Performance of Microbicides for the Preservation of Vegetable Tanned Leather

by
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Abstract

In the tanning industry, the deterioration of leather due to the development of fungi is of great concern. Some fungi metabolize important substances in leather, causing serious damage such as pigmented stain that is difficult to remove, defects, surface roughness, and loss of physical and mechanical resistance, which affect the quality of the final product. This work evaluated the performance of five microbicides conventionally used in the leather industry, against different fungi. Microbicides were applied during the tanning process with vegetable tannin in the fatliquoring step. Accelerated microbiological tests (plating and tropical chamber) were performed. The results revealed a low antifungal capacity of selected microbicides when applied at an offer of 0.2% (mass hide base) fungicides. Treatment with OIT+BMC/water at an offer of 0.75% showed satisfactory fungal protection against different fungi tested and proved to be the most suitable for the preservation of vegetable tanned leather.

Introduction

Leather, whose basic raw materials are skins or hides of animals, is an intermediate industrial product with numerous applications in different manufactoring sectors such as footwear, clothing, upholstery, and many artifacts. Raw hides are basically constituted of water, which accounts for nearly 60% to 70% of their weight, and approximately 30% of proteins that are biopolymers formed from polypeptides chains. The main protein in hides is collagen, which represents one third of the total protein of mammals.[1,2,3] Hides and leathers, due to the high humidity associated with their fats and proteins, may serve as metabolic substrates for microorganisms, especially bacteria and fungi. These microorganisms use the nutrients present in substrates as food and energy sources and are capable of contaminating the hides at any leather processing stage from slaughter to ready processed leather.[4,5]

During biodeterioration of hides and leathers, microorganisms produce two types of enzymes called peptidases and proteinases. These enzymes hydrolyze fats, proteins and carbohydrates into lower structures, which are readily metabolized by bacteria and fungi.[6,7] Among the most common genera of contaminating fungi are *Aspergillus*, *Penicillium* and *Trichoderma*.

If microbial contamination is not prevented, mainly in the beamhouse operations of leather processing in tanneries (where the cleaning and preparation of the hide happens) and during the tanning steps, the finished product may have a variety of defects affecting its quality, such as undesirable pigmentation, dyeing and finishing non-uniformity, fatty acids, surface roughness and a decrease in physical-mechanical properties which reduce the value of leather and leather goods. In addition to the financial losses, there are also indirect losses of rework, namely the cost of labor and chemicals, and downgrading of finished leather.[8,9]

Strategies are used to control microorganisms in order to reduce or eliminate this problem, including the use of microbicides. Microbicide prevent the development and growth of fungi in pickled hides, tanned leathers (from chromium or vegetable tannin) and finished products during their storage and shipping.[10,11] The proper use of the microbicide itself requires proper selection of the active principle, analysis of process formulation, and determination of quantity and appropriate timing of microbicide addition to the formulation in terms of handling, packaging and storage of leather.

As a measure of effectiveness, microbicides should be demonstrated to have the following properties: high activity; broad antimicrobial spectrum; compatibility with leather and with process liquors; stability on leather; non-discoloring; environmentally acceptance; low toxicity to humans and other warm-blooded animals; and cost effectiveness.[12]

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The use of pentachlorophenol (PCP), which was widely used as a leather preservative, has been restricted due to contaminants present in the technical-grade product (the most toxic being tetrachlorodibenzodioxin—TCDD), the environmental problems, and/or aggressiveness in handling. For this reason, it is of interest to devote efforts to find and develop other effective microbicides with lower environmental and toxicological impacts.[11,13]

Most fungicides used in leather industry fall into two chemical families:[14] the phenolic family including 4-chloro-3-methylphenl (PCMC) and ortho-phenylphenol (OPP); and the heterocyclic family including 2 (thiocyanomethylthio)-benzothiazole (TCMTB), n-octilisotiazolinona (OIT), and 2-mercaptobenzothiazole (MBT).

The present study evaluates the ability of five selected antifungal agents commonly used in the leather industry to preserve vegetable tanned leather against mold caused by four fungi.

Materials and Methods

The performance of five microbicides usually used in the leather industry for hide and leather preservation was evaluated. These were applied during tanning process with vegetable tannin in the step of fatliquoring. Their fungicidal action was tested by accelerated microbiological plating and incubation in a tropical chamber to visually verify the presence and growth of fungus as well as the damage caused to the leather, which was observed by scanning electron microscopy images (SEM). Furthermore a comparative study of other dosages was realized for two selected microbicides.

Materials and Application of Microbicides to Leather

The antimicrobial capacity (fungicide action) of the microbicides 2-thiocyanomethylthio benzothiazole (TCMTB); 2-n-octyl-4-isothiazolin-3-one (OIT 5%); Oil dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol-2-ylcarbamate (OIT+BMC/oil); Aqueous dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol-2-ylcarbamate (OIT+BMC/water) and 2-n-octyl-4-isothiazolin-3-one (OIT 9.3%) against growth of the following different fungi types *Aspergillus niger*, *Aspergillus flavus*, *Penicillium herguei* and *Penicillium chrysogenum*, was evaluated since these are described in the literature as being accounted among the responsible for damage during the leather processing.[5,15] The fungi were provided by the Laboratory of Biochemistry and Applied Microbiology, Institute of Food Science and Technology (ICTA), UFRGS.

Fungi were inoculated in Petri plates containing the culture medium potato dextrose agar (Oxoid) and incubated for seven days at 28°C. A spore suspension (10^5 spores/mL) of each fungus was prepared with aqueous solution, the spore counting was carried out using Neubauer chamber. The same culture medium (potato dextrose agar) was used for determination of the leather samples resistance to fungal attack by test plating.

The tests were performed using six pieces (approximately 40 g) of pickled hide in the experiments of vegetable tanning. Five pieces were treated with the five tested microbicides and one was used for the control test without the addition of a microbicide. The tanning experiments were carried out in drums (rotating reactor) separately. The quantities (%) of chemical products used in the formulation for the tanning were based on the mass of the hide (Table I).

Initially 0.2% offer of five tested microbicides were added. The results of these tests showed poor preservative action, so that additional tests were carried out with offers of 0.5% and 0.75% of microbicides TCMTB and OIT+BMC/water to verify the proper offers of these microbicides.

Accelerated Microbiological Tests

Accelerated microbiological tests are needed to observe and quantify the growth of microorganisms in hides and leather. These tests should be conducted under ideal conditions to favor the growth of microorganisms in an attempt to simulate the performance of biocides in real conditions. The accelerated tests commonly used in laboratories for testing microbicides are test plating and incubation in a tropical chamber.[16]

Plating Test

The fungal growth control was performed following the ASTM D4576-08[17] Standard on sterile plates using potato dextrose agar (PDA) as culture medium. After solidification of the medium, one drop of spore suspension ($1x10^5$ spores/ml) for each selected fungus was inoculated directly onto the leather sample and one drop was placed onto the culture medium at a distance of 45 mm

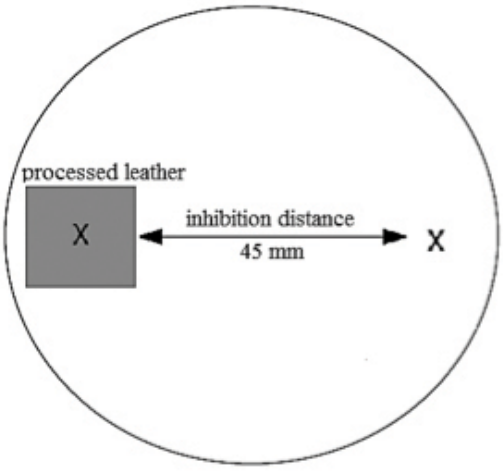

Figure 1. Inoculation points (X) of spore suspension.

as shown in Figure 1. The plates were incubated at 28°C. Fungal growth was observed weekly until 28 days by visual inspection. The parameter called "inhibition distance" (Figure 1) was considered as being the maximal distance on the plate where the growth of the microorganism was prevented due to the microbicide activity.[7] Fungal growth was also visually verified on the surface of the leather sample itself. Additional tests were done in tropical chambers to compare with the plating results.

Incubation in a Tropical Chamber

The tropical chamber method is a simple one that would be easy to do in tanneries, for instance, and allows a large number of samples to be tested and evaluated concurrently. It consists of a chamber constructed based on descriptions of ASTM D7584–10 and kept at a controlled temperature of 30°C, with 100% humidity and highly contaminated with fungi.[18]

Table I
Formulation of tanning process with vegetable tannin.

Step	Quantity (% of hide mass)	Chemical	Time/ Process control
Pickle	200	Water	
	5	Sodium chloride	
	0.2	Formic acid (1:10)	
	0.2	Sulfuric acid (1:10)	30 min
Preconditioning	1.2	Sodium sulfate	120 min pH < 3
			Drain off
Tanning	100	Water	
	10	Vegetable tannin (Mimosa Extract)	60 min
	10	Vegetable tannin (Mimosa Extract)	60 min
	10	Vegetable tannin (Mimosa Extract)	120 min
Fatliquoring	50	Water	
	7	Sulfited fatliquoring agent	
	6	Sulfated fatliquoring agent	
	0.2 *	Microbicide**	60 min
Fixation	100	Water	
	1	Formic acid (1:10)	20 min
			Drain off

*With the exception of the control sample (0%) and offers 0.5% and 0.75%.
**TCMTB, Isothiazolin, OIT+BMC/oil, OIT+BMC/water, OIT.

Leather samples (Table I), measuring 90 mm x 30 mm, were taken in duplicate and placed into the tropical chamber to assess fungal growth on their surfaces. In this case, the *Aspergillus niger* fungus was the contaminant tested. The upper surfaces (grain side) and lower surfaces (flesh side) of samples treated with 0.2% microbicide were evaluated. The percentage of contaminated surface was measured after 28 days of testing.

Scanning Electron Microscopy (SEM)

Morphological modifications of the leather sample surface due to leather deterioration caused by *Aspergillus niger*, *Aspergillus flavus,* and *Penicillium herguei* were evaluated using scanning electron microscopy (SEM).

Results and Discussion

Table II shows the inhibition distance (ID) and fungal growth on the surface (FGS) of different samples of vegetable tanned leather after 28 days of testing. The fungal growth on the surface is the percentage of the leather sample that has been contaminated with fungus.

Figure 2 shows the growth of different fungi, *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, and Penicillium herguei* on the surface of the tanned leather samples. In this test, a greater distance of inhibition (values near 45 mm) indicates a greater degree of protection provided by the microbicide. All samples used as controls (that were not treated with a microbicide) had fungal growth on their surfaces and, in just two weeks of testing, there was no growth inhibition (measured as an inhibition distance of 0 mm). The control samples contaminated with *Aspergillus niger, Aspergillus flavus,* and *Penicillium herguei* showed 100% of the surface covered with fungi by the end of the test.

There was low antifungal ability of microbicides used at an offer of 0.2% when the vegetable tanned leather was contaminated with *Aspergillus niger* and *Aspergillus flavus* because after 28 days of testing, all samples were already contaminated and some showed 100% surface contamination, as demonstrated in Figures 2 (a) and 2 (b). A low antifungal capability for TCMTB applied at 0.05% to vegetable tanned leather has been previously reported[7], where the surface contamination by *Aspergillus niger* reached 40%, and the surface contamination by *Trichoderma harziabum* reached 9% after 20 days of testing. Samples of wet-white leather treated with TCMTB at 0.15% also showed surface contamination by *Aspergillus niger* and *Trichoderma viride*.[19]

For the leathers infected with *Penicillium herguei* in three samples treated with OIT+BMC/oil, OIT+BMC/water, and OIT 9.3%, no growth on the surface was observed at the same concentration. For samples contaminated with *Penicillium chrysogenum*, the ones treated with TCMTB, OIT+BMC/oil, and OIT+BMC/water had no fungal growth during the time of the experiment.

Figure 3 shows the decrease in distance of inhibition by the plating test during the 28 days of the experiment. The absence of bars in these figures means that the distance between the sample and the fungus growth was null.

The distances of inhibition against different fungal species decreased over the course of the experiment for all strains. For samples treated with TCMTB, OIT 5%, OIT+BMC/oil, OIT+BMC/water, and OIT 9.3% contaminated with *Aspergillus*

Table II

Inhibition distance (ID) and fungal growth on the surface (FGS) after 28 days on vegetable tanned leather samples.

Sample/ Microbicide	*Aspergillus niger*		Aspergillus flavus		Penicillium herguei		Penicillim *chrysogenum*	
	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)
Control	0	100	0	100	0	100	0	50
TCMTB	0	74	0	100	0	7	16	0
Isothiazolin	0	93	0	100	0	5	0	5
OIT+BMC/oil	0	14	0	100	24	0	20	0
OIT+BMC/water	0	88	0	100	21	0	19	0
OIT	0	77	0	100	2	0	0	2

niger and *Aspergillus flavus*, a lack of inhibition distance (0 mm) was reached within 14 days of testing. For samples contaminated with *Penicillium chrysogenum* and *Penicillium herguei,* distances of inhibition became stable after 21 days for all samples treated with microbicide.

According to the results above, it is assumed that microbicides are not effective against the selected fungi, therefore a comparative study was conducted with higher offers of 0.5% and 0.75% of TCMTB and OIT+BMC/water microbicides. Figures 4, 5, 6, and 7 show the images of the plating test of samples of

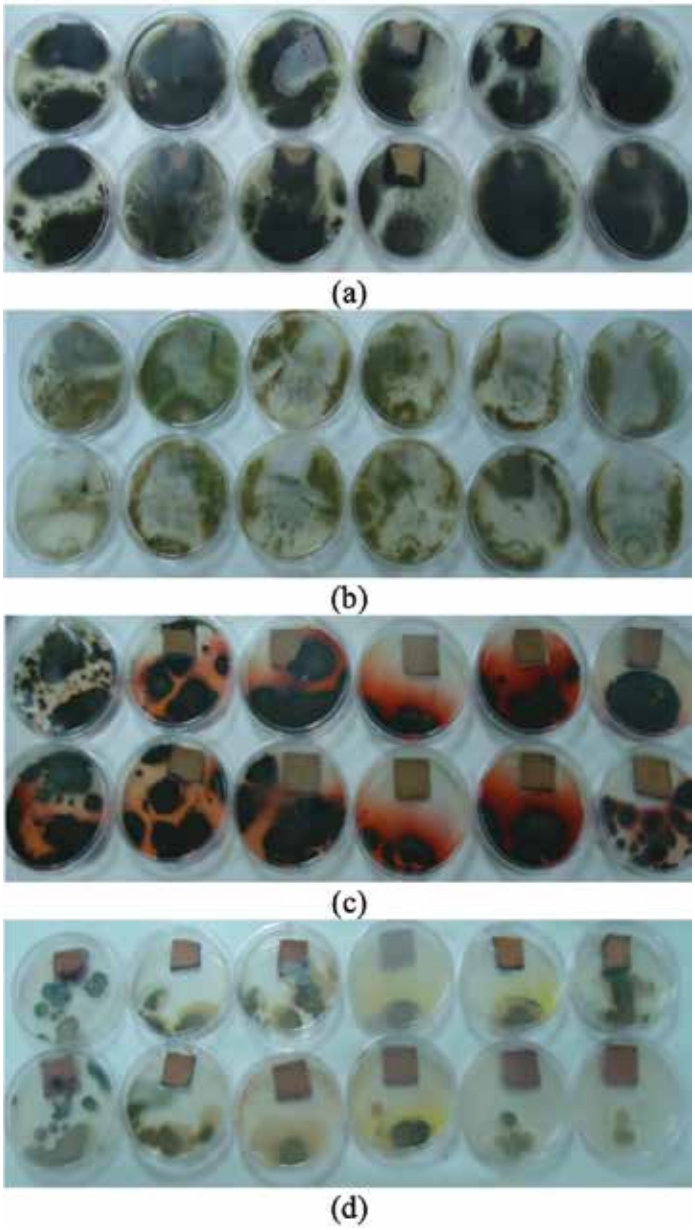

Figure 2. Contamination of vegetable tanned leather, from left to right are the sample preserved with 0,2% of the microbicides: control, TCMTB, Isothiazolin, OIT+BMC/oil, OIT+BMC/water and OIT for the fungal species (a) *Aspergillus niger,* (b) *Aspergillus flavus,* (c) *Penicillium herguei* e (d) *Penicillium chrysogenum.*

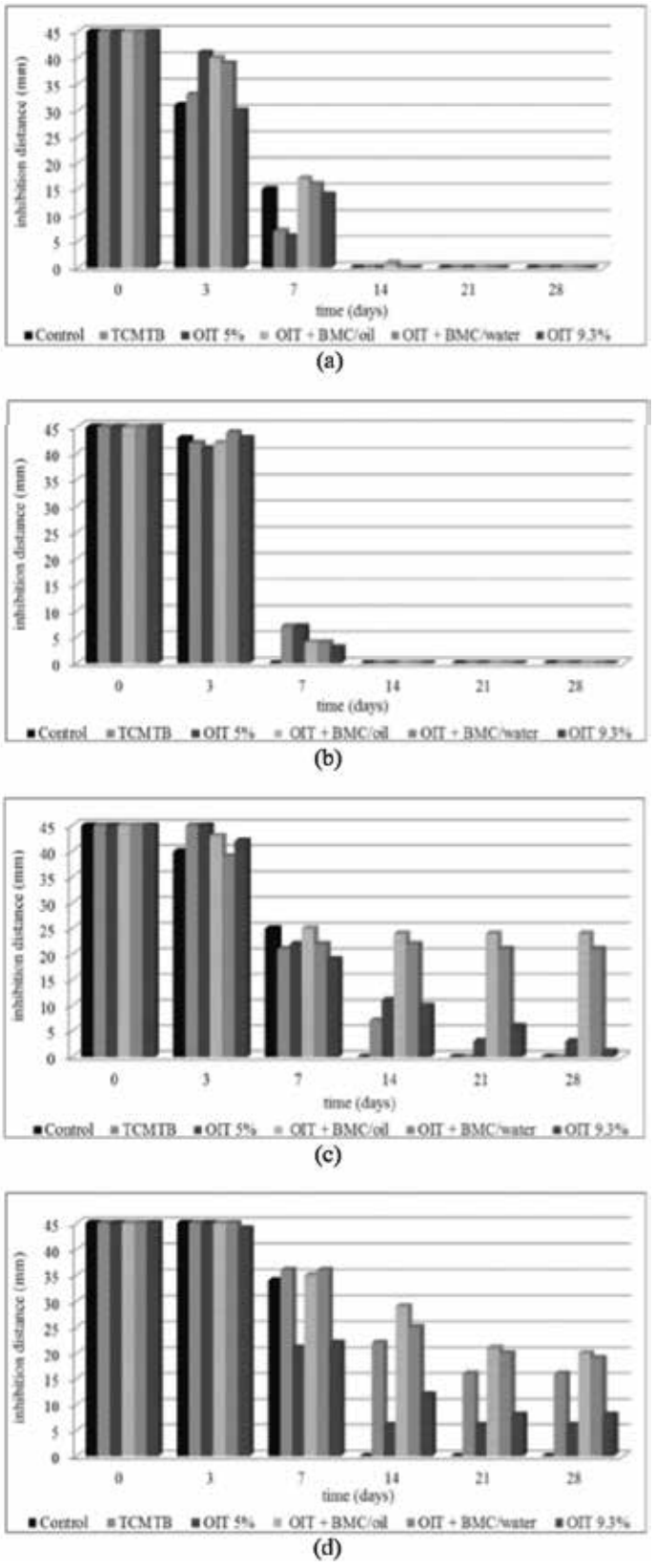

Figure 3. Decrease of the inhibition distance of vegetable tanned leather preserved with 0.2% microbicide and contaminated with (a) *Aspergillus niger,* (b) *Aspergillus flavus*, (c) *Penicillium herguei* and (d) *Penicilium chrysogenum.*

vegetable tanned leather contaminated with *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Penicillium herguei*, respectively.

It was observed that even 0.75% of TCMTB was not sufficient to inhibit the growth of *Aspergillus niger* and *Aspergillus flavus* on the leather sample surfaces. Treatment with OIT+BMC/water at 0.5% could not prevent the growth of only *Aspergillus niger*, however at an offer of 0.75%, no fungal growth was evident in any contaminant tested, showing that this dose was sufficient to prevent the growth of the fungi.

Table III presents the results of the experiment carried out in the tropical chamber where fungal growth observed in the samples was quantified. Values represent the percentage of surfaces (grain side and flesh side) covered by the *Aspergilllus niger* fungus after 28 days of testing. As expected, both areas of the samples of leathers used as a control (without antimicrobial protection) were contaminated with *Aspergillus niger*. The five samples of leather treated with 0.2% TCMTB, OIT 5%,

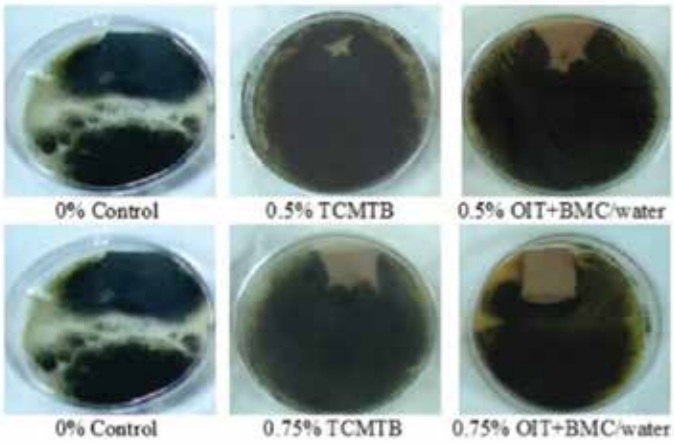

Figure 4. Growth of *Aspergillus niger* in samples of vegetable tanned leather preserved with 0.5% and 0.75% microbicide offers.

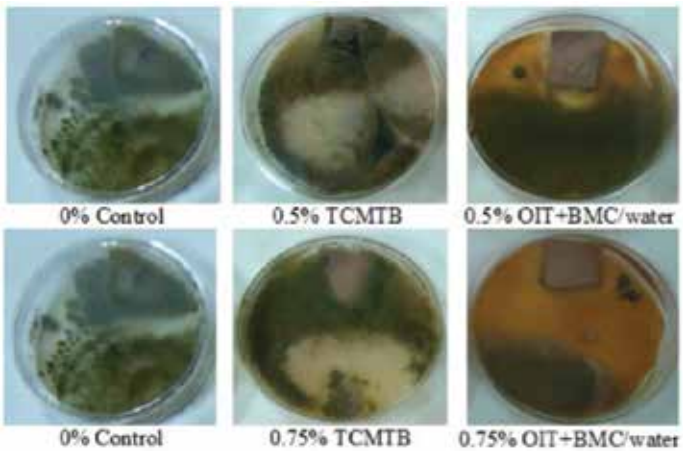

Figure 5. Growth of *Aspergillus flavus* in samples of vegetable tanned leather preserved with 0.5% and 0.75% microbicide offers.

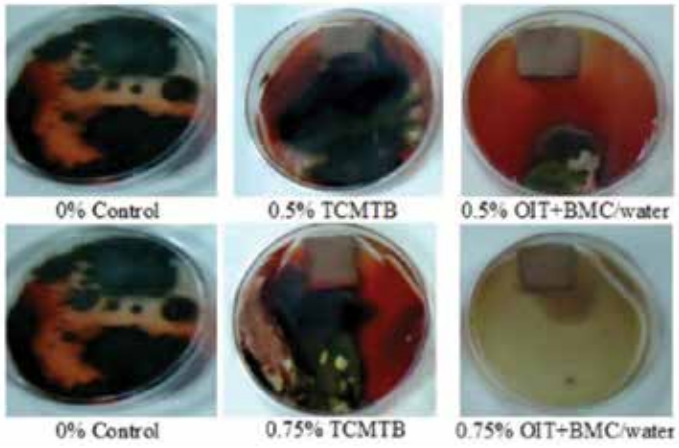

Figure 6. Growth of the fungus *Penicillium herguei* in samples of vegetable tanned leather preserved with 0.5% and 0.75% microbicide offers.

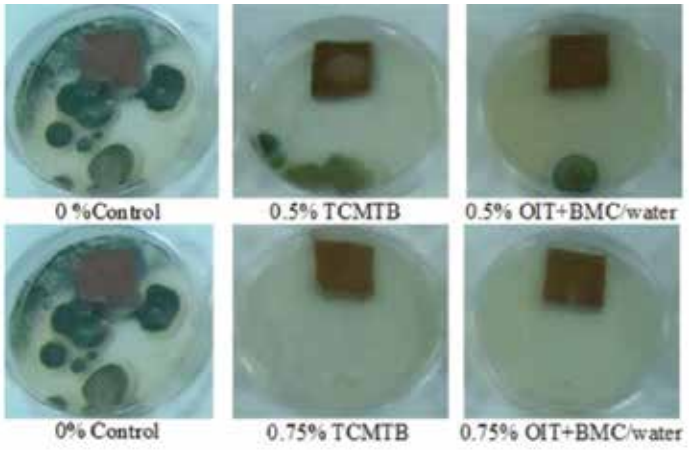

Figure 7. Growth of *Penicillium chrysogenum* in samples of vegetable tanned leather preserved with 0.5% and 0.75% microbicide offers.

Table III
Surface contamination of leathers in the tropical chamber test in samples preserved with 0.2% microbicide offer.

Sample/ Microbicide	Contaminated leather surface (%)	
	Grain side	Flesh side
Control	80	91
TCMTB	14	26
Isothiazolin	40	71
OIT+BMC/oil	40	45
OIT+BMC/water	20	34
OIT	80	90

OIT+BMC/oil, OIT+BMC/water, and OIT 9.3% showed fungal growth on both sides. It was observed that the flesh side was more susceptible to fungal growth than the grain side, which may be due to the greater fiber exposure.

Some samples, such as those treated with OIT, showed high percentages of contamination with values of surface contamination close to the samples that have not undergone any microbicide treatment. The microbicides evaluated demonstrated low antifungal capacity when applied at the present concentrations in this type of leather. These observations showed compatibility with the action of microbicides found in the plating test.

It is more difficult to protect vegetable leathers against fungal growth compared to chrome leathers, because vegetable extracts (polyphenols combined with carbohydrates) provide to the fungi a direct nutrient in the form of simple sugars.[7] In accelerated microbiological tests in tropical chamber reported[11], it was observed that fungi that commonly attack the leather including *Aspergillus* and *Penicillium* species in samples of wet-blue treated with TCMTB (0.1%) and OIT (0.35%) had superficial growth in the tenth week (5%) and in the third week (5%) of the experiment, respectively.

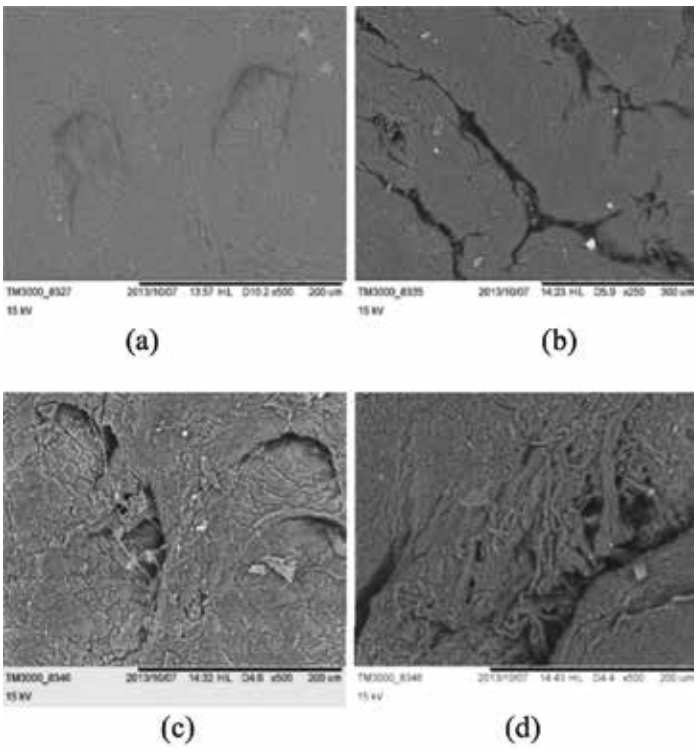

Figure 8. Images of samples of leathers tanned with vegetable tannin (a) uncontaminated, (b) contaminated with *Aspergillus niger,* (c) contaminated with *Aspergillus flavus* and (d) contaminated with *Penicillium herguei*

The images obtained by SEM (Figure 8) show the biodeterioration of leather caused by microorganisms' digestion to satisfy their nutritional needs. The non-contaminated sample (Figure 8a) shows an intact smooth surface with well-defined pores without fiber exposure. However, the deteriorated samples show irreparable damage to the leather, like rough surfaces and exposed fibers, compromising the physicochemical characteristics and mechanical properties of the material.[20] This shows the importance of the correct use of microbicides in leather processing to ensure the quality of the final product.

Conclusions

From the evaluation of the results obtained and discussed in this paper, it is concluded that the proper use of microbicide is the first requirement for the protection of leather, which is of fundamental importance for the leather industry. The five microbicides, when applied at an offer 0.2%, were shown to have low antifungal capacities against *Aspergillus niger* and *Aspergillus flavus*. It was observed that in some applications of microbicides in leathers tanned with vegetable tannin, there was not adequate antimicrobial protection, indicating that there is a necessity to use new methods such as increasing the offers of microbicides, mixing the substances with different active principles, or searching for new alternative microbicides.

When the tests were performed with higher offers of TCMTB and OIT+BMC/water, treatment with 0.75% TCMTB was not sufficient to inhibit fungal growth, however, treatment with 0.75% OIT+BMC/water inhibited growth of the four fungal contaminants. Therefore, OIT+BMC/water was the most suitable microbicide for preservation of vegetable tanned leather against the attack of *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum,* and *Penicillium herguei* fungi.

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