Bacterial and Fungal Damage in Leather

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Abstract

Microbial degradation leads to significant loss of quality and economic value by the tanner. This damage cannot be reversed as it involves the degradation of the collagen and elastin fibers, which are important proteins in the leather-making process. It is, therefore, important to carefully monitor the after-slaughter, curing, beamhouse and post-tanning processes to prevent this type of damage. This is only possible if you can identify the signs and/or defects caused by bacteria and fungi as early as possible and put in place corrective measures to halt any further or future damage. This study evaluated various techniques for identifying bacterial and fungal damage in leather, including visual inspection, smell, feel, microscopy, and culturing techniques. Samples of cured hides and leather obtained from different sources in the USA were subjected to these techniques to determine the presence of these microbes or to identify their damage. The results highlight various defects and indicators that point to various microbial causes. A combination of visual inspection, microscopy, and culturing techniques can provide hides and skins sellers, packers, and tanners with reliable and accurate identification techniques for identifying early signs of damage. While microscopy was sufficient to observe fungal growth and bacterial damage, culturing was more reliable for identifying the bacterial causative agents. This study highlights the importance of implementing routine inspections and monitoring to prevent continued microbial damage to hides to ensure the quality of the leather.

Introduction

Leather is a natural material made from the stabilization of the collagen structure in hides/skins from animals using tanning agents.[1] The tanning process transforms a product that would otherwise be deemed as waste into a versatile and arguably, the most sustainable material for use in footwear, fashion, automotive and furniture industries among many other uses. Over the years, leather has been a preferred material for these uses because of its superior strength properties, durability and longevity, breathability properties, superior feel and comfort, repairability, and resistance to abrasion among others.[2]

Raw hides and skins, however, like many other natural products, are prone to microbes and putrefaction if left unattended after slaughter. Approximately 60-70% of an unpreserved hide's weight is water while the other 30% are fats and proteins, making it a good breeding ground for microorganisms due to the sufficiency of nutrients and the presence of the ideal growing conditions of pH, temperature and moisture.[3,4] Microorganisms such as bacteria and fungi, flies, larvae, beetles, rodents and other insects will soon after slaughter begin to encroach onto the hides thus the need for immediate preservation, commonly referred to as curing. The increasing demand for full-grain leather and aniline finishes underscores the need to ensure there is minimal damage to the grain of the hide before, during and after processing.

Biodeterioration in leather

The hide/skin *in vivo* contains natural saprophytic bacteria kept in control by the animal's metabolic defense system.[5] However, this balance is lost *in vitro* and the process of biodeterioration starts immediately after slaughter. These bacteria degrade and remove dead tissue in the living animal, but upon death, they cause autolysis which is the self-hydrolysis of the collagen fibers.[3] Other opportunistic bacteria from the environment also start growing on the flayed hide and multiply rapidly causing putrefaction. It is, therefore, paramount that necessary precautions are taken at the different stages of leather processing that are susceptible to bacterial damage.

Curing aims to destroy any active bacteria, prevent bacterial activity, or prevent bacterial contamination. Curing after the onset of bacterial action might kill all the active bacteria but leave behind the secreted enzymes and hence putrefaction will continue. Various techniques can be employed to achieve these functions including but not limited to; salting (brining, dry-salting, wet-salting), pickling, chilling and freezing. Salting, which entails saturating the hide structure with Sodium Chloride, is the most common curing method in North America, Europe and other temperate climates.[5] Use of marine salts is limited due to the presence of impurities that encourage the growth of halophilic bacteria.

Hides and skins undergo a series of processing stages before they are referred to as finished leather. The beamhouse encompasses all preparatory and cleansing stages of leather making before tanning is done. Soaking is the first stage of processing whose aim is to rehydrate the hides to facilitate subsequent processing. Putting the hides in water for prolonged periods increases the chances of bacterial activity, especially when coupled with elevated temperature (above 22°C).[3] Various bacterial species including *Staphylococci* spp., *Bacillus* spp., *Micrococcus* spp., *Pseudomonas* spp., *Corynebacterium* spp., and *Moraxella* spp. have been reported to cause putrefaction in raw hides and skins, with over 90% of them being gram-positive.[6] These microbes can be controlled using chemicals that kill bacteria

and prevent their breeding (*bactericides*) and those that stop their active life (*bacteriostats*).[7]

After the beamhouse processes, is tanning. Tanning is the preservation and conversion of the raw hide or skin into a stable material (leather), using various agents such as Chromium, Vegetable extracts, Aldehydes and Oils, making it resistant to bacterial attack and heat damage.[5] After tanning, several post-tanning processes (Retanning, Dyeing, Fatliquoring, Drying and finishing) are carried out to impart the properties of feel, color and softness as per the intended use of the leather. Fungi are the most common microbes that cause defects in tanned and finished leather as the fiber structure is stabilized and less likely to be damaged by bacteria. Molds, yeasts and filamentous fungi such as the genus *Aspergillus* and the genus *Penicillium* are the most frequent causes of defects in tanned leather.[8] Use of fungicides in the tanning liquor should help to control or avoid damages caused by fungi.

Impact of biodeterioration on the quality of leather

Microbial damage has been known to cause significant losses across the leather value chain. Not only do they lead to financial losses, but also significantly lower the quality of the leather and the final product. Microbes have been reported to cause uneven grain and grain damage, undesirable pigmentation and uneven dyeing, non-uniform finishing, looseness and pippiness, reduced physical and mechanical properties.[4,9] Although microbial degradation in leather has widely been studied, little work has been published on the identification of defects caused by microbes to facilitate correct decision-making in the tannery or curing premise to nip the issue in the bud. This study will highlight some techniques and quick giveaway signs that will indicate to the tanner that the damage on the hides, skins or leather was caused by microbes and therefore guide them to make necessary adjustments to their process to control and/or eliminate the problem. The overall hypothesis in this study is that microbial damage can be identified through various culturing techniques, smell, visual inspection and microscopy to facilitate easy control and treatment to promote the quality of leather produced. This hypothesis was tested here.

Materials and Methods

Materials

Various rawhide, brine-cured hide and leather samples used for this study were obtained from the Leather Research Laboratory (University of Cincinnati), Ohio, USA. These samples had been previously acquired from various slaughter and cure premises, packers, tanneries, and manufacturers in the United States of America for purposes of research and testing.

All microbiological media were procured from HACH Company (analytical instruments, test kits, and reagents manufacturer and distributor). These include the Biological Activity Reaction Tests (BART) kits and Paddle Testers. Various test kit manufacturers and distributors such as LaMotte, US Water Systems, Bore Saver, Cannon Water Tech., Geoquip among others, supply these types of kits. HACH was randomly selected for the acquisition of test kits for this study. All reagents, chemicals and equipment used in this study were of laboratory or analytical grade.

Methods

Culturing Techniques

Biological Activity Reaction Tests (BART)

This test was used to evaluate the presence of bacteria on a brine-cured hide suspected to be undergoing microbial degradation. The biological activity reaction test is a water testing system for nuisance bacteria and can involve several different tests. These tests detect the activity (aggressivity) of nuisance bacteria by the time lag (TL, measured in the number of days from the start of the test to when a reaction is observed). The longer the TL before the observation of activity, the less aggressive the bacteria are in that particular sample.

Sterile water was added to hide samples (3 × 3 inches) and sonicated for 60 minutes in a conical flask. The extract from the pooled samples was removed and introduced into the different BART test tubes for growth. The tubes were placed in a dark environment at room temperature for 8 days. The presence of bacterial growth (observed through color change in the medium) was checked in the test tube daily. These test kits were used in this study to evaluate the presence of Acid Producing Bacteria (APB), Heterotrophic Aerobic Bacteria (HAB), Iron related bacteria (IRB), Sulphate reducing Bacteria (SRB) and Slime forming Bacteria (SFB) on brine-cured hides that were suspected to show signs of putrefaction. The liquid media in the BART test kits was examined by eye for turbidity, color change, formation of sediments and slime formation. Controls were set up with sterile water in place of the hide extract.

Paddle Test

The paddle test is a semi-quantitative screening that easily detects contamination by coliform bacteria on a substrate, in this case, brine-cured hides suspected to be contaminated. The paddle is a double-sided slide attached to the vial cap. Each side of the slide is used to perform a separate test (Coliform side-clear and fungi side-red). Both sides of the paddle were pressed against the solid surface of the flesh and grain sides of the hide samples and then incubated at 30˚C for 48 hours before observation for total microbes. A positive test results if colonies are observed on the paddle. The colony density is then compared to the colony density chart (Appendix 1) to determine the quantity of the colonies in the original sample. After an extra 24 hours, the plates were considered negative if no growth was observed. All the paddles were examined by eye for growth and colony morphology and any changes in the medium. Controls were set up by incubating paddles at the same conditions without exposing them to the hide.

Visual examination

The general condition and presence of defects on the raw, brine-cured, wetblue and crust samples were examined visually, through touch and smell. Visual evidence was captured using a NIKON Coolpix A900 camera in the form of photographs.

Microscopic examination

All the samples were examined and photographed using an Olympus professional research-grade microscope. A Thermoscientific Apreo C Scanning Electron Microscope (SEM) was used for higher magnifications and to differentiate microbial stains from those of other sources such as metals. All the leather microscopy and identification were done in accordance with the ISO 17131 method.[10]

Results and Discussion

Culturing Techniques

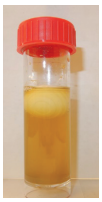
Culturing is an essential tool that can be used to identify microbial growth in hides and leather. This technique involves the cultivation of microorganisms present in the sample, allowing for the detection and quantification of specific microorganisms present. The results obtained from culturing techniques, such as the Paddle Test and Biological Activity Reaction Test (BART) Qualitative Test, provide valuable information on the presence and types of microorganisms present in the hide or leather, which can be used to identify and control microbial growth or damage.

Bart Qualitative Test

The BART Qualitative Test is a rapid test that can detect the presence of total bacteria, total coliforms, and *E. coli* in a sample. The test uses a specialized medium that changes color in the presence of these microorganisms. The results of the test are qualitative, meaning that it can detect the presence of microorganisms, but it cannot provide an exact count. Table I outlines the results from the brine-cured hide sample. The results indicated that Iron related Bacteria,

Table I

Biological Activity Reaction Test Results After 8 Days

Type of Bacteria Tested	Sample A Control- Sterile water	Sample B Hide Sample	Result
Iron Related Bacteria (IRB)			POSITIVE RESULT A brown slime ring or foam around the ball.
Slime Forming Bacteria (SFB)			POSITIVE RESULT Presence of a cloudy slime solution and a gel-like ring at the base of the tube.
Sulphate Reducing Bacteria (SRB)			POSITIVE RESULT A black slime ring beneath the ball A black slime growth at the base of the tube
Acid Producing Bacteria (APB)			POSITIVE RESULT Bleaching of purple to bands of yellow
Heterotrophic Aerobic Bacteria (HAB)			POSITIVE RESULT Complete bleaching of the blue color

Slime forming Bacteria, Sulphate reducing Bacteria, Acid-producing Bacteria and Heterotrophic Aerobic Bacteria were present in the hide sample. The Heterotrophic Aerobic Bacteria were more aggressive as they had tested positive by the third day. These types of bacteria require oxygen to survive and do not produce their own food, instead oxidize other sources of organic carbon, in this case, the hide matter, as their source of nutrition. The abundance of nutrient sources in the hide matter explains the aggressiveness and speed of multiplication of these particular bacteria.

Iron-related bacteria (IRB) were also present in the sample. These bacteria obtain their energy through oxidation and reduction of iron compounds present in the salt-cured hide. These bacteria can be divided into two main groups: iron-oxidizing bacteria and iron-reducing bacteria. Iron-oxidizing bacteria (FeOB) are aerobic or microaerophilic microorganisms that can oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) and use it as a source of energy while Iron-reducing bacteria (FeRB) are anaerobic microorganisms that can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and use it as a source of energy.[11,12] IRB produce a yellow, orange, red, or brown bacterial slime.[13] This was the indicator for a positive result in the test for IRB.

Slime-forming bacteria produce a slimy polymeric substance called slime or biofilm, without the need for Iron or Manganese like IRB. The slime is composed of extracellular polymeric substances (EPS) which can include polysaccharides, proteins, and lipids.[14] EPS can provide a protective matrix for the bacteria and also allow them to adhere to surfaces. The growth of these bacteria was observed as a cloudy cluster suspended in the liquid medium and a gel-like ring around the ball, and at the base of the tube.

The hide sample also tested positive for Sulphate Reducing Bacteria (SRB). SRB have the ability to reduce sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S), with the unmistakable "rotten egg" odor.[13,15] This process can happen in the presence of organic matter, in this case, the hide. SRB produced a dark slime that was deposited as a ring beneath the ball and also at the base of the tube in the positive test. Usually, SRB are outnumbered by other microbes because of their slow growth properties and Carbon preference.[15]

Acid-producing bacteria, as the name suggests, produce acid as a byproduct of the fermentation of carbohydrates as part of their metabolic process.[16] These bacteria break down sugars present in the hide and to an extent, the hide structure leading to loss. The acidity from these bacteria caused the bleaching/yellowing of the medium in the positive result. The different bacteria present in this sample are possibly extremophiles due to their ability to grow at high salt concentrations at curing (halophiles) and some even persist through the high liming pH (alkaliphiles). The use of uncontaminated salt and the application of a biocide during curing and soaking should help eliminate these bacteria from hides that test positive for these microbes. All control setups tested negative.

Paddle Test

The Paddle Test is a quantitative test used to detect the presence of microorganisms. It is based on the principle of microbial growth. The sample is placed on a paddle with nutrient agar and then incubated for a specific period at optimum parameters. After the incubation period, the bacteria present in the sample grew and formed colonies on the agar as shown in Table II. The number of colonies formed was used to estimate the number of microorganisms present in the original sample against a given scale (Appendix 1) as shown in Table III and Table IV. The paddle test revealed the presence of Aerobic Bacteria and Mold on the sample. The red side of the paddle is rich in carbohydrates and other nutrients that Mold/yeast requires to grow. After incubation, the Mold present in the sample grew to form colonies on the agar. The number of colonies formed was then used to estimate the number of Mold present in the original sample.

A positive result was recorded for bacterial and mold growth on the brine-cured hide sample. The grain side of the brine-cured hide recorded approximately 1000 bacterial colonies while the flesh side recorded 10^5 CFU (colony forming unit). No fungal growth was observed on the grain side of the hide while about 100 colonies were observed from the flesh sampling area. The high number of bacterial colonies suggests that a wide variety of microorganisms are present in the hide sample. More colonies (bacterial and fungal) were observed on the flesh side of the hide, indicating that the flesh side may be more conducive to microbial growth than the hair side. This is due to the direct access to nutrients and hide moisture to support their growth. These types of bacteria (aerobic) take nutrition from other sources of organic carbon in the presence of Oxygen.[17] This leads to the degradation and decay of the hide. The bacteria present in these samples are also possibly extremophiles due to their ability to grow at high salt concentrations at curing and persist through the high liming pH. Proper curing, storage and treatment with biocides and fungicides should help prevent bacterial and mold growth on raw hides thus preventing damage. All control setups tested negative.

Visual Inspection and Microscopy

In addition to culturing techniques, visual inspection, smell, and microscopy can also be used to identify microbial degradation in hides and leather. Visual inspection involves looking at the surface of the hide or leather for signs of degradation, discoloration, slime formation or other changes that may indicate microbial growth. The smell can also be used to detect microbial degradation. Some microorganisms produce characteristic odors, such as musty, moldy or sour smells, that can indicate their presence. The presence of unpleasant odors may indicate the presence of microbial growth on the hide or leather. Microscopy can be used to examine the leather in more detail. A sample can be taken and observed

Table II

Paddle Test Results After The 48 Hr. – Incubation Period

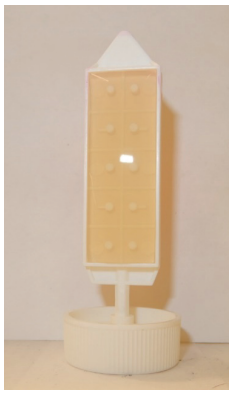
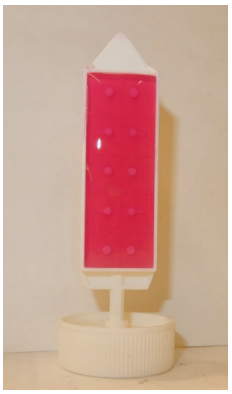
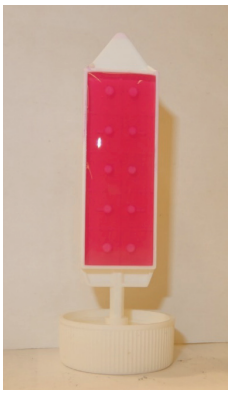
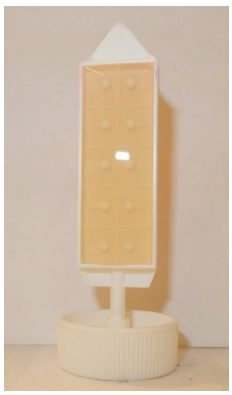
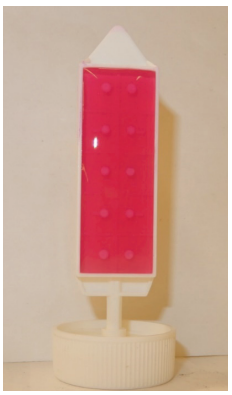
Side of Hide Sampled	Total Aerobic Bacteria		Yeast And Mold	
	Control (Negative Result)	Sample (Positive Result -Bacterial Growth)	Control (Negative Result)	Sample (Negative Result)
Grain Side				
Flesh Side				

Table III

Total Aerobic Bacteria Testing Results After 48 Hours

Observed (Estimated) Level of Contamination on Brine Cured After 48 Hours (Total Aerobic Bacterial Growth)		
Grain side		Flesh Side
Bacterial Colony Density	1000 (10^3)	100,000 (10^5)

Table IV

Yeast And Mold Testing Results After 5 Days

Observed (Estimated) Level of Contamination on Brine Cured-Hide After 5 Days (Yeast And Mold Growth)		
Grain side		Flesh Side
Yeast and Mold Colony Density	NONE	100 (10^2)

under a microscope to look for the presence of microorganisms or characteristic damages or stains caused by them. This method can also be used to identify some specific types of microorganisms present. This study examined multiple hide and leather samples for signs and defects that can be identified through smell, visual inspection, or microscopy to identify the presence of bacterial growth and damage on a hide or leather to facilitate the choice of the right mitigation measure.

Identification of Bacterial Damage

Foul smell and Staling

The sampled brine-cured hides had a vivid putrid odor that emanated from the bag in which the hide was stored. This obnoxious odor was a clear indication that the hide was undergoing putrefaction. The flesh side of the hide was observed to have a slimy coating on its surface, with a brownish color, a slimy texture, and an unpleasant odor. Putrefaction is the digestive action on the hide structure caused by enzymes secreted by bacteria as they find nourishment from the hide substrate.[4,7] These enzymes quickly hydrolyze the hide's proteins, fats and carbohydrates into forms that can be readily metabolized by the microbes. These bacteria cause extensive degradation of the collagen leading to the release of byproducts that create a foul odor and even attract maggots.[18] Curing should subsequently be done as soon as possible as it has been shown that a twenty-four-hour delay in curing will result in observable grain damage in the resultant leather.[3]

This staling process is characterized by an increase in heat due to this activity, especially in a bundled or piled lot. Staling may occur when there is delayed curing, inadequate curing, or contamination during curing, or, in the case of cured hides, it occurs when they are in poor and prolonged storage conditions.[19] It is an accepted general rule that foul smell, hair slip and heating are the best warning signs the tanner has that staling has occurred or is occurring. Hides showing these signs should either be resalted or put into the process immediately. It is also good practice to wash the hides immediately after flaying to remove the body heat as soon as possible to prevent autolysis and slow bacterial growth.

Hairslip

Hairslip is the loosening of the hair from the hide due to bacterial damage and is known as the first sign of putrefaction.[20] When these hide samples were handled, patches of hair and the epidermis slipped off the grain of the hide as shown in Figure 1.

Hairslip is often accompanied by a very sensitive grain whereby the grain easily rubs away during processing. The rubbing leads to a dull grain and blotchy appearance after finishing.[7] In more advanced stages where there are rotten spots in the hide, grain slip can be observed which affects larger portions of the epidermis.[19] Previous studies have reported that the most common putrefying bacteria in

Figure 1. Hairslip on a rawhide

Green and salted hides are the *Staphylococcus* sp. *Micrococcus* sp. and *Bacillus* sp. while *Pseudomonas* sp., *Proteus* sp. and *Escherichia* sp. have been reported to cause hairslip and perforation.[3,21] The *Bacillus* sp. seems to be the biggest concern posing the greatest danger of damage.[3] This degradation persists into the soaking stage and the use of biocides is paramount to inhibit further bacterial growth. Hides with hairslip or grain slip should be handled with care and not be subjected to aggressive agitation as the grain is already weakened. Such hides should be processed as soon as possible to prevent further damage.

In hides that have adhering, fat, flesh, dirt and dung, cure penetration is significantly retarded by the presence of extraneous materials as shown in the sample in Figure 2. This is referred to as "Improper After-cleaning".[19] The curing may be delayed sufficiently to be favorable to autolytic and bacterial damage as observed in the sample in Figure 2 which was characterized by extensive hairslip discussed above.

Figure 2. Extraneous Fat and Flesh on a rawhide

Figure 3. Maggot Infestation on a rawhide

After flaying, there is often fatty tissue and meat that remains attached to the hide. This process is known as after-cleaning.[19] In an ideal slaughtering process, the flaying should remove all of these materials, however, in many cases, it is necessary to remove these materials before curing the hide. This is because heavy layers of fatty tissue can impede cure penetration and delay the curing process, making it more susceptible to damage from bacteria and autolysis. Similarly, meat left on the hide can also impede salt penetration and can cause the hide to rot where it is attached, as it readily decomposes.[19]

If the rawhide is not handled properly, the number of microbes on it can significantly increase, leading to significant damage to the raw material.[22] Dirt favors bacterial breeding on the hide. The presence of dirt, dung and blood creates an environment that is favorable for the breeding of microbes. This can lead to maggot infestation as observed in the sampled hide shown in Figure 3. *Maggot* infestation is a condition in which the fly *maggots* feed off and develop in a dirty, polluted, or unattended environment or decomposing matter/tissues, in this case, the hide.[23]

Maggot infestation on a hide is a clear sign of degradation and bacterial damage. Maggots feed on organic matter and if they are present on the hide, they can eat away at the hide causing holes and grain damage. Such hides should be processed as soon as possible employing the use of biocides and disinfectants to slow down further damage.

Red heat

The sampled hide in Figure 4 showed extensively widespread red patches on the flesh side. This is another sign that the hide is degrading. Red heat occurs due to extremely halophilic archaea.

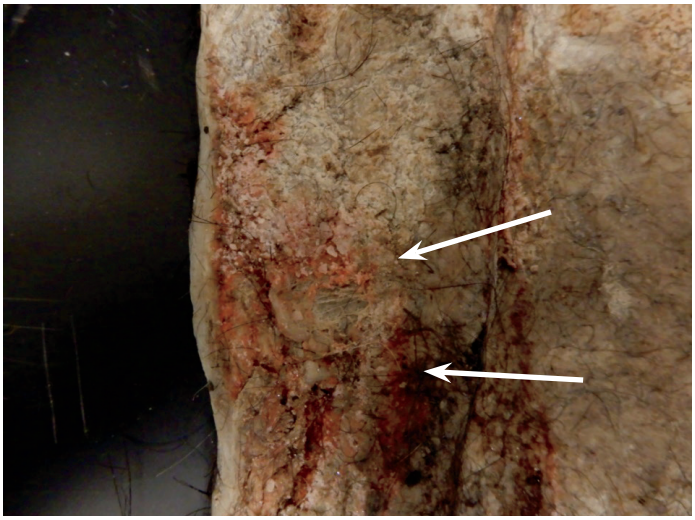


Figure 4. Red coloration caused by extremely halophilic archaea

The presence of extremely halophilic archaea gives rise to red or colored spots on the flesh side of the hide and this is an indicator that putrefaction has occurred.[7,21] These types of bacteria are adapted to living in salty environments. The utilization of marine salts, contaminated salts, or salts that have been previously used can increase the likelihood of halophilic bacterial growth.[3,19] The use of such salts can cause a higher risk of bacterial growth and putrefaction, which can be detrimental to the preservation of the hide. To avoid this, it is recommended to use clean and fresh salts to prevent this damage.

Enlarged hair follicles

Initial stages of bacterial damage involve the attack of the hair root section by the proteolytic enzymes produced by bacteria. This leads

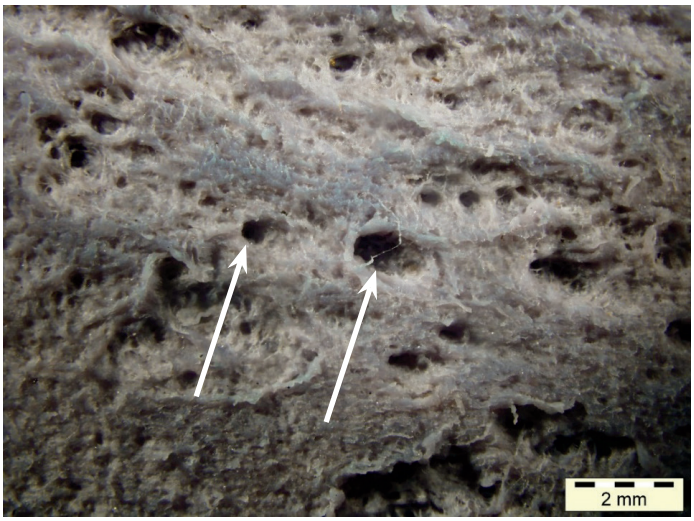


Figure 5. Enlarged hair follicles due to bacterial degradation

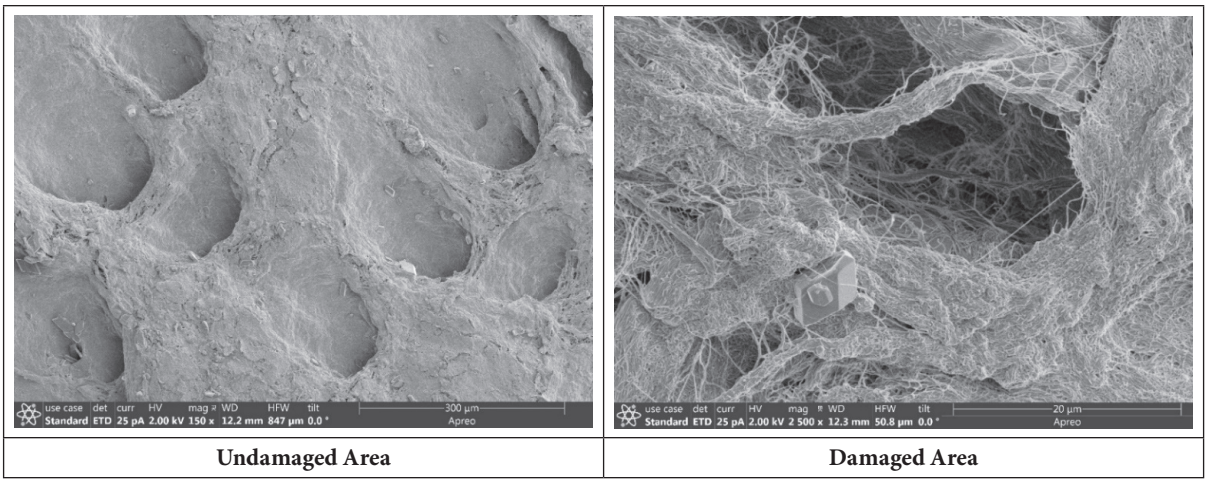


Figure 6. SEM Images of hair follicles

to the wide opening and darkening of the follicles and loosening of the hair shafts thus, hairslip. This degrades some of the hair follicles, causing them to become enlarged and darkened as shown in the sampled hide in Figure 5.

This condition is known as the 'Pin-prick effect' and is a clear indication of the ongoing bacterial degradation in the lot. The SEM image in Figure 6 shows the follicle degradation by the proteolytic enzymes distorting the grain pattern.

Circular grain damage

Another indication of bacterial damage is the occurrence of circular damage on the grain of the hide. The sampled wetblue leather was characterized by a range of small to midsized circular pits on the grain as observed in Figure 7. Putrefaction begins with a single bacterium, which replicates to form a circular colony.[24] Proteolytic enzymes exuded by the colony cause circular pits and groves that expand as the colony grows as observed on the wetblue sample.

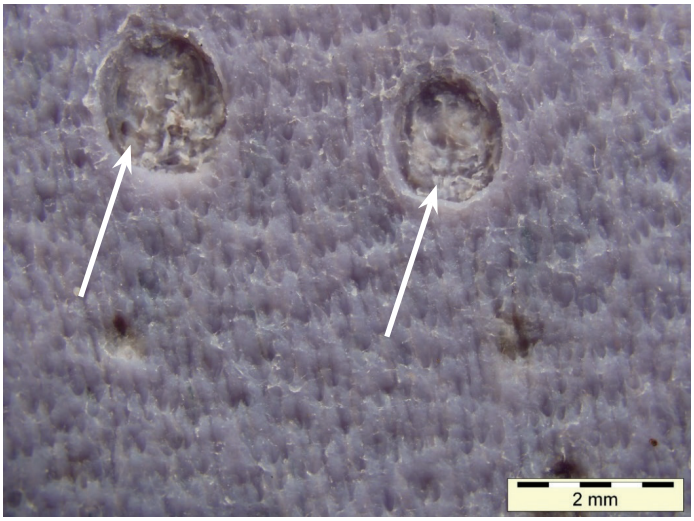


Figure 7. Circular Grain Damage due to bacterial degradation

Figure 8. Holes due to bacterial degradation

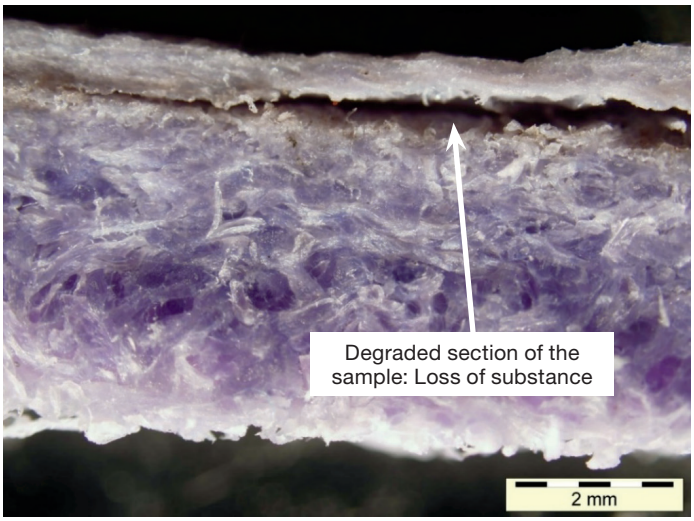


Figure 9. Cross-sectional view of a degraded wetblue sample

Holes

At advanced stages of the damage, the pits and groves are converted to holes as shown in the sample in Figure 8. The presence of these holes is an indication that microbial degradation is at its advanced stages and that the hide structure is wasting away. This is especially common after the soaking process which is designed to rehydrate the hide and remove the curing salt which creates a conducive environment for bacterial growth and degradation.[18,22] These bacteria, with multiple proteolytic and collagenolytic abilities, grow and multiply fast by producing proteolytic enzymes whose function is to convert substrates from the hide to a form that their cells can absorb leaving holes in the hide.[18,24]

Loss of substance

Loss of the hide substance is also an indication of bacterial action. The grain and the junction between the grain and the corium are the regions that are most susceptible to damage from these bacteria and enzymes. Putrefaction causes degradation of the fibers at the grain-corium junction which leads to loss of substance.[3] This is characterized by spaces between the grain and the dermis of the leather structure as shown in the cross-sectional view of the sampled wetblue in Figure 10. At advanced stages, this would lead to blistering, which is the complete delamination and peeling of the grain layer from the corium as shown in Figure 11. This loss of substance is reflected in the final leather by flankiness (loose and flaccid), pipiness, taint, veininess and poor break.[19]

Identification of Fungal Damage

This study examined multiple hide and leather samples for giveaway signs and defects that can be identified visually or through microscopy to confirm the occurrence of fungal damage. The presence of mold or other microorganisms can often be seen as discoloration or staining on the surface of the hide.

After tanning, the hide is less susceptible to degradation by putrefactive bacteria. Molding and fungal growth are common in

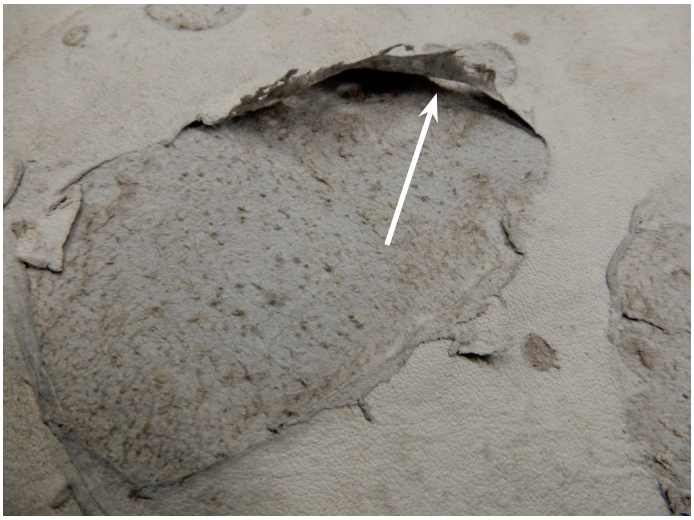


Figure 10. Grain Peeling due to bacterial damage

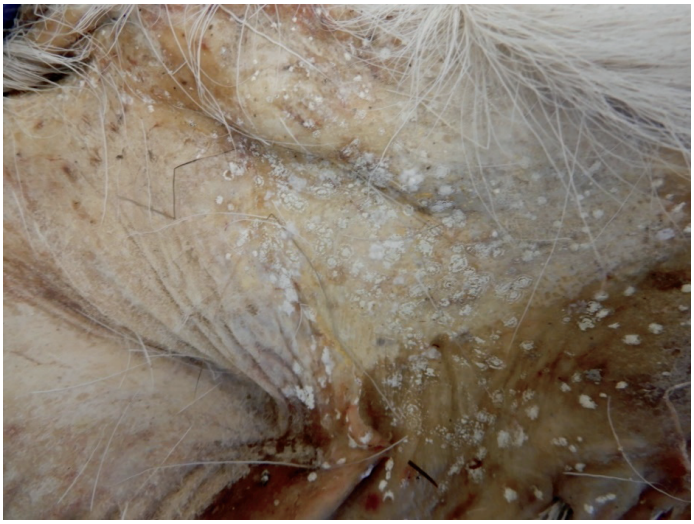


Figure 11. Fungal growth on a rawhide

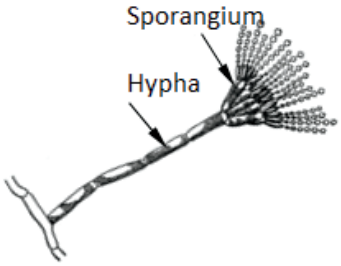


Figure 12. Typical Mold structure

wetblue, crust and even finished leather that has been stored in moist or highly humid environments. This, however, does not exclude raw and pickled hides, which are stored in these conditions, and will also have fungal growth as shown in Figure 11. The sampled hide had been packed in a plastic bag for three weeks, locking in moisture and thus encouraging fungal growth.

Visual and microscopic examination of the hide sample revealed mold growth since the fungal hyphae (long filamentous structures) and sporangia (bulbous spore-forming bodies) observed as white spots on the hide matched the structure of a typical Mold illustrated in Figure 12.

A wetblue sample was also visually examined for fungal growth and microscopic images were captured. The wetblue, previously stored in a plastic bag, was characterized by a moldy smell, colored stains as well the presence of fungal hyphae as shown in Figure 13. Similar studies have been published and associated this kind of damage to *Aspergillus*, *Penicillium*, *Paecilomyces*, *Scopulariopsis*, *Trichoderma* and *Rhizopus* sp. [21] This is a clear indication of fungal growth which leads to staining of the grain surface of the leather.

Further SEM analysis of the sample clearly showed the fungal structures of the mold as shown in Figure 14 and Figure 15.

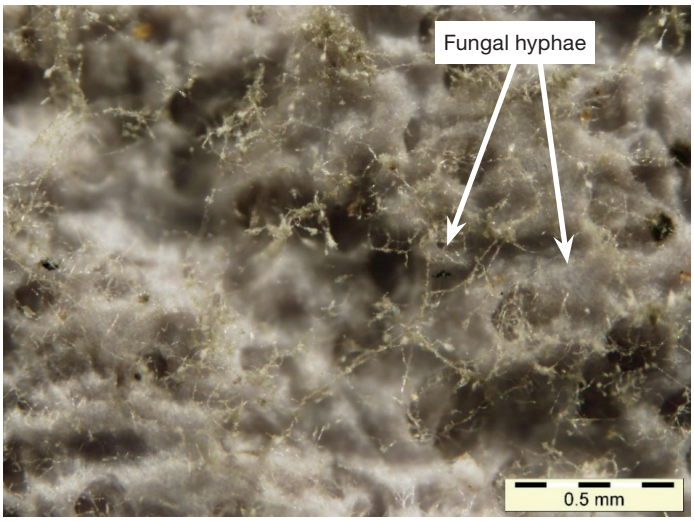


Figure 13. Mold Growth on wetblue

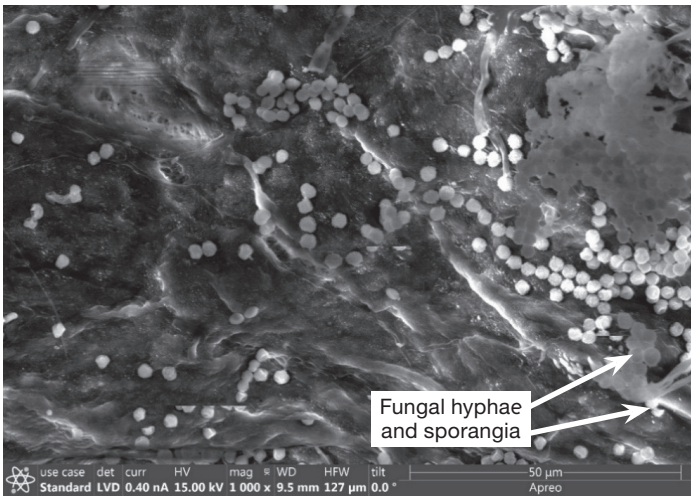


Figure 14. SEM analysis for Mold growth

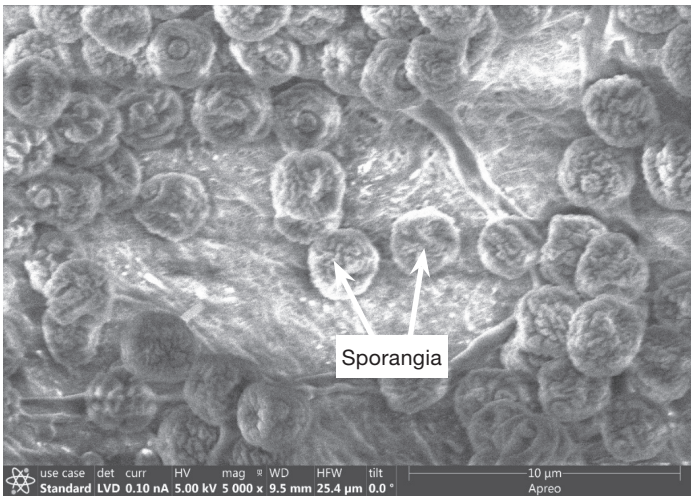


Figure 15. SEM analysis for Mold growth

Stained Leather Surface

Fungal growth can also be identified by their characteristic stains on wetblue, crust and finished leathers. These leathers have a blotchy appearance and in some cases, visible hyphae and sporangia as in the sample finished leather in Figure 16. If the drying process is too slow and the leather is left in a humid and warm environment, such as drying chambers with poor air circulation, there will be rapid fungal growth on the leather.

This biodeterioration will be observed as colored spots in various shades; grey, dark-brown, yellow-green, green, and brown-green as observed in Figure 16. These types of damage are associated with various fungal species including *Penicillium rugulosum*, *Penicillium glaucum*, *Penicillium funiculosum*, *Paecilomyces variotii*, *Aspergillus ochraceus* and *Aspergillus wentii.*[21] Occurrence of red spots on wet-blue leather is also an indicator of fungal growth. The red spots have been identified to be caused by various fungal species which include *Penicillium purpurogenum*, *Penicillium klebanii*,

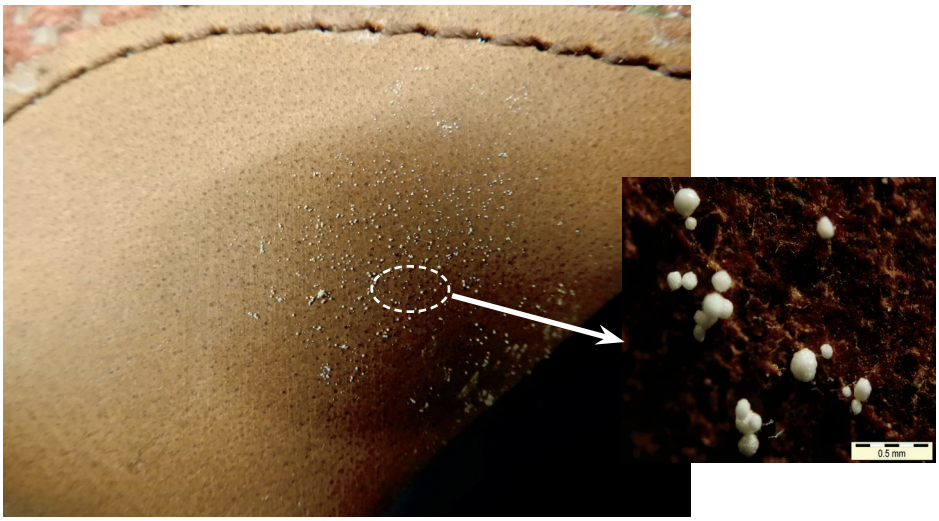


Figure 16. Fungal Growth on Finished leather

Penicillium roseopurpureum and *Penicillium aculeatum*.[21,25] The presence of phosphates, fatliquors, ammonium salts and other organic compounds in the tanned and retanned leather matter tend to promote the growth of fungi. Vegetable tannins contain polyphenols and carbohydrates in form of simple sugars which offer direct nutrients to fungi making vegetable-tanned leathers more susceptible to fungal growth compared to chrome-tanned leathers.[4] In vegetable tanning solutions, they grow on the surface causing fermentation of tanning agents.

Yeast Spots

Yeast growth may occur in leather that has been stored or shipped over a long period. On wetblue, the yeast growth areas are dark green as their by-products change the chrome complex color.[19] Mounds of yeast cells subsequently grow on the grain surface, as shown on the sample in Figure 13. This inhibits dye penetration and distribution in the subsequent processing stages leading to a patchy and botchy crust and finished leather.

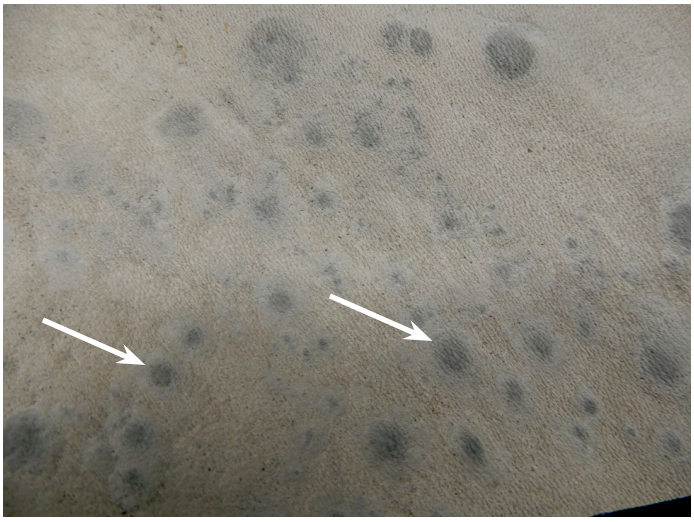


Figure 17. Mounds of Yeast growth areas on wetblue leather

Fungi are common when insufficient fungicide is used or when the microbes become tolerant to the fungicide. Previous studies have suggested a regular (every three months) change of fungicide is good practice to control fungal growth and damage.[19] Mold growth is prevalent in finished leather with a moisture content of 15% and above.[19] Therefore, sufficient drying and the use of a disinfectant are encouraged during the application of all aqueous finish materials to protect the protein binders from microbial degradation.

Conclusion

In conclusion, this study demonstrated that visual inspection, microscopy, and culturing techniques are effective for the identification of bacterial and fungal damage in leather. These techniques can be used alone or in combination to accurately detect and identify different types of damage and their causes at different stages, allowing for timely and effective treatment and prevention of further damage. The results of our study highlight the importance of routine monitoring and inspection of hides from slaughter, through the various processing stages, for the preservation of the leather quality and prevention of bacterial and fungal infections. Future research could focus on optimizing and validating these techniques for use in different working environments, as well as developing more rapid and cost-effective methods for the detection of microbial damage in leather.

Acknowledgments

The author acknowledges the Leather Research Laboratory for their collaboration and support towards this study and leather research in general. Special thanks to the laboratory's staff Cietta Fambrough and Michael Kelley who were very instrumental in this work.

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Appendix

Appendix 1: Paddle Test

Table 1 shows the density of bacterial colonies. **Table 2** shows the density of yeast and mold colonies. Compare the colonies on the paddle tester to the images in **Table 1** and **Table 2**. Select the image that is most similar to the colonies on the paddle tester, then use the density values above the image.

Table 1 Bacterial colony density

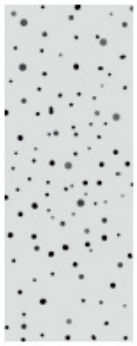
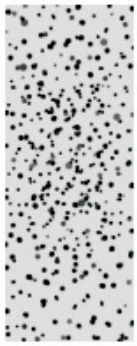
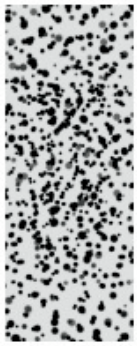
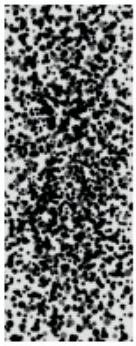
100 (10^2)	1000 (10^3)	10,000 (10^4)	100,000 (10^5)	1,000,000 (10^6)	10,000,000 (10^7)
					

Table 2 Yeast and mold colony density

100 (10^2)	1000 (10^3)	10,000 (10^4)	100,000 (10^5)	1,000,000 (10^6)