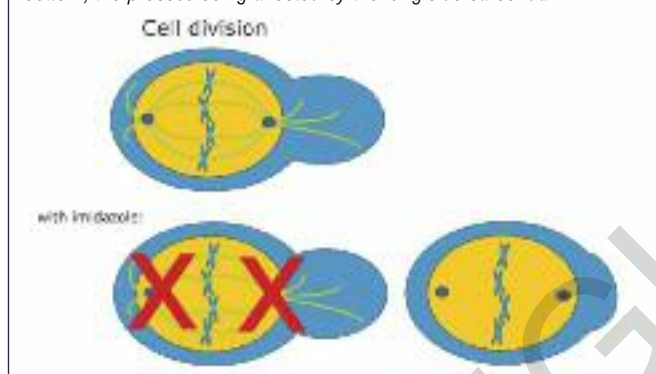


Fig. 10: Fungal cell division process. Top is the normal process and, bottom, the process being affected by the fungicide carbendazim.



component of the eukaryotic cytoskeleton which is involved in structural support, intracellular transport and DNA segregation. The action of imidazole compounds disrupts microtubule assembly, preventing appropriated cell division, and thus resulting in the mal-segregation of chromosomes during cell division (Fig. 10).

CMK (4-chloro-3-cresol) acts specifically on the cell membrane and inactivates intracytoplasm enzymes by forming unstable complexes. The lipophilic molecules are trapped by the membrane phospholipids. The following processes are involved (Fig. 11):

- If CMK concentration is low, the cell constituents (nucleic acids, glutamic acid) are liberated in the external media;
- If CMK concentration is high, the disinfectants inhibit permeases, thus causing denaturation of the proteins and lysis of the cell membrane¹⁴.

IPBC – mechanism of action

Carbamate fungicides, such as 3-iodo-2-propynyl butylcarbamate (IPBC), disrupt the formation of fungal cell walls by interfering with synthesis of phospholipids and fatty acids, as demonstrated in Figure 12. They also affect mycelial growth, spore production and germination. Also, IPBC is considered an iodophore, since it is composed of iodine complexed with a solubiliser, acting as 'free' iodine. Therefore, this compound is corrosive to exposed membranes.

Part two of this paper will be published in *World Leather* December 2019-January 2020. ☺

Fig. 11: Representation of the mechanism of action of CMK fungicide.

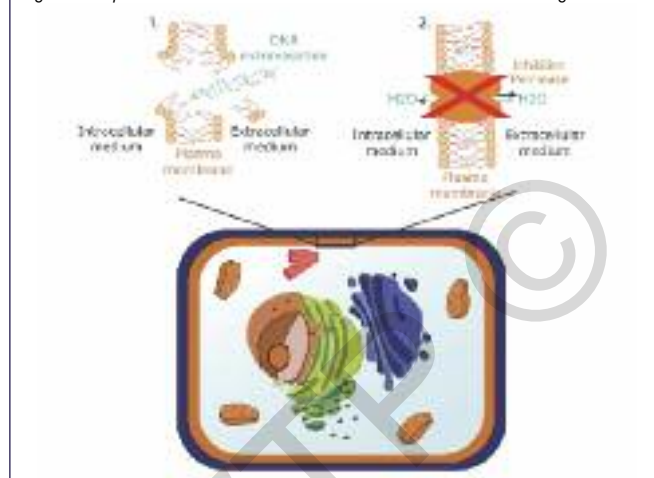


Fig. 12: Representation of the mechanism of action of IPBC fungicide.



References

1. Orlita, A.: *Biodeterioration in leather industry*, Elsevier publishing co., 1968
2. Orlita, A.: *Microbial biodeterioration of leather and its control: a review*, *International Biodeterioration & Biodegradation*, 53(3):157-163, 2004
3. Lindner & Neuber: *Preservation in the Tannery*, *International Biodeterioration*, 26, 195-203, 1990
4. Rathore et al.: *Isolation, Screening and relative capacity of fungi which causes infestation of finished leather*, *Int.J.Curr.Microbiol.App.Sci*, 2013
5. Sharma & Sharma: *Succession of mycoflora on finished leathers during storage*, *Defence science journal*, 1979
6. Stakishatite & Lugauskas: *Microscopic fungi that damage synthetic leathers and polyvinyl chloride films*, 1981
7. Zugno et al.: *Fungal Growth on wet blue: Methods to Measure Impact on Leather Quality*, *Buckman Laboratories International*, 2011. Available at: www.aaqtc.org.ar/congresos/china2009/oralPresentations/1-2.pdf [Accessed: May 2019]
8. Paulus W.: *Directory Of Microbicides For The Protection Of Materials: A Handbook*, Kluwer Academic Publishers, 2004
9. *Journal Leather Technology Centre*. Nov/Dec, 2007.
10. *Doctor Fungus*: Available at: <http://powersite3.com/drffungus/antifungalpharmacology/>
11. Erwig & Neil: *Interactions of fungal pathogens with phagocytes*, *Nat Rev Microbiol*, 2016
12. *NCBI Databank*. Available at: <https://www.ncbi.nlm.nih.gov/>
13. Williams, T.M.: *The Mechanism of Action of Isothiazolone Biocides*, *NACE International*, 2007
14. Maris, P., *Modes of action of disinfectants*, *Rev Sci Tech.*, 1995.