Performance of Antimicrobial Agents for the Preservation of Chrome Leather

by
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Abstract

Fungal growth in leathers causes significant damages like stains, surface roughness and loss of physical-mechanical resistance. This raises the need to develop control strategies by the use of antimicrobial agents. Considering the improving processes with regard to the use of antimicrobial agents added in skins and leathers to prevent their contamination by fungi, this study aims to evaluate the performance of six antimicrobial agents conventionally used in the leather industry, against four different fungi. These agents were applied during the chrome tanning process. Accelerated microbiological assays (tests plating and incubation on tropical chamber) were performed, as well as sorption and wash-out testing of three selected antimicrobial agents by UV/VIS spectrophotometry and verification of surface biodeterioration through SEM. Antimicrobial agents 2-thiocyanomethylthio benzothiazole (TCMTB) and Aqueous dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol-2-ylcarbamate (OIT+BMC/water) showed antifungal capacity against different fungi tested applied in concentration of 0.2% (weight leather base). In the tropical chamber test, for the five samples of wet-blue leather treated with antimicrobial agents the growth of fungus was not observed. From the antimicrobial agents subjected to sorption testing and wash out, the TCMTB based antimicrobial agent presented rapid and high sorption in wet blue leather and also has resistance to washing.

Introduction

All parts of an animal are subjected to decomposition when the animal dies. For hides, if they are not preserved, the biodegradation process will begin quickly. Biodegradation is an important negative factor for aesthetics and functional properties of organic materials such as hide, leather and products made from it.

The hides have high moisture and are a rich source of fats and proteins that may serve as feed substrates for microorganisms, especially bacteria and fungi. These microorganisms use the nutrients in the skins or hides as source of food and energy production and they can infect the hides from slaughter (skinning) until the processed leather.[1,2] During the processing of leather in tanneries in first stages the hide is attacked by bacteria like *Bacillus subtilis*, *Escherichia coli*, *Micrococcus spp.*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. When the hides are tanned, fungi such as *Penicillium spp., Aspergillus spp., Trichoderma spp., Rhizopus spp.* and *Mucor spp can grow on the hides.*[3,4,5]

The enzymes secreted by microorganisms react with the hides. Hence, fats, proteins and carbohydrates are broken down into smaller structures that are easily metabolized by microorganisms. Fungal growth on wet-blue (chrome tanned leather) may lead to a variety of defects in the final leather such as undesirable pigmentation, dyeing and finishing non-uniformity, fatty acids and the decrease of strength properties due to the degradation of collagen.[6] Therefore, the appropriate use of antimicrobial agents are necessary to prevent biodeterioration during hide preservation and leather manufacturing.

Antimicrobial agents may have specific action on a specific type of microorganism, as bactericides against bacterial attack, and fungicides that confer resistance to fungi, or may have broad spectrum of action, providing resistance to microorganism without distinction. The choice of an antimicrobial agent with broad activity is required when the issue comprises different types of microorganisms or when they become resistant to conventional treatments. In some cases, a synergistic association of different active principles gives good action against microbial communities.[7,8]

In the leather industry the fungicides represents about 15% of the costs of wet-blue processing chemicals.[8] As measure of effectiveness of fungicides they should demonstrate to have the following properties: high activity; broad antimicrobial spectrum; compatibility with leather and with and chemicals products used in the process; stability on leather; non-discoloring; environmentally acceptability; low toxicity to

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humans and other warm-blooded animals and cost effectiveness.[9] Effective use of antimicrobial agent involves selecting the active principles, the properly formulation, timing and amounts added in the processing baths of leather.

In the 70's, the hides and leather were preserved with chemicals such as compounds containing mercury and chlorinated phenols. Preservatives based on pentachlorophenol (PCP) were widely used as leather preservative[8,9,10] owing to its dual functions as a bactericide and fungicide and also its low cost. However, due to the toxicity of PCP, mostly due to contaminants present in the technical-grade product (the most toxic being the tetrachlorodibenzodioxin -TCDD), poor biodegradability and the damage caused to the environment, its use and marketing were restricted.[11,12] Antimicrobial agent is basically composed of three components:[13] active ingredient - responsible for antimicrobial action of the compound prepared; solvents - used to emulsify the active principle (the main solvents are water, hydrocarbons and glycols); and surfactants - responsible for optimal emulsion of the active ingredient, solvent stability of formulated product and the preferred distribution of the active principle in the application of the same in leather processing.

Antimicrobial agents generally used in the leather industry are classified into two chemical families of phenolic and heterocyclic compounds. The phenolic compounds are 4-cloro-m-cesol (PCMC) and ortho-phenylphenol (OPP) and the heterocyclic compounds are 2-thiocyanomethylthio benzothiazole (TCMTB), n-octilisotiazolinona (OIT) and 2-mercaptobenzothiazole (MBT).[14]

The phenolic antimicrobial agent is formulated from synthetic molecules of ortho-phenylphenol and para-chloro-meta-cresol, presenting themselves in the form of sodium or potassium salts in different concentrations. These chemicals are completely miscible in water and do not present problems of chemical stability.

They also exhibit good absorption of active ingredients by the hides and are stable to pH variations. The mechanism of action of phenolic antimicrobial agent is the reversible adsorption of phenolic molecules onto the cytoplasmic membrane of microorganisms which initially inhibits the transport of nutrients and eventually deteriorates and penetrates the cell wall, leading to microbial death.[15] The phenolic molecules are not consumed in this process and as they desorb, remain able to act on other cells of microorganisms still alive. Most of these antimicrobial agent present health risks because they are carcinogenic, and risks to the environment by straining the ecosystem.

The main heterocyclic antimicrobial agent used in the leather industry is TCMTB, which gained importance with the prohibition of import and export of articles treated with PCP. This antimicrobial agent is an electrophilic substance that chemically reacts in an irreversible way with nucleophilic substances, being consumed in this reaction, so the resulting molecules are no longer TCMTB. Some advantages of the application of TCMTB are: excellent antimicrobial action, suitable in particular for the preservation of wet blue; highly efficient when preservation of leather for a longer time is required solubility in water; good stability in acidic medium and no interference to operation of wasted water from biological treatment.[16]

The aim of this study was to analyze the use of preservatives by evaluating the capacity of six selected antifungal formulations used to preserve wet-blue leather against attack by four different fungi species.

Materials and Methods

The performance of six different antifungal agents usually used in the leather industry for hide and leather preservation was evaluated. These were applied during chrome tanning process. The fungicide action was tested by microbiological plating and incubation in tropical chamber to verify visually 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, spectrometric tests (UV/VIS spectrophotometry) were conducted to investigate the sorption and desorption of antimicrobial agents when the wet-blue leathers were subjected to washing.

Materials and Application of Antimicrobial Agents to Leather

The antimicrobial agents (product with single active ingredient or mixture) used in the trials, following listed, are commercial products provided by Brazilian chemical companies.

1. TCMTB (2-thiocyanomethylthio benzothiazole): Active ingredient 30%; Inert ingredients 70%.

2. OIT 5% (2-n-octyl-4-isothiazolin-3-one): Active ingredient 5%; Inert ingredients 95%.

3. OIT+BMC/oil (Oil dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol - 2-ylcarbamate): not provided by the company.

4. OIT+BMC/water (Aqueous dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol - 2-ylcarbamate): not provided by the company.

5. OIT 9.3% (2-n-octyl-4-isothiazolin-3-one): Active ingredient 9.3%; Inert ingredients 90.7%.

6. PCMC (4-chloro-3-methylphenol): Active ingredient 71%; Inert ingredients 29%.

The antimicrobial capacity (fungicide action) of the selected antimicrobial agents was evaluated against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium herguei* and *Penicillium chrysogenum*, since they are described in the literature as being accounted among the responsible for damage during the leather processing.[2,17] 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 potato dextrose agar (Oxoid) and the plates were incubated for seven days at temperature of 28°C. A spore suspension of each fungi was prepared with aqueous solution, the spore counting was carried out using Neubauer chamber. The inoculum suspension was adjusted to 10^5 spores/mL. The same culture medium (potato dextrose agar) was used for determination of the leather samples resistance to fungal attack by the test plating.

The tests were performed using a pickled bovine hide of 22 kg and thickness of 3 mm. The hide pieces used in the experiments were taken from the butt part. The hide pieces were first treated in a new pickle conditioning treatment.

In the plating test, pieces of approximately 40 g were use in the experiments of chrome tanning. Six of them were treated with the six antimicrobial agents and one was the control test without addition of antimicrobial agents. 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% concentration of six tested antimicrobial agents were added. Additional tests were carried out with 0.1% and 0.4% concentrations of antimicrobial agents TCMTB, OIT+BMC/water and PCMC.

Plating Test

Samples of 25 mm x 25 mm of the obtained leather (Table I) were analyzed in duplicate to the determination of fungal attack resistance (ASTM D4576-08)[18] for the four selected fungi. This assay was performed by microbiological plating in 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 used as contaminant was inoculated directly on both the leather sample and one drop on the culture medium at distance of 45 mm as shown in Figure 1.

The plates were incubated at the temperature of 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

TABLE I
Formulation of chrome tanning.

Step	Quantity (% of hide mass)	Chemical	Time/ Process control
Pickle conditioning treatment	200	Water	
	5	Sodium chloride	
	0.2	Formic acid (1:10)	
	0.2	Sulfuric acid (1:10)	30 min pH < 3
Tanning	9	Basic chromium salt	120 min
	0.2 *	Antimicrobial agents **	180 min
Basification (chrome fixation)	0.5	magnesium oxide	360 min pH (3,8 - 4,2)
			Drain off

* With the exception of the control sample (0%) and concentrations of 0.1% and 0.4%.
**TCMTB, OIT 5%, OIT+BMC/oil, OIT+BMC/water, OIT 9.3% and PCMC.

antimicrobial activity.[19] It was also visually verified the fungal growth on the surface (FGS) of the leather sample itself.

Incubation in a Tropical Chamber

The tropical chamber test consists in incubating wet-blue leather samples with humidity (100%) and temperature (30°C) and highly contaminated with fungus based on descriptions of ASTM D7584-10.[20]

The leather samples treated with 0.2% concentration of TCMTB, OIT 5%, OIT+BMC/oil, OIT+BMC/water, OIT 9.3% (Table I) measuring 90 mm x 30 mm were taken in duplicate and placed into the tropical chamber to assess the fungal growth on the surface, during 28 days of testing. In this case, the fungus *Aspergillus niger* was the contaminant tested. The samples treated with antimicrobial agents were evaluated by the upper surface (grain side) and lower surface (flesh side) and percentage of contaminated surface was observed at the end of the test (28 days).

Sorption and Wash Out

Different concentrations of the antimicrobial agents TCMTB, OIT+BMC/water and PCMC were prepared, and the absorbance was measured at the wavelength of 290 nm, 279 nm and 285 nm, respectively, by UV/VIS spectrophotometer.

The standard curve of different antimicrobial agents was obtained from the concentrations and the absorbance.

Wet-blue (previously tanned) measuring 6.0 cm x 4.0 cm (approximately 30 grams), were put into the drum together with 100% water and 0.2% and 0.5% antimicrobial agents. An aliquot of 1mL float was collected at predetermined times from 0 to 60 minutes and analyzed through UV/VIS spectrophotometer. The quantities (%) of water and antimicrobial agent used were based on the weight of the pieces

Then, for the wash-out test, the wet-blue samples were removed and placed in another drum with 200% water, added for washing. To measure the percentage of antimicrobial agent desorbed by wet-blue, at predetermined times from 0 to 60 minutes, 1mL float was collected and analyzed through UV/VIS spectrophotometer. Sorption and washout of antimicrobial agents in wet-blue leather was calculated referring to the concentration absorbance standard curve.

It was estimated therefore, to the three antimicrobial agents in different times, the percentage of antimicrobial agents desorbed (by washing) after the test relative to the amount absorbed.

Scanning Electron Microscope Images

The wet-blue leather samples after plating test were subjected to scanning electron microscope (SEM) studies to assess morphological modifications of the leather sample surface grain due to deterioration of the leather caused by fungal growth. Uncontaminated samples and contaminated samples by *Aspergillus niger*, *Aspergillus flavus* and *Penicillium herguei*, were examined using a scanning electron microscope JEOL JSM 6060 (SEM).

Results and Discussion

Plating Test

The results of the inhibition distance and fungal growth on the surface of different samples of wet-blue leather after 28 days of

Table II

Inhibition distance (ID) and fungal growth on the surface (FGS) on wet-blue leather samples after 28 days.

Sample/ antimicrobial agents	Control		TCMTB		OIT 5%		OIT+BMC/oil		OIT+BMC/ water		OIT 9.3%		PCMC	
	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)	ID (mm)	FGS (%)
Aspergillus niger	0	100	1	0	2	0	2	4	9	0	0	0	0	49
Aspergillus Flavus	0	100	0	0	0	30	0	68	38	0	0	49	0	93
Penicillium herguei	0	62	25	0	22	0	45	0	45	0	31	0	0	0
Penicillium chrysogenum	0	59	28	0	25	0	45	0	45	0	26	0	22	0

testing are presented in Table II. In these tests the inhibition distance (ID) is assessed by the maximum distance (45 mm) from the leather where no fungal growth occurs. The closer are the values from 45 mm, greater is the protection provided by the antimicrobial agent. The fungal growth on the surface (FGS) is the percentage of the leather sample that has been contaminated with fungus. Figure 2 shows the growth of the various fungi *Aspergillus niger*, *Aspergillus flavus*, *Penicillium herguei* and *Penicillium chrysogenum* on wet-blue leather surface.

All samples used as control (without antimicrobial agent) had fungal growth on its surface and in just three weeks of test there was no inhibition (distance of 0 mm). The control samples contaminated with *Aspergillus niger* and *Aspergillus flavus,* showed 100% of the surface covered of fungi by the end of the test.

According to the results the antifungal protection provided by both antimicrobial agents TCMTB and OIT+BMC/water was remarkable because no growth of fungus on these respective leather surfaces was detected. The antimicrobial agents OIT+BMC/water showed a inhibition distance larger than TCMTB. Antifungal antimicrobial agent capability of TCMTB applied (0.1%) to wet-blue leather contaminated with *Aspergillus niger* is reported[19], where the inhibition zone was 4 mm not showing surface contamination after 90 days of testing.

Larger resistance of the fungus *Aspergillus flavus* to OIT 5%, OIT+BMC/oil, OIT 9.3% and PCMC was observed, and the contaminated surface percentage in the samples reached levels of 30%, 68%, 49%, 93%, respectively, after 28 days of testing (Table II and Figure 2).

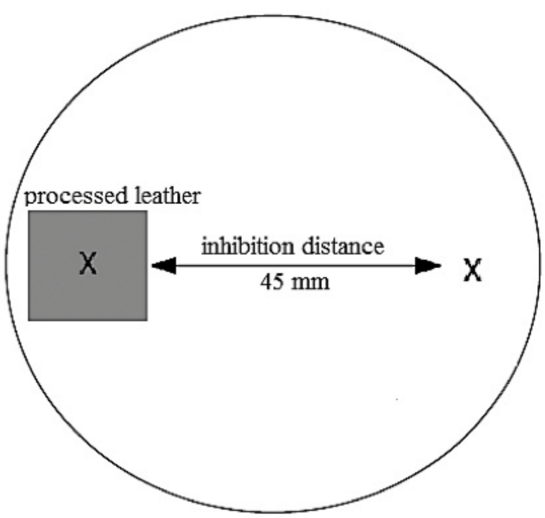

Figure 1. Inoculation points (X) of spore suspension.

Figure 3 shows the decrease the inhibition distance of for plating test, analyzed at predetermined periods, during the 28 days. The absence of bars charts in these figures means that the distance between the sample and the fungus growth was zero. For samples contaminated with *Aspergillus niger* and *Aspergillus flavus*, greater distances of inhibition were observed for wet-blue leathers treated with the OIT+BMC/water antimicrobial agent. The distances of inhibition reached a minimum value approximately 14 days after test beginning for *Aspergillus niger*, and approximately 21 days for *Aspergillus flavus*, remaining constant until the end of the test. For samples contaminated with *Penicillium herguei* and *Penicillium chrysogenum*, greater distances of inhibition were observed for those treated with OIT+BMC/oil and OIT+BMC/water, remaining at maximum (45 mm) until the end of the test (28 days).

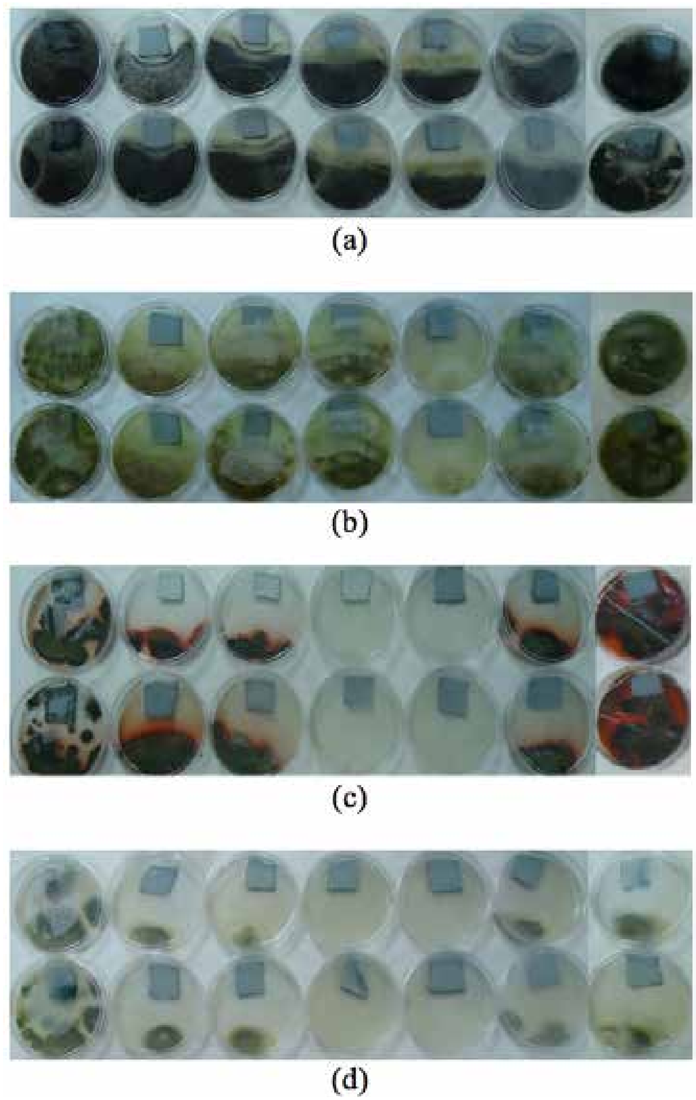

Figure 2. Plating of the fungal (a) *Aspergillus niger*, (b) *Aspergillus flavus*, (c) *Penicillium herguei* and (d) *Penicillium chrysogenum*, on wet-blue leather samples, from left to right: Control, TCMTB, OIT 5%, OIT+BMC/oil, OIT+BMC/water, OIT 9.3% and PCMC

Besides the plating test performed on samples of wet blue with 0.2% concentration, additional tests were performed with different concentrations (0.1% and 0.4%) of TCMTB, OIT+BMC/water and PCMC antimicrobial agents, to check the effect of these in comparison to the initial concentration of 0.2%. In these tests, the 0.1% concentration of all antimicrobial agents showed an unsatisfactory resistance when samples were contaminated with *Aspergillus niger* and *Aspergillus flavus*, where the growth of these fungi occurred largely on the leather surface. When the samples were contaminated with *Penicillium herguei* and *Penicillium chrysogenum*, only the PCMC based antimicrobial agents showed no antifungal effective protection in the same concentration. In samples treated with a concentration of 0.4% every antimicrobial agents were effective (not superficial fungal growth was observed after 28 days trial), but the TCMTB e OIT+BMC/water antimicrobial agents showed maximum distance of inhibition (45 mm), indicating that there is no need for greater concentration than the initial study with 0.2%.

Incubation in a Tropical Chamber

As expected, high percentages surfaces of wet-blue leather without antimicrobial agent treatment (control samples) were contaminated on both grain (70%) and flesh (85%) sides with *Aspergillus niger*. In these samples the beginning of contamination appeared already in the first seven days. No growth of *Aspergillus niger* was observed on either the grain or flesh side of the antimicrobial agents treated leathers after 28 days exposure in the tropical chamber; these observations corroborate the plating test results.

Samples of wet blue leather treated with 2-thiocyanomethylthio benzothiazole - TCMTB (0.1%) and 2-n-octyl-4-isothiazolin-3-one - OIT (0.35%) were incubated in tropical chamber along with spores of fungi, including species of *Aspergillus* and *Penicillium*, for 16 weeks. Superficial mold growth was only observed at the tenth week of testing of samples treated with TCMTB (5% contamination) and at the third week for samples treated with OIT (5% contamination).[11]

Sorption and Wash Out

Figures 4 and 5 show the sorbed percentage by wet-blue leather of TCMTB, OIT+BMC/water and PCMC antimicrobial agents. At 0.2% and 0.5% concentrations of antimicrobial agents, a greater sorption capacity was observed for TCMTB antimicrobial agent, reaching levels of 94.20% after 15 minutes of immersion for a concentration of 0.2%, and 92.56% after 30 minutes of immersion for an 0.5% concentration. In a 0.2% concentration, the maximum sorption capacity of antimicrobial agent OIT+BMC/water and PCMC was 77.11% in just 5 minutes and 63.74% in 60 minutes of immersion, respectively. For a 0.5% concentration of these antimicrobial agents, a higher sorbed percentage of antimicrobial agents by wet-blue was observed, being 89.24% for OIT+BMC/water after 5 minutes and 76.94% after 60 minutes of immersion for PCMC.

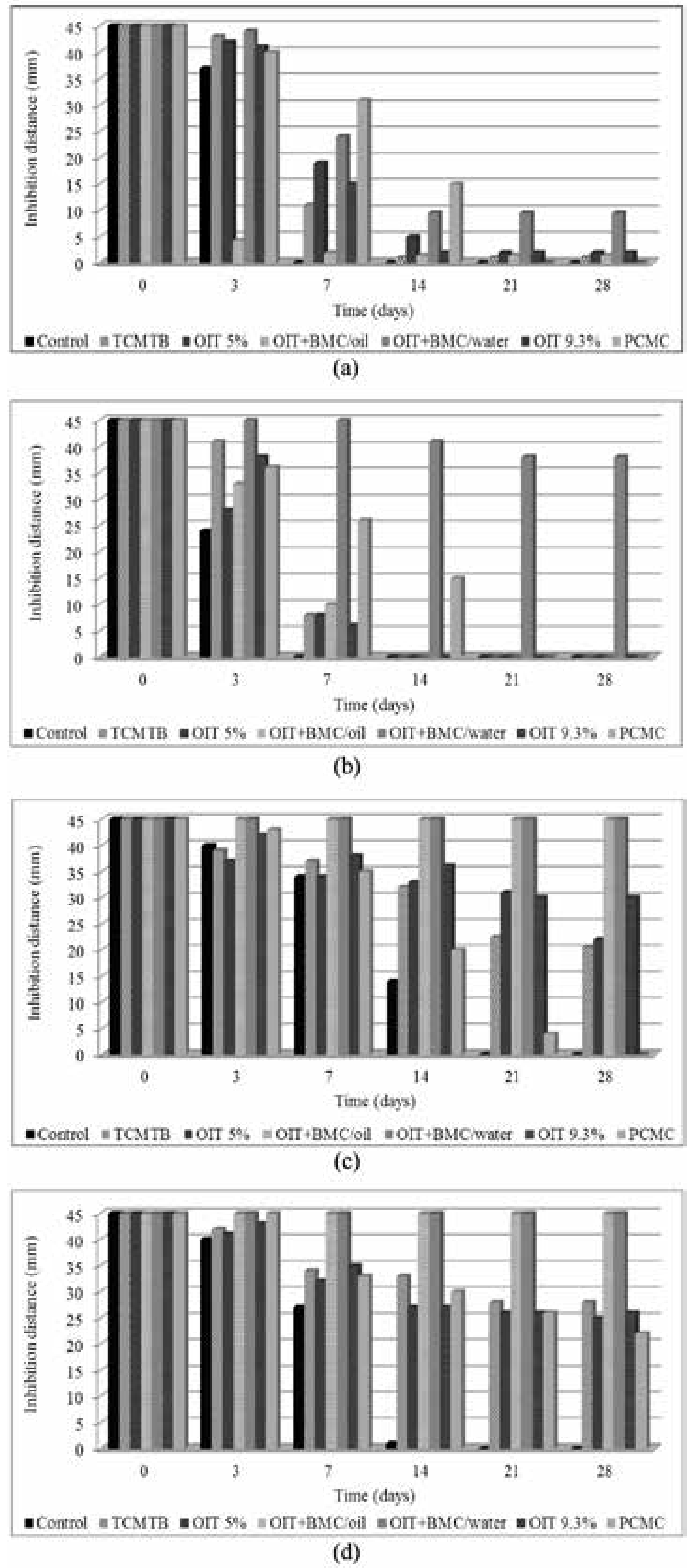

Figure 3. Decrease of the inhibition distance of wet-blue leather preserved with 0.2% antimicrobial agent and contaminated with (a) *Aspergillus niger,* (b) *Aspergillus flavus*, (c) *Penicillium herguei* and (d) *Penicilium chrysogenum.*

Regarding the sorption kinetics, similar behavior to all antimicrobial agent concentrations was observed. That is represented on the curves of Figures 4 and 5, where it was found that after certain test time equilibrium was reached. In this equilibrium, the percentage of antimicrobial agent sorbed by wet-blue leather remains virtually unchanged.

After sorption, the test leathers were subjected to wash out test. Figures 6 and 7 show the curves of the desorption kinetics of antimicrobial agents TCMTB, OIT+BMC/water and PCMC, respectively, by wet-blue leather when it was subjected to washing. For different concentrations of TCMTB, the percentage of antimicrobial agent that the leather releases when subjected to washing is relatively low, remaining virtually constant after 10 minutes of immersion. The highest amount of desorbed antimicrobial agent was observed after 30 minutes, and it was 12.68% for a concentration of 0.2% and 7.52% for a concentration of 0.5%. For leathers treated with OIT+BMC/water and PCMC, the amounts of antimicrobial agent desorbed remained in elevation with time of trial, not reaching the equilibrium. The percentage of released antimicrobial agent was considered high, since after 60 minutes of immersion the desorbed percentages observed for concentration of 0.2% and 0.5% of two different antimicrobial agents were 23.11% and 17.94% for the OIT+BMC/water, and 51.49% and 18.35% for the PCMC antimicrobial agent. Differently from TCMTB antimicrobial agent, the curves of desorption of antimicrobial agents OIT+BMC/water and PCMC does not reach equilibrium. In this case, the maximum desorption is observed when wet blue is subjected to washing at the end of the test after 60 min. This indicates that the chromium tanned leather, processed with the addition of OIT+BMC/water and PCMC antimicrobial agents should not be subjected to washing for a long time.

In previous study, Changqing and collaborators[21] verified that the absorptivity of isothiazolinone for wet-blue is about 45% and is practically unchanged after 15 minutes for different initial concentrations, (0.05%, 0.10%, 0.25%, and 0.50%). The washout rate of isothiazolinone for wet-blue is relatively high, from 51% to 58% for concentrations of 0.10%, 0.25%, 0.50% and about 74% for concentration of 0.05%. Since it was easily washed out from the wet-blue, isothiazolinone should be added at the later stage of chrome tanning and the treated leather should be preserved without further washing.

Scanning Electron Microscope Studies

The SEM images (Figure 8) show the attack of fungi on the grain surface of the leather after 28 days of test. According to the SEM

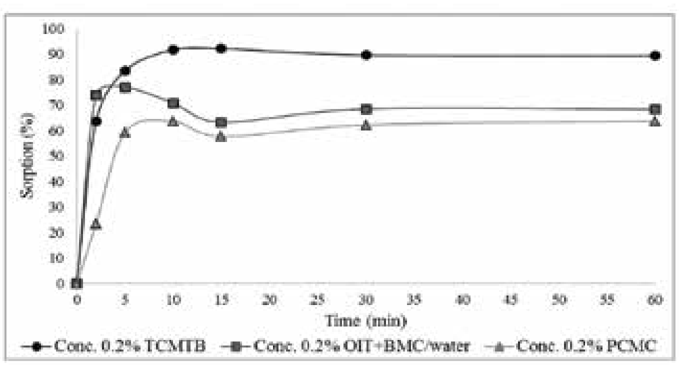

Figure 4. Sorption of antimicrobial agents TCMTB, OIT+BMC/water and PCMC (initial concentration of 0.2%) by wet-blue leather.

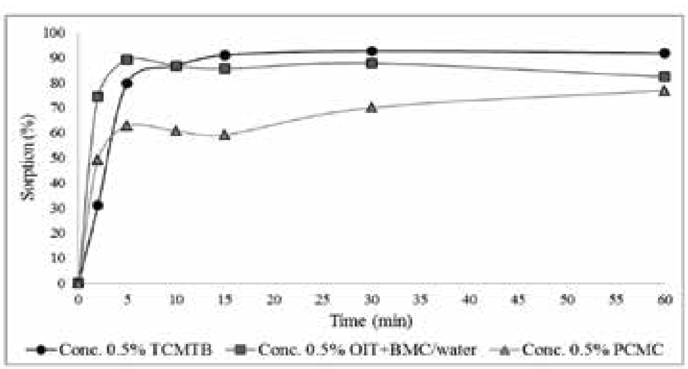

Figure 5. Sorption of antimicrobial agents TCMTB, OIT+BMC/water and PCMC (initial concentration of 0.5%) by wet-blue leather.

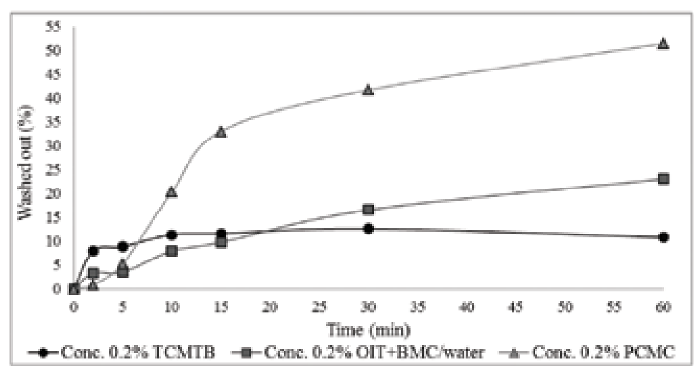

Figure 6. Washing out of antimicrobial agents TCMTB, OIT+BMC/water and PCMC (initial concentration of 0.2%) by wet-blue leather.

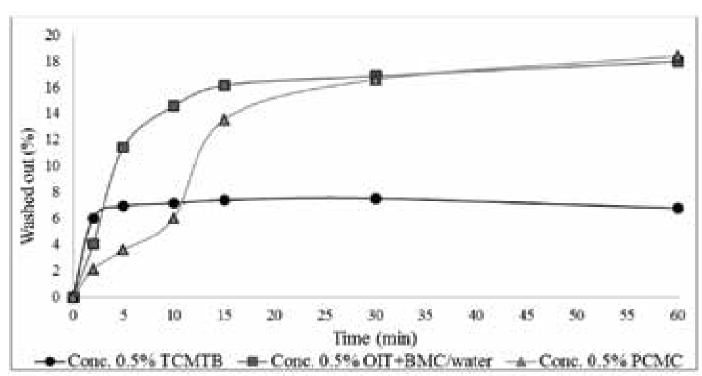

Figure 7. Washing out of antimicrobial agents TCMTB, OIT+BMC/water and PCMC (initial concentration of 0.5%) by wet-blue leather.

images it can be seen that the non-contaminated sample shows an intact smooth surface with well-defined pores. On the other hand, the deteriorated samples show rough surfaces due to the attack of fungi which use this material (hide, leather) to supply their nutritional needs compromising the physicochemical characteristics and mechanical properties of the material.[22,23] This shows the importance of the correct use of antimicrobial agents in leather processing, to ensure the quality of the final product. These results differ from previous work[24] where when wet-blue leather samples were subjected to biodegradation test there was no change in the grain surface. But in this work[24] there was no nutrients added.

Conclusions

Among the six antimicrobial agents conventionally used in the leather industry and studied in this work, two of them, TCMTB and OIT+BMC/water when applied in wet-blue leather, revealed higher antifungal capacity against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium herguei* and *Penicillium chrysogenum*. The OIT+BMC/oil antimicrobial agent at the indicated concentration used did not show antifungal capacity against the growth of fungi *Aspergillus flavus* and *Aspergillus niger*. The antimicrobial agents OIT 5%, OIT 9.3% and PCMC had intermediate performance compared to the others one already mentioned.

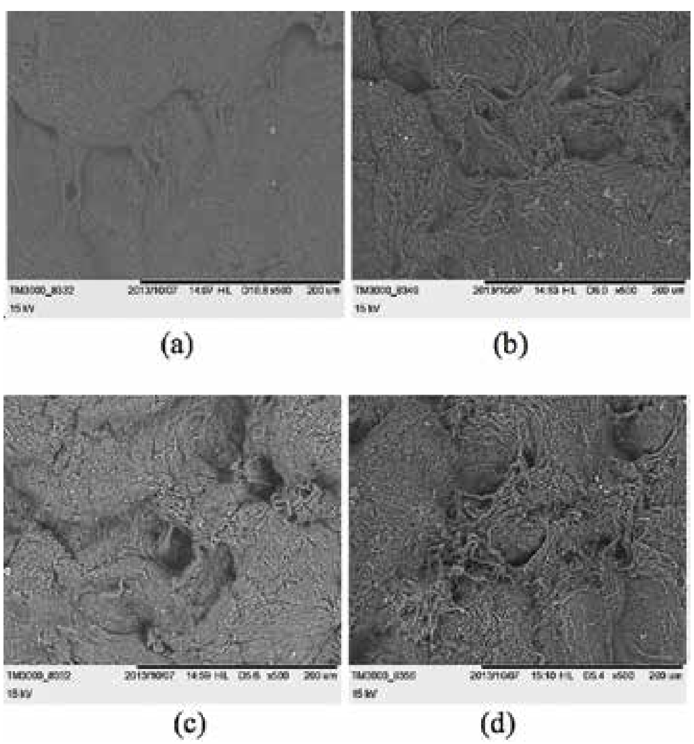

Figure 8. SEM images of the wet-blue leather samples (a) control (no fungal attack), (b) contaminated with *Aspergillus niger*, (c) contaminated with *Aspergillus flavus* and (d) *Penicillium herguei*.

In sorption test and wash-out with TCMTB, PCMC and OIT+BMC/water antimicrobial agents, the one based on TCMTB was quickly sorbed by wet-blue and only a small percentage was left in the residual bath. The percentage of the antimicrobial agent desorbed by the leather subjected to washing was relatively low, indicating that the antimicrobial agent has a resistance to washing, which is not observed for the leathers treated with the PCMC and OIT+BMC/water antimicrobial agents.

The bioterioration of fungi caused clear damage on the leather surface compromising the quality of leather, which leads to devaluation, and rejection of products on the market.

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